Final Report

National Agriculture Innovation Project (NAIP)
Indian Council of Agricultural Research, New Delhi

Utilization of seabuckthorn in the healing and prevention of gastric erosions and ulcers in animals

Principal Investigator

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Executive Summary

To study the prophylactic and therapeutic efficacies of seabuckthorn (SBT) and to ascertain the more effective treatment regimen for management of gastric ulcerations and erosions (GUE) in dogs, a number of different studies were undertaken. First a more dependable non-fatal experimental model of GUE was developed for dogs and then an effective and reliable endoscopic monitoring system was developed to evaluate the progression of GUE and healing process which obviated the need of killing laboratory animals to record similar observations as practice earlier. Later systematically the therapeutic efficacy of seabuckthorn seed oil was verified and its effective doses were determined for GUE management in dogs. Though the prophylactic efficacy of SBT seed oil could not be established in the present model of GUE in dogs and hence no further studies were undertaken in this direction. However, the therapeutic efficacy of SBT oil was further comparatively evaluated *vis-e-vis* routinely used allopathic drugs for GUE in a series of follow-up studies. The combinations of most useful allopathic drugs and seabuckthorn oil were also evaluated and their synergistic therapeutic effects were discovered first time in world. Thus a more effective, therapeutic regimen for management of GUE in dogs was established and recommended by this study. A number of other treatment combinations involving SBT seed oil and different herbal preparations were also initiated with an objective to further reduce the GUE healing time in dogs, but such studies are still underway.

Part-I: General Information of Sub-project

1. **Title of the sub-project: Utilization of seabuckthorn in the healing and prevention of gastric erosions and ulcers in animals**
2. **Sub-project code:**
3. **Component: 2**
4. **Date of sanction of sub-project: 9 June2008**
5. **Date of completion: March 2014**
6. **Extension if granted, from July 2012 to March 2014**
7. **Duration of the sub project: 5 years 9 months**
8. **Total sanctioned amount for the sub-project: 42.089 (Plus institutional charges)**
9. **Total expenditure of the sub-project: 40.03776**
10. **Consortium leader:**

Principal Investigator	**Dr SP Tyagi,** Associate Professor
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Telephone Number	01894-235287 (R), 230357 (O), 230351-874 (Work), 9418471225
Fax no.	01894-230327 (Dean office)
Email	sptyagivet@gmail.com
Name of Co- Principal Investigators	1. Dr AC Varshney, Professor 2. Dr Adarsh Kumar, Associate Professor 3. Dr Amit Kumar, Assistant Professor

11. **List of consortium partners:**

	Name of CPI/ CCPI with designation	Name of organization and address, phone & fax, email	Duration (From-To)	Budget (` Lakhs)
CPI				
CCPI1				
CCPI2				
CCPI 3				
CCPI 4				

CPI-Consortia Principal Investigator; CCPI-Consortia Co-Principal Investigator

12. **Statement of budget released and utilization partner-wise (` in Lakhs):**

	PI Name, designation & address)	Total budget sanctioned	Fund released (up to closing date)	Fund utilized (up to closing date)
PI	**Dr SP Tyagi**	**42.089**	**42.089**	**40.058**
Total				

Part-II: Technical Details

1. Introduction

A number of plant extracts and preparations had repeatedly shown to possess both anti-inflammatory and anti-gastroulcerative activities in different animal models (Tyagi 2006). Such facts are very important for management of gastric ulcerations though such feat is unthinkable to achieve with current range of routinely used allopathic anti-inflammatory drugs. Seabuckthorn plant was one such example and its oil had shown anti-gastroulcerative (Tyagi, 2006), hepatoprotective (Cheng, 1992), anti-cancerous (Li and Liu, 1991), anti-lipemic and anti-arrhythmic (Fengming, 1989) properties. Many scientists like Zhou *et al.* (1994), Che *et al.* (1998), Xing *et al.* (2002) and Suleyman *et al.* (2001) reported the therapeutic and preventive efficacy of the seabuckthorn oil in gastric ulcers in laboratory animals like rats and rabbits. Tyagi (2006) evaluated the prophylactic and therapeutic efficacy of seabuckthorn (*Hippophae rhamnoides*) seed oil in gastric ulcerations in dogs and reported that seabuckthorn seed oil had therapeutic and some limited prophylactic efficacy for dexamethasone-induced GUE in dogs. However, more intensive research studies were needed to ascertain the most effective doses of seabuckthorn preparations or its combinations with other drugs having better efficacy for prevention as well as treatment of gastric ulcers and erosions in animals. Hence, the present work was undertaken.

2. Overall Sub-project Objectives

1) To study therapeutic efficacies of seabuckthorn oil in gastric ulceration and erosions in animals.
2) To study prophylactic efficacies of seabuckthorn oil in gastric ulceration and erosions in animals.
3) To develop seabuckthorn oil based ulcer prevention/treatment formulations.

4) Sub-project Technical Profile

SN	Activities	Verifiable indicators	1st year	2nd year	3rd year	4th year	5th year	6th year
1.	Recruitment of contractual staff	Actual recruitment	√					
2.	Procurement of equipment	Procured equipment	√					
3.	Procurement of other operational items	Procured operational items	√	√	√	√	√	√
4.	Renovation of experimental animal house	Actual renovation completion report	√					
5.	Renovation of lab/operation theatre and office	Actual renovation completion report	√					
6.	Collection of Indian varieties of seabuckthorn from its natural habitat	Actual purchase receipts, stocks	√	√	√	√		
7.	Extraction/procurement of seabuckthorn oil	Actual purchase receipts, stocks	√	√	√	√		
8.	Procurement of experimental animals	Actual purchase receipts, animal maintenance records	√	√	√	√	√	√
9.	Maintenance of animals under standard managerial conditions		√	√	√	√	√	√
10.	Pilot trials for creation of experimental models of gastric ulcerations and erosions (GUE)	Detailed research reports	√	√				
11.	Studies on therapeutic efficacy of seabuckthorn in GUE	Detailed research reports			√			
12.	Studies on prophylactic efficacy of seabuckthorn in GUE	Detailed research reports			√	√		
13.	Studies on therapeutic efficacy of seabuckthorn combinations in GUE	Detailed research reports				√	√	√
14.	Repeat studies on above aspects,	Detailed research reports			√	√	√	√

	if required							
15.	Development of formulation for GUE	Detailed research reports					√	√
16.	Final project report	Final report						√

Broad experimental protocol for different sets of research trials:

1. Pilot trials for improving experimental model of gastric ulceration and erosions (GUE) in dogs.
2. Studies on comparative evaluation of the therapeutic efficacy of Seabuckthorn seed oil *vis-e-vis* routinely used allopathic drugs for GUE in dogs
3. Studies on therapeutic efficacy of different doses of Seabuckthorn seed oil alone and in combination with famotidine for GUE in dogs
4. Studies on therapeutic efficacy of Seabuckthorn seed oil in combination with sucralfate and misoprostol for GUE in dogs
5. Studies on comparative evaluation of prophylactic efficacy of Seabuckthorn seed oil *vis-e-vis* routinely used allopathic drugs for GUE in dogs
6. Studies on therapeutic efficacies of herbal extracts alone and other combinations of seabuckthorn seed oil for GUE in dogs.

Expected output/outcome: Seabuckthorn based therapeutic and prophylactic treatment regimen for management of GUE in dogs.

5) Baseline Analysis

SN	Previous relevant studies done before start of this sub-project	
1.	**Studies related to determination of the risks of GUE in dogs due to variety of reasons**	Stanton and Bright (1989), Dow (1990), Maruoka *et al.* (1993), Forsyth *et al.* (1998), Rohrer *et al.* (1999) and Boston *et al.* (2003)
2.	**Studies related to effect of routine medicines in the treatment or prevention of GUE in dogs**	Okabe *et al.* (1978), Boulay *et al.*(1986), James *et al.*(1986), Johnston *et al.*(1995), Jenkins *et al.*(1991), Ward (2000) and Davis *et al.* (2003).
3.	**Studies related to effect of SBT on GUE in rats and dogs**	Zhou (1986), Che *et al.* (1998), Suleyman *et al.* (2001), Xing *et al.* (2002) and Tyagi (2006).

As per the research done till the start of this sub-project, the GUE was well recognized disease entity in dogs but its management was considered difficult. Seabuckthorn seed oil had been found efficacious in the treatment of GUE in rats and dogs to some extent. In one study, the SBT oil was reported to be even better that routinely used medicines for GUE in rats. However, no controlled study was undertaken before to comprehensively verify the comparative efficacies of SBT oil vis-e-vis other standard GUE drugs. In fact even standard drugs for management of GUE had also not been investigated in details in dogs.

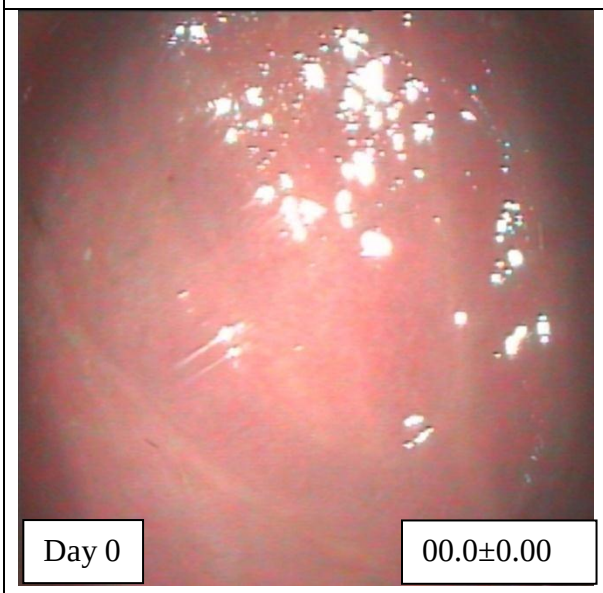
6) Research Achievements with Summary

COMMON EXPERIMENTAL RESEARCH SCHEDULE:

Selection and management of experimental animals: Requisite prior permission for whole experimentation was duly obtained from institutional animal ethics committee. Average sized apparently healthy adult mongrel dogs weighing 15-25 kg were utilized. They were acclimatized in the college kennel for a period of 15 days under standard managerial condition prior to the start of the trial. These dogs were vaccinated with anti-rabies vaccine (Raksharab @ 1ml/ dog SC), dewormed with Praziplus @ 1 tab / 10 kg body weight (BW). They were also given ectoparasiticidal bath with carbaryl 10% (Notix, Petcare) and treated with Inj. Ivermectin (Neomec, Intas Pharmaceuticals, India) @ 200mcg/kg BW SC, for ectoparasites. All animals were regularly exercised and fed a uniform commercial adult dog complete diet (Pedigree meat rice and Pedigree chicken vegetable or Nutripet, Petcare, India) twice daily along with water access throughout the day.

Creation of non-fatal GUE: This model of gastric ulceration and erosions (GUE) was standardized in pilot trials. For this Inj. dexamethasone was administered in all the dogs @ 1mg/kg, IV, b.i.d. until there was endoscopic evidence of GUE ulcer index reaching to 7 on two consecutive endoscopic observations or 8 in a single observation occasion as per Tyagi (2009) (Table 1). In trails where therapeutic efficacies of different test drugs/SBT oil were evaluated, the day '0' was considered the day on which above GUE index was achieved and the test drugs were started. Whereas, in trials where prophylactic efficacies were evaluated, the day '0' was the initial day of the dexamethasone administration because the test drugs/SBT oil were also started simultaneously.

Plate 1: Endoscopic view of gastric mucosal surface of dogs along with their GUE index during creation of GUE

<table>
<tr>
<td> 
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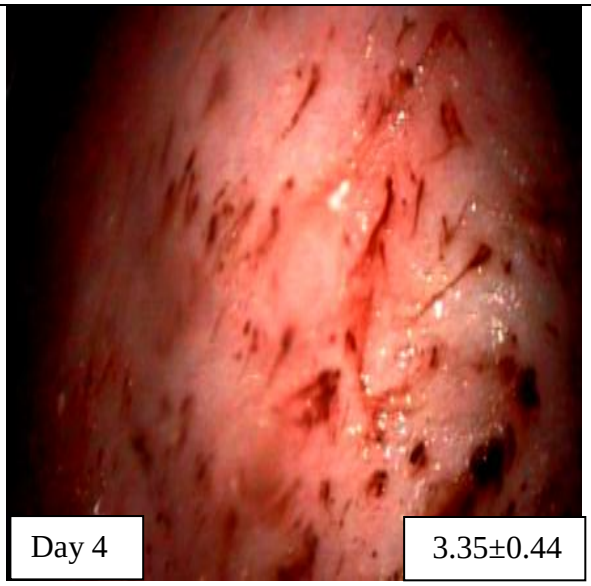
<td> 
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<tr>
<td>Apparently normal healthy mucosa with shiny and transparent mucous layer.</td>
<td>Multifocal, superficial lesions with adherent blood clots. Thin mucous layer showing engorged blood vessels</td>
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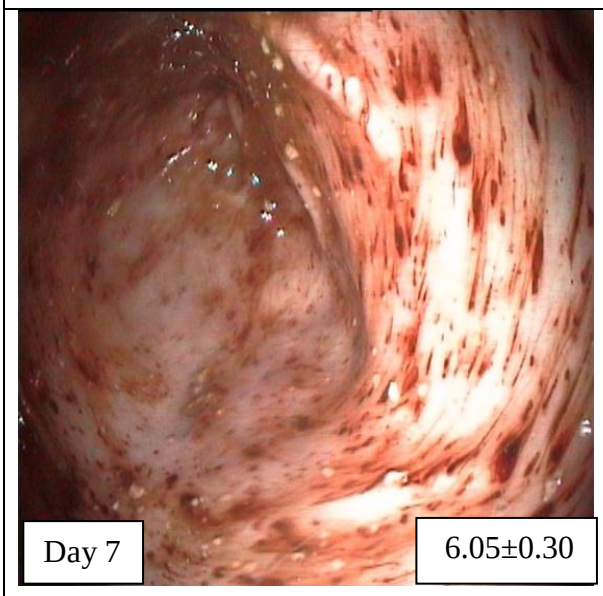
<tr>
<td> 
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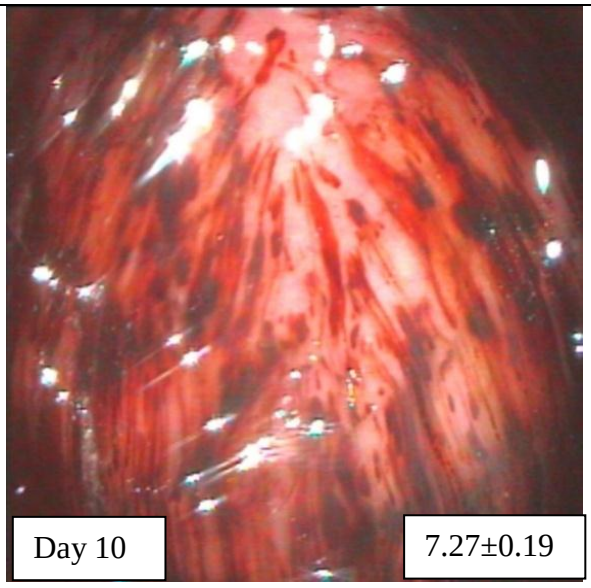
<td> 
</td>
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<tr>
<td>Multifocal punctate as well as linear gastric lesions with active hemorrhagic base.</td>
<td>Large, deep, diffuse lesions with adherent blood clots covering most of the mucosa</td>
</tr>
</table>

Observations: Development of GUE and their progress of healing were evaluated and compared on the basis of clinical, haematological, faecal occult blood test (FOBT) and endoscopic observations on every third day till complete healing of GUE lesions. Biochemical parameters were done on weekly basis to see any adverse effect on liver or kidney. Clinical parameters were rectal temperature (ºF), heart rate (/min), respiration rate (/min), colour of mucous membrane (cmm) , body weight (kg), variations in appetite, vomiting, colic, melena, diarrhea, constipation, any change in hair coat and skin or any other behavioral change in dogs. Hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC) and Differential leukocyte count (DLC) were used as hematological parameters along with Aspartate transaminase (AST), Alanine transaminase (ALT), Total protein (TP), Blood urea nitrogen (BUN) and Creatinine (CRTN) used as biochemical parameters.

Gastro-endoscopic examination: GUE index was determined on the basis of the number of gastric lesions and severity scoring system as per Table 1 developed during pilot trials of the project. For this, the dogs were kept off food for 12 hours and off water for 4-6 hours before the procedure. Then dogs were anesthetized using Xylaxine @ 2mg/kg BW and Ketamine @ 10 mg/kg BW given intramuscularly. Endoscopic examination of their stomach was performed using a 9 mm o.d. flexible fibre optic gastro-duodenoscope (Karl Storz, Germany) with a 1.5 metre working length after intubating the animal and keeping them in right lateral recumbency. During gastro-endoscopic examinations all the areas of stomach namely fundus, gastric body and pylorus were examined for GUE lesions.

Table 1.	**Description**
Score	**Gastric lesion number scoring system**
0	• No lesions
1	• 1-2 localized lesions
2	• 3-5 localized lesions
3	• 6-10 lesions
4	• >10 lesions/very large/diffuse lesion
Score	**Gastric lesion severity scoring system**
0	• No blood clots
1	• Free floating or adherent smaller blood clots with no detectable haemorrhage base
2	• Adherent smaller blood clots with active haemorrhage base • Apparently superficial smaller focal mucosal erosion (<3mm) with or without active haemorrhage • Apparently superficial linear mucosal erosion without active haemorrhage • Sub mucosal haemorrhages or erythmatous mucosa • Adherent larger blood clots without active haemorrhage base
3	• Apparently superficial larger focal mucosal erosion (>3mm) with or without active haemorrhage • Linear erosions with active bleeding • Adherent larger blood clots with active haemorrhage base • Apparently deeper mucosal lesions without haemorrhage
4	• Apparently deeper mucosal lesion /ulcer with adherent large blood clots or with active haemorrhage

Net gastric–ulcerations-erosions (GUE) index= Gastric lesion number score+ Gastric lesion severity score.
*In case of mixed lesions as per above description, a higher score was assigned.

Preparation of SBT Oil: Fresh seabuckthorn (*Hippophae rhamnoides turkestanica*) seeds were procured from their natural habitat from 'Distt. Lahaul' (Himachal Pradesh, India). These seeds were dried and subjected to cold mill-press method to obtain oil in sufficient quantity.

Statistical Analysis: Wherever required, the statistical analysis of data was carried out using analysis of variance (ANOVA) using Students-Newman-Keuls test (intragroup comparison) and Dunnett's test (intergroup comparison) of Instat software (Graphpad) at 5 % and 1% level of significance.

The detailed research summary is as follows-

1. Pilot trials for creation of experimental model of gastric ulcerations and erosions:

The study was done in two phases. In first phase, a series of trials were conducted using 12 dogs. They were divided into four equal groups. Intravenous/intramuscular injections of different non-steroidal and steroidal drugs were administered twice a day for variable periods up to 22 days.

Table 2: Details of different groups

Group I	Dexamethasone @ 1 mg/kg BW
Group II	Prednisolone @ 1 mg/kg BW
Group III	Meloxicam @ 1 mg/kg BW
Group IV	Ketoprofen @ 2 mg/kg BW

Results and Discussion:

Clinical observations:

Group I dogs showed reduced appetite after the first week of injections and became anorectic towards the end of second week. Vomiting was noted once at 13th day in one dog. Melena was recorded in two dogs while one dog defecated loose pungent stool after the first week of injection for 3 days. Moderate melena continued till 16-19 days. Drug was discontinued at day 13 in one dog and at day 16 in two dogs in this group. Similar finding are also reported by Dogra *et al.* 2013 and Gupta 2012.

Group II dogs showed no sign of anorexia. Mild melena was recorded in one dog at day 6 and 7. Vomiting was recorded only once at day 7 and day 10 in two different dogs. Drug was discontinued at day 19. Reto *et al.* 2008 reported gastric mucosal lesions in dogs treated with dexamethsone and prednisolone at various doses for treatment of acute intervertebral disc disease which were not responsive to omeprazole at 0.7 mg/kg orally once daily, or misoprostol at 2 µg/kg orally 3 times daily.

Group III dogs showed anorexia from day 3; severe vomiting in one and moderate vomiting in other two dogs were observed from day 3-4. Moderate melena from 4th day and severe melena from day 9 was also observed. Drug was discontinued at day 4 in two dogs and at day 10 in one dog. One of the dogs died due to duodenal rupture at day 5. The post-mortem examination of this animal revealed severe duodenal ulcer accompanied with hemorrhagic enteritis and peritonitis. However, gastric lesions were absent. Trevor *et al.* 2006 reported five canine cases of gastrointestinal (GI) perforation and septic peritonitis associated with the routine use of meloxicam and advised to use meloxiacam with caution in routine clinical practice.

Group IV dogs showed no signs of anorexia or vomiting, though one dog showed slight reduction in feed intake from day 3 onwards. Melena was noted at day 4 in one dog and at day 15 in another dog. Injection was stopped after 22 days. Narita *et al.* 2005 also reported the adverse effects of long-term administration of ketoprofen observed in the study were not clinically important in healthy dogs

Weight loss was observed in all groups which ranged from 1-5kg. The mean weight loss recorded was 3, 2.6, 3.5 and 2.5 kg in group I, II, III and IV respectively. The weight loss was due to decrease in food intake and it was less in group IV as there was least effect on appetite in this group of animals.

The mean heart rate decreased till day 10 and then increased in group I, II and IV but in group III, the mean heart rate increased from the base value up till day 13. The mean respiration rate slightly fluctuated

but was within the normal range in all the groups. The mean rectal temperature decreased towards the end of trial but was within the normal range in all groups.

Haematological observations:

The mean Haemoglobin (Hb) levels decreased gradually till day 14 in all the groups and then showed increasing trends. The packed cell volume (PCV) also decreased from the base value till day 14 in group II and III and up to day 21 in group I. Group IV dogs show fluctuation in the mean PCV. Mean Total erythrocyte count (TEC) decreased till day 7 in group III and IV and till day 14 in group I (which was significant) and group II. Thereafter, TEC gradually increased in all the groups. These finding are also reported by Dogra *et al.* 2013, Thakur 2011, Gupta 2012 and Thakur 2013 who studied effect of various gastric ulcer healing drugs in different dosage and frequency for treatment of dexamethsone induced non-fatal gastric ulcers in dogs. The decrease in Hb, PCV and TEC is due to continuous bleeding in gastrum of the dogs due to effects of various steroidal and non-steroidal anti-inflammatory agents used in the study.

Table 3: Mean Hb, PCV, TEC and TLC of different groups of dogs at different intervals of time.

Day	Hb(g%)				PCV (%)			
	Groups				Groups			
	I	II	III	IV	I	II	III	IV
0	13.4	14	10.8	11.73	49.66	46	42	43.3
7	13.26	12.86	10.5	9.93	52.33	45.3	39	33.66
14	10.46	12.66	8	10.9	39.66	45	35	46.66
21	10.8	13.6		13	34.66	53		42
Day	**TEC($\times 10^6$)**				**TLC($\times 10^3$)**			
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
0	5.67	7.6	5.14	6.24	26.8	17.6	18.10	19.12
7	4.1	5.95	3.73	4.35	39.13	20.05	22.22	20.05
14	4.07*	5.42	4.79	4.89	32.45	16.66	61.30	20.45
21	4.56	8.77		3.38	30.84	11.05		22.26

*P<0.05 when compared with the baseline values within the group

Mean total leucocyte count (TLC) increased at day 7 and then decreased in group I and II dogs. Group III and IV dogs showed increase till day 14 and 21 respectively. Significant increase in neutrophils was observed in group I dogs at day 7 and day 21 and at day 14 in group II. Gradual decrease in mean neutrophils were seen in group II and IV but were insignificant statistically. Significant reductions in mean lymphocytes were observed in group I and II at day 7, 14 and 21. Monocytes, eosinophils and basophils remained within the normal range in all the dogs.

Table 4: Mean neutrophils and lymphocytes of different groups of dogs at different intervals of time.

Day	Neutrophils (%)				Lymphocytes (%)			
	Groups				Groups			
	I	II	III	IV	I	II	III	IV
0	59.33	57.66	65.33	70	31.33	36.66	22	22
7	80*	67	60	66	10.6*	24.66*	34	20
14	76.66	76.66*	56	64.66	16*	19.33*	32	17
21	90*	56		67	8*	35		19

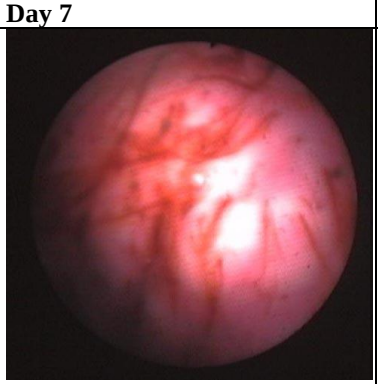
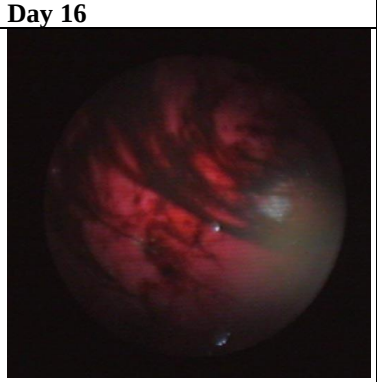
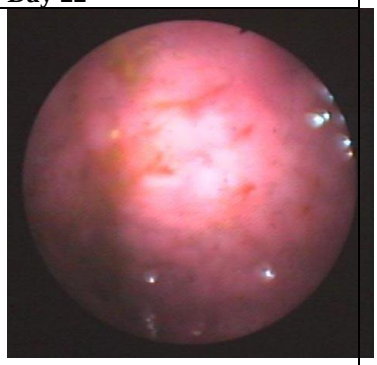
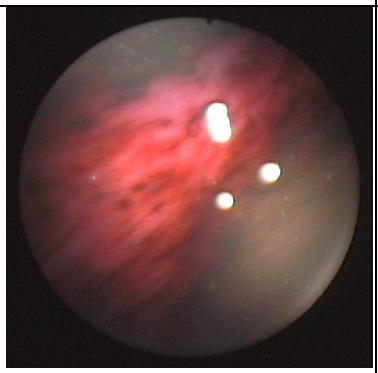
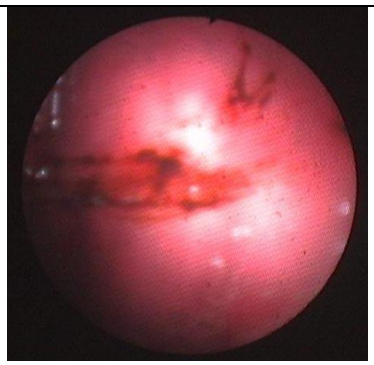
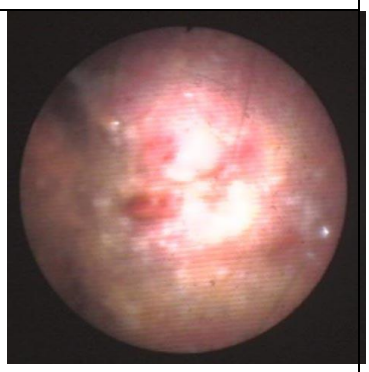
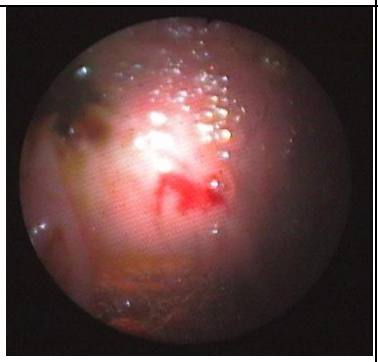
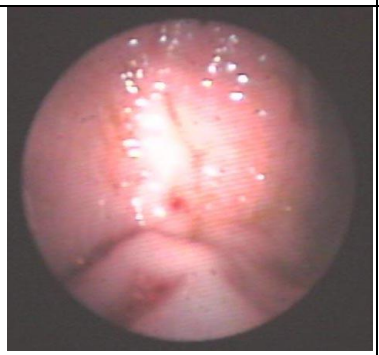
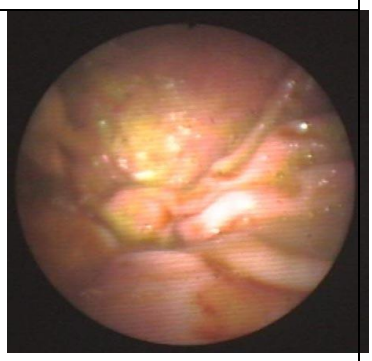
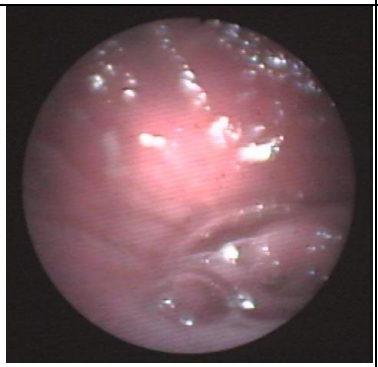
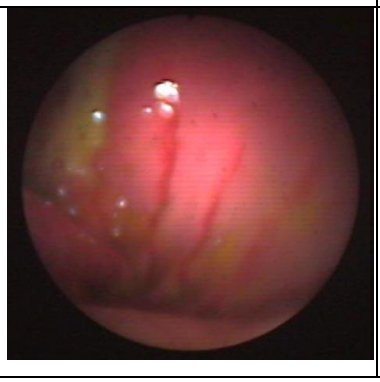
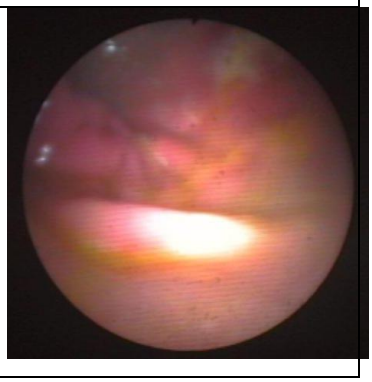
*P<0.05 when compared with the baseline values within the group

Biochemical observations:

Throughout the trial the mean AST, ALT, BUN and CRTN values remained within the normal range and no animals showed any abnormal clinical signs as well which might be suggestive of hepatic or renal involvement.

Gastro-endoscopic observations:

Plate 2: Representative endoscopic appearance of gastric mucosa in different groups at different time intervals

	Day 7	Day 16	Day 22
Gr I			
Gr II			
Gr III			
Gr IV			

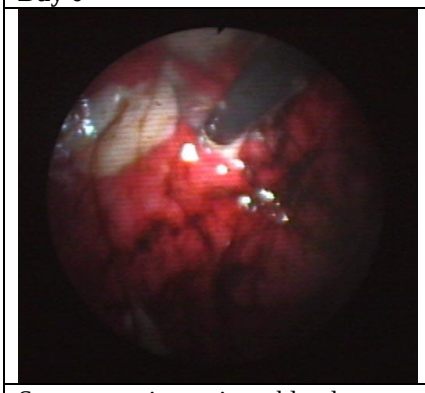
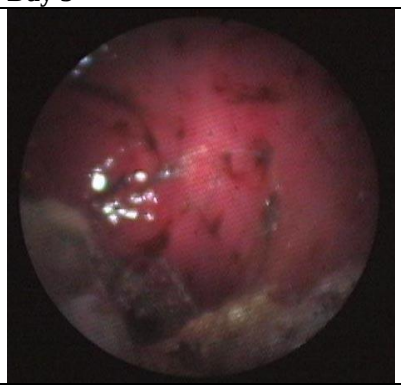
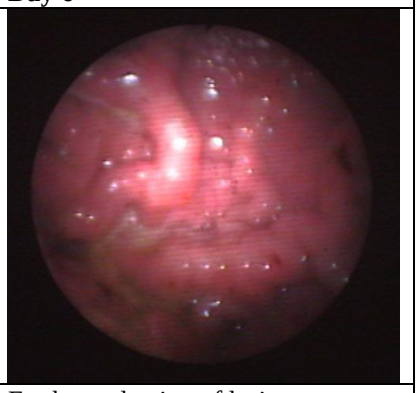
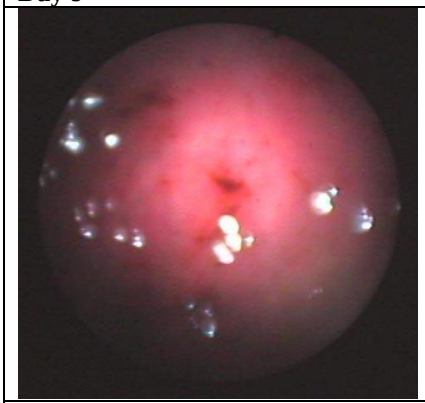
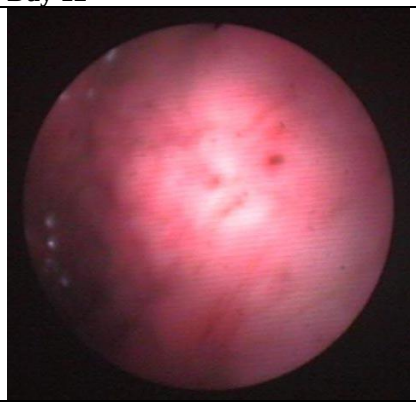
Group I animals showed gradually increasing severity of gastric lesions starting from the very first endoscopic observation i.e. day 4 after initiation of drug. The desired severity of gastric lesions corresponding to 7/8 gastric index reached in two animals on day 10, 1 animals on day 13 and 1 animal on day16th. Such gastric lesions were in general consisted of multiple linear as well as focal mucosal

defects of variable shapes and depths. The lesions were generally larger and widely distributed all over the gastrum i.e. fundus, body and pylorus. Large adherent multiple blood clots as well as fresh blood was also observed inside stomachs in such dogs. The mucosa was severely hyperemic and the mucus layer was appreciably very thin. Such findings are more or less similar to those reported by Tyagi (2006), however he used dexamethasone single a day for 16 days uniformly in all dogs. By increasing the frequency of dexamethasone in the present study from single a day to twice a day, the desired GUE index could be achieved in a shorter duration.

Following discontinuation of dexamethasone after achieving the desired GUE index, the spontaneous healing also occurred in a gradual and predictable manner in all the animals of group I within 12-15 days. In group II the results were inconsistent as 2 animals showing severe degree of gastric lesions on day 7 showed reduced severities on the following intervals whereas remaining 2 animals did not develop desired GUE index even up to day 21 just like all the animals of group IV. The group III animals showed different degrees of gastric ulcerations but none achieved desired GUE index.

In second phase of the study, 3 dogs were utilized and administered Prednisolone @ 2 mg/kg IM/IV b.i.d, but the results still remained inconsistent. Hence, it was concluded that Dexamethasone @ 1 mg/kg BW leads to the development of GUE index of 7-8 in all the animals in a reliable and repeatable manner.

Plate 3: Spontaneous healing of GUE lesions in group I after discontinuation of dexamethasone

Day 0	**Day 3**	**Day 6**
		
Severe gastric erosions, blood streaks running all over the stomach	Reduced severity of lesions	Further reduction of lesions
Day 9	**Day 12**	
		-
Mild lesions present at few regions at pylorus	Very mild lesions left to recover	

2. Studies on comparative evaluation of the therapeutic efficacy of seabuckthorn seed oil *vis-e-vis* routinely used allopathic drugs for GUE in dogs

20 dogs divided into five equal groups were utilized in this study. The animals were treated with following drugs (Table 5) twice a day respectively till complete healing of GUE.

Table 5: Details of treatment in different groups

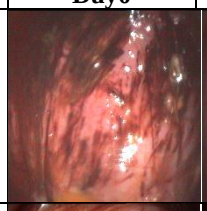
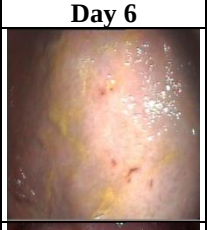
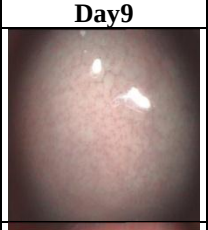
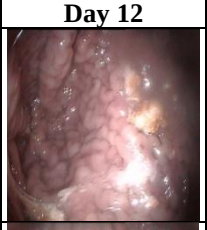
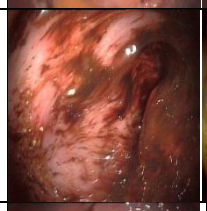
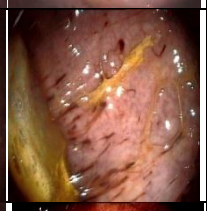
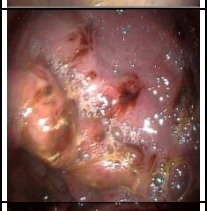
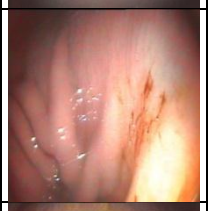
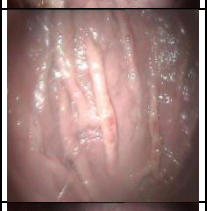
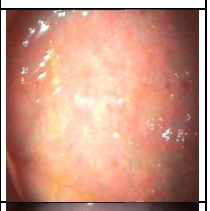
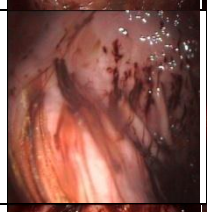
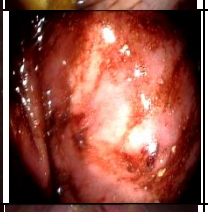
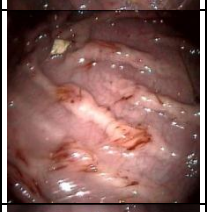
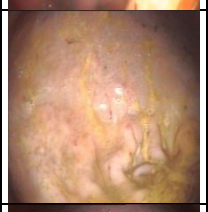
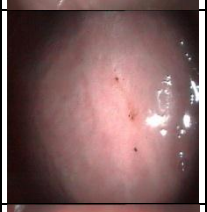
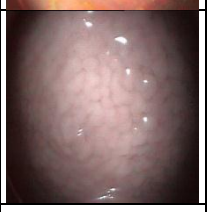
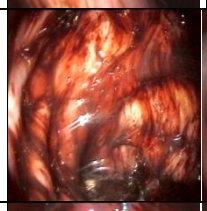
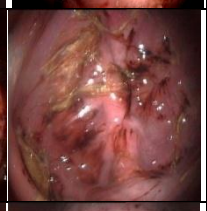
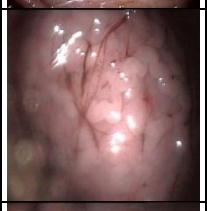
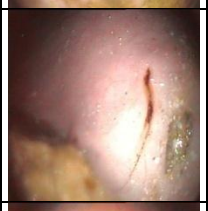
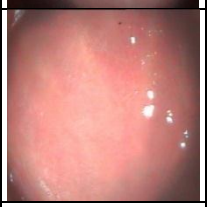
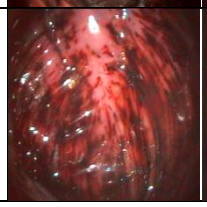
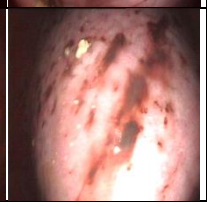
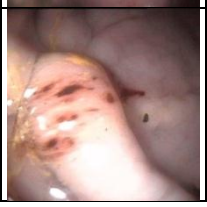
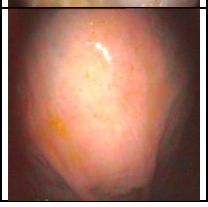
Group I	Lansoprazole (Lanzol-30, Cipla, India) @ 1.5mg/kg PO bid
Group II	Sucralfate (Sparacid, Dr. Reddy's Laboratories, India) @ 1g/10kg PO bid
Group III	Misoprostol (Misoprost-200, Cipla, India) @ 10µg/kg PO bid
Group IV	Famotidine (Famtac, Piramal Healthcare, India) @ 1mg/kg PO bid
Group V	Seabuckthorn oil @ 5ml PO bid

Results & Discussion:

Gastro-endoscopic observations:

On day 0, the GUE indices were 8.00±0.00, 7.75±0.25, 7.25±0.25, 7.75±0.25 and 7.50±0.29 in group I, II, III IV and V respectively. The GUE indices decreased gradually in all the groups after the start of treatment, however the healing occur fastest in SBT oil treated group followed closely by famotidine treated group. The restoration of protective mucus layer was also fastest in these groups.

Plate 4: Endoscopic view of gastric mucosal surface of dogs in different groups at various observation intervals

Groups	Day0	Day 3	Day 6	Day9	Day 12	Day 15
G I Lans						-
G II Sucral						
G III Miso						
G IV Famo						-
GV SBT oil					-	-

The complete healing of GUE lesions occurred in Seabuckthorn oil treated group in 7.50 days as compared to 8.25 days in group IV, 9.00 days in group I, 10.50 days in group III and 13.50 days in group II (Plate 3). Overall the average healing time was considerably lesser in the above test groups except group II when compared with the average spontaneous healing time of GUE lesions as observed during pilot trials.

Such observed gastric ulcers healing property of SBT is in agreement with previous studies on rats and dogs (Jiang *et al.* 1989; Mironov*et al.* 1989; Xiao *et al.*1992; Zhou *et al.* 1994; Che*et al.* 1998; Suleyman*et al.* 2001; Xing *et al.* 2002, Tyagi 2006 and Xu*et al*. 2007) and humans (Qiu and Qiao 1997 and Nikitin*et al*. 1989). It has been found effective against various kinds of gastric ulcers induced by physically necrotizing agents, NSAIDs or stress. Jiang *et al.* (1989) identified an anti-ulcer component of SBT oil i.e. β-sitosterol- β -D-glucoside which significantly decreased the size of the ulcer area in their studies in certain kinds of ulcers.

Table 6: GUE indices of dogs of different groups at various observation intervals (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Group I	8.00 ±0.00	5.00** ±0.00	2.00** ±0.71	0.66** ±0.66(n=3)	0.00 ±0.00(n=1)	–
Group II	7.25 ±0.25	5.25** ±0.25	3.50** ±0.29	2.75** ±0.48	0.50** ±0.50	0.00 ±0.00(n=1)
Group III	7.75 ±0.25	6.75 ±0.25	3.25* ±1.25	3.33* ±1.33(n=3)	1.00** ±0.00(n=3)	0.00 ±0.00(n=1)
Group IV	7.50 ±0.29	4.75 ±0.63	2.25** ±1.32	1.00 ±1.0(n=2)	0.00 ±0.00(n=1)	–
Group V	7.75 ±0.25	5.00 ±0.82	2.50** ±1.44	0.00 ±0.00(n=2)	–	–

*($p<0.05$), **($p<0.01$)

Clinical observations:

The rectal temperature, respiration rate and heart rate did not vary much with the base values and remained within normal physiological limits throughout the period of study in all the groups. No statistical difference was observed between various groups at any observation intervals.

A marked improvement in appetite was observed in all the animals during treatment. Most of the animals started showing improvement 3 days after the start of treatment but two animals continued with decreased appetite till 9th day in group II. Towards the end of the study all the animals had regained their normal appetite. During treatment no vomiting and diarrhoea were observed in any of the animals but melena was observed till day 3 in group V, day 6 in group I and group IV whereas, it continued to be seen till 9th day in group II and group III. The severity of melena gradually decreased towards the end of study in all the groups.

A non-significant gain of body weight (restoration towards normal) in dogs of all groups was observed. By the end of observation period, the maximum gain in body weight was 3.64 %, 4.64 %, 4.22 % 0.87 % and 6.44 % in groups I, II, III, IV and V respectively. Therefore, weight gain was highest in group V followed by group II, group III, group I and then group IV. Greater weight gain in Seabuckthorn oil group can be attributed due to faster healing of GUE lesions (as evidenced endoscopically), early restoration of normal appetite and rapid normalization of digestive processes.

Fecal occult blood test (FOBT)

The faecal occult blood test was strongly positive in all the groups at day 0. Thereafter, the strength of FOBT reactions gradually decreased but varied within and in between various groups. On all the instances, a direct correlation was observed between detection of blood clots or gastric lesions endoscopically and a corresponding FOBT reaction. No false positive or false negative reaction was observed at any intervals. This indicated that faecal occult blood test is proficient in diagnosing smaller quantities of blood in faeces in cases of subclinical GUE in dogs. Gilson *et al.* (1990) also reported that

faecal occult blood tests could detect quantities of blood that were smaller than those required to cause melena. Rohrer *et al.* (1999) too reported detection of occult blood in high percentage of dogs (9/10) in which gastric haemorrhages was evident after administration of methyl-predinisolone sodium succinate.

Hematological Parameters

In general, a gradual rise in Hb, PCV and TEC levels was observed from 0 day till the end of study in all the groups except group II. However, the rises were statistically insignificant within as well as in between groups. In group II, Hb, PCV and TEC continued to drop till 6th day but started rising thereafter. PCV improved earliest in group V followed by group I, IV and lastly II and III.

Table 7: Haemoglobin, packed cell volume and total erythrocyte counts of different groups at various observation intervals (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Hb (g/dl)						
Group I	11.05 ±0.82 (n=4)	12.17 ±0.67 (n=4)	12.77 ±0.69 (n=4)	12.93 ±0.96 (n=3)	14.3 ±0.00 (n=1)	_
Group II	10.75 ±1.27 (n=4)	10.05 ±1.38 (n=4)	9.75 ±1.01 (n=4)	10.6 ±0.83 (n=4)	11.9 ±0.36 (n=4)	13.4 ±0.00 (n=1)
Group III	8.73 ±1.41 (n=4)	9.97 ±1.24 (n=4)	10.05 ±1.29 (n=4)	9.3 ±0.40 (n=3)	9.64 ±0.38 (n=3)	9.8 ±0.00 (n=1)
Group IV	11.63 ±0.42 (n=4)	11.83 ±0.56 (n=4)	11.95 ±1.89 (n=4)	9.15 ±3.65 (n=2)	6.5 ±0.00 (n=1)	_
Group V	12.80 ±0.53 (n=4)	13.10 ±0.64 (n=4)	13.83 ±0.65 (n=4)	14.45 ±0.25 (n=2)	_	_
PCV (%)						
Group I	29.15 ±2.01 (n=4)	31.73 ±1.42 (n=4)	34.25 ±1.56 (n=4)	35.13 ±1.33 (n=3)	35.20 ±0.00 (n=1)	_
Group II	27.90 ±3.20 (n=4)	26.62 ±2.84 (n=4)	25.45 ±1.87 (n=4)	28.23 ±1.22 (n=4)	31.25 ±0.92 (n=4)	36.40 ±0.00 (n=1)
Group III	23.35 ±3.80 (n=4)	26.38 ±3.32 (n=4)	29.42 ±4.27 (n=4)	26.07 ±2.48 (n=3)	26.73 ±2.71 (n=3)	22.00 ±0.00 (n=1)
Group IV	29.98 ±1.03 (n=4)	30.63 ±1.74 (n=4)	28.05 ±5.24 (n=4)	20.00 ±3.8 (n=2)	19.80 ±0.00 (n=1)	_
Group V	32.28 ±1.71 (n=4)	33.70 ±1.85 (n=4)	35.55 ±1.94 (n=4)	38.20 ±1.40 (n=2)	_	_
TEC (X10^{12}/L)						
Group I	4.18 ±0.22 (n=4)	4.65 ±0.17 (n=4)	4.99 ±0.07 (n=4)	5.20 ±0.17 (n=4)	4.92 ±0.00 (n=1)	_
Group II	3.95 ±0.56 (n=4)	3.73 ±0.61 (n=4)	3.54 ±0.50 (n=4)	3.94 ±0.37 (n=4)	4.44 ±0.26 (n=4)	5.12 ±0.00 (n=1)
Group III	3.48 ±0.56 (n=4)	3.85 ±0.38 (n=4)	4.08 ±0.26 (n=4)	3.92 ±0.47 (n=3)	4.27 ±0.32 (n=3)	3.95 ±0.00 (n=1)
	4.37	4.48	4.56	3.46	2.32	_

Group IV	±0.23 (n=4)	±0.31 (n=4)	±0.82 (n=4)	±1.44 (n=2)	±0.00 (n=1)	
Group V	4.89 ±0.34 (n=4)	5.00 ±0.31 (n=4)	5.11 ±0.35 (n=4)	4.97 ±0.99 (n=2)	_	_

TLC and granulocytes decreased in all the groups over different observation intervals but the decrease in TLC was significant on 6th, 9th, and 12th day in group II, on 3rd, 6th, 9th, and 12th day in group III and on 3rd and 6th day in group IV and V.

Table 8: Total leukocyte counts (X 10^9/L) in different groups at various observation intervals (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Group I	25.10 ±4.33	23.30 ±2.05	17.87 ±3.79	17.33 ±2.54	13.2 ±0.00(n=1)	_
Group II	34.18 ±4.39	25.18 ±4.61	15.22** ±2.50	12.95** ±3.10	11.15** ±2.10	6.9 ±0.00(n=1)
Group III	31.95 ±4.68	21.83* ±2.59	11.63** ±2.61	11.47** ±3.34(n=3)	9.07** ±1.37(n=3)	9.1 ±0.00(n=1)
Group IV	28.85 ±1.95	18.23* ±5.00	11.23** ±1.38	10.65 ±1.25(n=2)	11.80 ±0.00(n=1)	_
Group V	28.03 ±1.98	21.73* ±1.40	14.48** ±1.84	7.85 ±1.05(n=2)	_	_

*(p<0.05), **(p<0.01)

Similarly, significant decrease in granulocytes was observed on 6th, 9th, and 12th day in group III. Towards the end of study lymphocytes and monocytes increased in all the groups but increase in lymphocytes was significant day 6th and 9th in group V only. No statistically significant variations however, were observed within different groups in TLC and DLC.

Table 9: Differential leukocyte count (%) in dogs of different groups at various observation intervals (Mean±S.E)

Granulocytes (%)

Days / Groups	0	3	6	9	12	15
Group I	88.73 ±1.01	83.65 ±0.64	74.92 ±3.69	71.23 ±5.67	86.60 ±0.00(n=1)	_
Group II	91.10 ±1.91	86.97 ±1.31	84.50 ±1.87	80.5 ±1.59	78.42 ±2.86	81.9 ±0.00(n=1)
Group III	88.57 ±1.55	84.43 ±2.86	79.90* ±2.14	75.5** ±1.15(n=3)	71.33** ±1.18(n=3)	67.00 ±0.00(n=1)
Group IV	85.48 ±3.68	87.05 ±3.00	78.28 ±3.42	65.00 ±7.10(n=2)	50.80 ±0.00(n=1)	_
Group V	89.18 ±0.79	84.40 ±3.36	80.33 ±2.28	76.4 ±9.60(n=2)	_	_

Lymphocytes (%)

Group I	8.73 ±1.06	13.57 ±0.81	21.45 ±3.16	18.93 ±0.07	11.20 ±0.00(n=1)	_
Group II	6.66 ±1.44	9.73 ±1.57	11.42 ±1.02	15.00 ±1.54	14.35 ±1.63	12.60 ±0.00(n=1)
Group III	8.40 ±1.21	12.43 ±2.82	16.45 ±2.08	20.20 ±1.21(n=3)	20.70 ±3.61(n=3)	28.60 ±0.00(n=1)

Group IV	10.58 ±2.15	10.03 ±2.75	17.18 ±2.70	28.70 ±7.80(n=2)	36.60 ±0.00(n=1)	_
Group V	8.35 ±0.55	12.83** ±1.41	16.35** ±0.64	20.85 ±4.15(n=2)	_	

*($p<0.05$), **($p<0.01$)

Biochemical parameters

AST level remained elevated than base values and decreased subsequently towards the end. The variations were however, insignificant in all the groups at various observation intervals. The patterns of variation in ALT values were again dissimilar in different groups. BUN and CRTN levels of dogs in all the groups did not vary much with the base values of day 0 and remained within normal physiological limits throughout the period of study.

Thus it was established that Lansoprazole, sucralfate, misoprostol, famotidine and Seabuckthorn oil are safe to administer in dogs as these drugs did not resulted into any adverse effect on haematological as well as biochemical parameters in any of the groups. Jensen *et al.* (1993) reported that lansoprazole @ 60 mg/day for 31 days did not produce any significant changes in haematological parameters in human patients with Zollinger-Ellison syndrome. Similarly, Hentschel *et al.* (1983) reported that hematological parameters were not affected by treatment with sucralfate @ 1 g, PO, thrice a day, in endoscopically diagnosed duodenal ulcer patients. Similarly, Tyagi (2006) reported a gradual increase in Hb, PCV and TEC following administration of Seabuckthorn seed oil, at the same dose rate used in the present study, in dexamethasone induced GUE in dogs.

Based upon the above observations, following conclusions were drawn –

- The overall therapeutic efficacy of Seabuckthorn seed oil in dexamethasone-induced gastric ulcerations and erosions in dogs is better than famotidine, lansoprazole, misoprostol and sucralfate.
- Lansoprazole, sucralfate, misoprostol, famotidine and seabuckthorn oil are safe to administer in dogs.
- Faecal occult blood test is quite sensitive in indirect assessment of haemorrhage occurring in gastric ulcerations and erosions in dogs but may show occasional false negative reactions.

3. <u>Studies on therapeutic efficacy of different doses of Seabuckthorn seed oil alone and in combination with famotidine for GUE in dogs</u>

20 average sized dogs divided in to following 5 equal groups were utilized in the study.

Table 10: Details of treatment in different groups

Group I (Test 1)	1 ml Seabuckthorn Oil + 4 ml liquid paraffin PO b.i.d.
Group II (Test 2)	2.5ml Seabuckthorn Oil +2.5 ml liquid paraffin PO b.i.d.
Group III (Negative Control I)	5 ml liquid paraffin PO b.i.d.
Group IV (Negative Control II)	No treatment
Group V (Test 3)	1 ml Seabuckthorn oil +4 ml liquid paraffin + Famotidine PO b.i.d.

Results and Discussion

Gastro-endoscopic observations:

Average number of days to bring down the GUE index to '0' was shortest in group V (combination of SBT oil and famotidine treated group) followed by group II (2.5 ml SBT oil), I, III and

IV (1.0 ml SBT oil, 5 ml liquid paraffin and negative control respectively). The complete healing of GUE lesions occurred in group V in 6.0 days followed by 9.00 days in group II and 10.5 days in group I, III and IV. The healing was qualitatively far better in group V and II as evidenced by rapid restoration of gastric mucus layer along with early healing of GUE lesions.

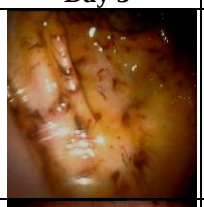
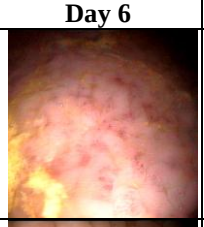
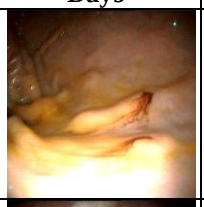
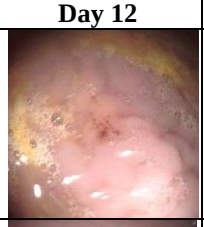
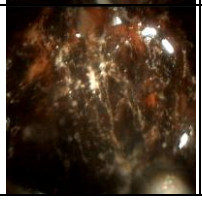
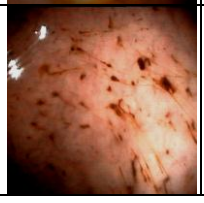
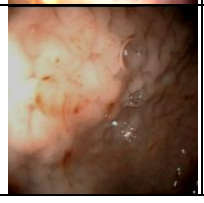
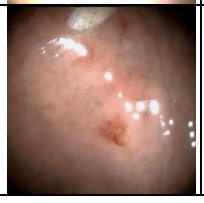
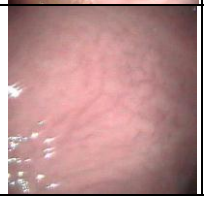
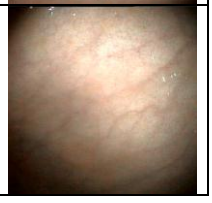
Table 11: GUE indices of dogs at various intervals (Mean± S.E.)

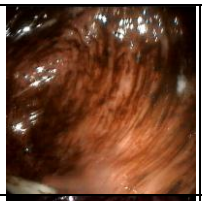
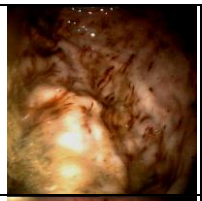
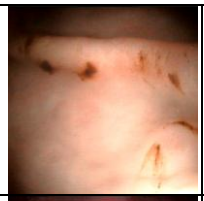
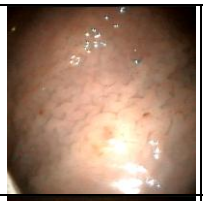
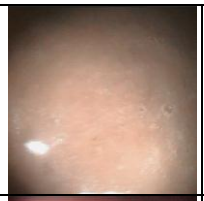
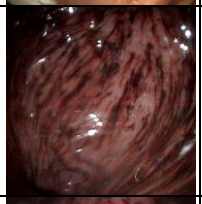
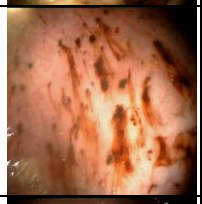
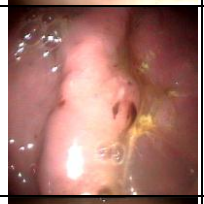
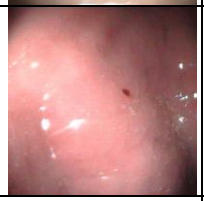
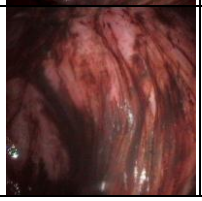
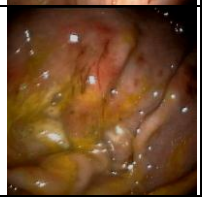
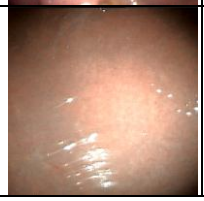
Days / Groups	0	3	6	9	12	15
Group I	8.0 ±0.00 **(n=4)**	4.0** ±0.00 **(n=4)**	2.25** ±0.85 **(n=4)**	1.33** ±0.33 **(n=3)**	1.00** ±1.00 **(n=2)**	0.00 ±0.0 **(n=1)**
Group II	8.00 ±0.0 **(n=4)**	4.75* ±1.1 **(n=4)**	1.50** ±0.5 **(n=4)**	0.67** ±0.67 **(n=3)**	0.0 ±0.0 **(n=1)**	
Group III	7.5 ±0.29 **(n=4)**	5.0** ±0.0 **(n=4)**	3.0[a] ±0.41 **(n=4)**	0.75** ±0.48 **(n=4)**	0.0 ±0.0 **(n=2)**	
Group IV	7.75 ±0.25 **(n=4)**	4.75* ±0.75 **(n=4)**	2.25** ±0.75 **(n=4)**	1.33** ±0.58 **(n=3)**	0.50 ±0.50 **(n=2)**	0 ±0.00 **(n=1)**
Group V	7.75 ± 0.25 **(n=4)**	3.00** ±0.71 **(n=4)**	0.0[b] ±0.0 **(n=4)**			

p<0.01 (**), p<0.05 (*), [a] Significant with [b] in between group (p< 0.01)

Dogra (2011) compared the therapeutic efficacies of different gastric ulcers medicines and seabuckthorn seed oil in dexamethasone-induced GUEs in dogs. She reported that GUE lesions healed in an average 7.5 days in the group of dogs treated with seabuckthorn seed oil given @ 5 ml PO b.i.d. followed by Famotidine (8.25 days), Misoprostol (10.5 days), Lansoprazole (9.0 days) and Sucralfate (13.5 days) in dogs. Tyagi (2006) also reported faster healing of GUE lesions in omeprazole and seabuckthorn oil treated groups of dogs in a slightly different experimental model of GUE in dogs. Earlier Seabuckthorn oil has been subjected to numerous gastric ulcer studies on rats and rabbits as well as humans (Jiang *et al.* 1989; Mironov *et al.* 1989; Xiao *et al.*1992; Zhou *et al.* 1994; Che *et al.* 1998; Suleyman *et al.* 2001; Xing *et al.* 2002; Xu *et al*. 2007; Qiu and Qiao 1997) and it was found effective in various models of gastric ulcers induced by physical necrotizing agents, NSAIDs or stress.

Plate 5 : Endoscopic view of gastric mucosal surface of dogs in different groups at various observation intervals

Groups	Day0	Day 3	Day 6	Day9	Day 12	Day 15
G I 1 ml SBT oil						
G II 2.5 ml SBT oil						

G III 5 ml Liquid paraffin						-
G IV No Treatmen t						-
G V 1 ml SBT oil + Famo				-	-	-

Clinical parameters

The rectal temperature, heart rates and mean respiration rates did not vary much with the base values and remained within normal physiological limits throughout the period of study in all the groups. No statistical difference was observed within or in-between various groups at any observation intervals.

A marked improvement in appetite was observed in all the dogs during treatment. Most of the dogs started showing improvement 3 days after the start of treatment but 6 dogs continued with decreased appetite till day 6. Towards the end of the study all the dogs had regained their normal appetite. During the study, 3 dogs had vomiting; 2 in group II on day 3 and 6 and 1 dog in group IV on day 3. The colour of the vomitus in all animals was yellow and no frank or clotted blood was seen. Melena was observed till 3 days in group V, till 6 days in group I, II, III and IV and till 9 days in 1 animal of group IV. Dogra (2011) also reported that melena was observed till day 3 in seabuckthorn treated dogs and upto day 6 in famotidine treated group.

A non-significant gradual regaining of lost body weight was observed in the dogs of all the groups. The gain in weight by day 9^{th} was 1.09%, 1.96 %, 0.69%, 1.97% and 3.83% in group I, II, III, IV and V respectively. Gain in weight was highest in group V (combination of 1 ml SBT oil and Famotidine). Regaining lost body weights during convalescent period of GUE is naturally expected because of improved appetite and digestion. Tyagi (2006) and Dogra (2011) have also observed the same. Further, rapid regaining of weight is indirectly indicative of better treatment efficacy. In the present study, the gain in body weight was faster in Group V where a combination of seabuckthorn seed oil and famotidine was used for treatment. In ancient Greece, seabuckthorn was used as a fodder to horses and resulted in rapid weight gain and a shiny coat for the horse. This, in fact, gave the name to the plant in Latin; '*Hippo*' meaning horse and '*phaos*' meaning to shine (Rongsen 1992). Since fluid volume changes are critical to the specialized stressor of haemorrhage (Sapolsky *et al*. 2000) so, as the degree of haemorrhage decreased over the period of time, degree of dehydration which may also be responsible for weight loss, decreased resulting in slow building up of body weight in all the groups.

Faecal occult blood test (FOBT)

The faecal occult blood test was strongly positive in all the groups at day 0. Thereafter, the strength of FOBT reactions gradually decreased but varied within and in between various groups. On all the instances a direct correlation was observed between detection of blood clots or gastric lesions endoscopically and a corresponding FOBT reaction. This indicated that faecal occult blood test is proficient in diagnosing smaller quantities of blood in faeces in cases of subclinical GUE in dogs. The same findings were reported by Thakur (2011) and Dogra (2011).

Haematological Parameters

In general, a gradual rise in Hb, PCV was observed from 0 day till the end of study in all the groups. When compared with day 0, significant increases in Hb levels were observed on day 9 in group I and V, day 12 in group I, II and IV, and day 15 in group IV.

Table 12: Haemoglobin of different groups at various intervals during phase II (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Group I	9.85 ±0.37 **(n=4)**	10.63 ±0.63 **(n=4)**	11.18 ±0.46 **(n=4)**	11.75* ±0.23 **(n=4)**	12.3** ±0.27 **(n=4)**	12.1 ±0.0 **(n=1)**
Group II	9.48 ±0.62 **(n=4)**	9.2 ±0.55 **(n=4)**	10.38 ±0.43 **(n=4)**	11.13 ±0.39 **(n=4)**	11.75* ±0.33 **(n=4)**	
Group III	10.7 ±0.83 **(n=4)**	11.1 ±0.76 **(n=4)**	11.65 ±0.70 **(n=4)**	11.93 ±0.69 **(n=4)**	12.35 ±0.74 **(n=4)**	
Group IV	9.93 ±0.62 **(n=4)**	10.50 ±0.41 **(n=4)**	11.10 ±0.45 **(n=4)**	11.48 ±0.51 **(n=4)**	12.13* ±0.21 **(n=4)**	12.60* ±0.30 **(n=2)**
Group V	10.2 ±0.43 **(n=4)**	10.98 ±0.3 **(n=4)**	11.35 ±0.32 **(n=4)**	12.13** ±0.36 **(n=4)**		

p<0.01 (**), p<0.05 (*)

Similarly PCV levels also increased gradually towards normal baseline levels in all the groups by the end of observation intervals. Increases in TEC levels were insignificant within or between groups at all observation intervals. In group II (*i.e*. 2.5 ml SBT oil group), recuperation in TEC started on day 6th rather than day 3rd as observed in other groups.

Table 13: Packed cell volume (%) of different groups at various intervals during phase II (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Group I	28.93 ±0.88 **(n=4)**	31.2 ±0.28 **(n=4)**	32.18* ±0.24 **(n=4)**	33.68** ±1.03 **(n=4)**	35.63** ±1.63 **(n=4)**	33.8 ± 0.0 **(n=1)**
Group II	27.98 ±1.61 **(n=4)**	28.3[b] ±1.65 **(n=4)**	30.45 ±1.12 **(n=4)**	32.45 ±0.52 **(n=4)**	33.2* ±0.62 **(n=4)**	
Group III	31.5 ±3.45 **(n=4)**	34.63[a] ±1.89 **(n=4)**	36.33 ±2.28 **(n=4)**	37.43 ±2.38 **(n=4)**	38.03 ±2.32 **(n=4)**	
Group IV	29.98 ±2.16 **(n=4)**	30.53 ±1.6 **(n=4)**	32.20 ±1.71 **(n=4)**	34.83 ±1.84 **(n=4)**	36.3 ±1.59 **(n=4)**	39.86*±1.16 **(n=2)**
Group V	29.78 ±1.5 **(n=4)**	31.95 ±0.96 **(n=4)**	32.83 ±0.81 **(n=4)**	34.5* ±1.34 **(n=4)**		

p<0.01 (**), p<0.05 (*)

[a] Significant with [b] in between group (p< 0.01)

Table 14: Total eythrocyte count (x10^{12}/L) of different groups at various intervals during phase II (Mean± S.E.)						
Days / Groups	**0**	**3**	**6**	**9**	**12**	**15**
Group I	3.76 ±0.27 **(n=4)**	4.24 ±0.29 **(n=4)**	4.36 ±0.25 **(n=4)**	4.54 ±0.23 **(n=4)**	4.91 ±0.25 **(n=4)**	4.27 ±0.0 **(n=1)**
Group II	4.24 ±0.29 **(n=4)**	3.97 ±0.33 **(n=4)**	4.16 ±0.37 **(n=4)**	4.62 ±0.16 **(n=4)**	4.72 ±0.17 **(n=4)**	
Group III	4.0 ±0.49 **(n=4)**	4.94 ±0.49 **(n=4)**	5.15 ±0.48 **(n=4)**	5.28 ±0.49 **(n=4)**	5.29 ±0.48 **(n=4)**	
Group IV	4.40 ±0.11 **(n=4)**	4.71 ±0.28 **(n=4)**	4.96 ±0.25 **(n=4)**	5.10 ±0.26 **(n=4)**	5.29 ±0.19 **(n=4)**	5.64 ±0.25 **(n=2)**
Group V	4.28 ±0.24 **(n=4)**	4.49 ±0.21 **(n=4)**	4.96 ±0.23 **(n=4)**	5.19 ±0.27 **(n=4)**		

TLC and granulocytes levels gradually decreased in all the groups to reach towards its normal base values by the end of last observation interval.

Table 15: Total leukocyte counts (X 10^{9}/L) in different groups at various intervals during phase II (Mean± S.E.)						
Days / Groups	**0**	**3**	**6**	**9**	**12**	**15**
Group I	27.75 ±1.73 **(n=4)**	22.08 ±1.68 **(n=4)**	16.8** ±2.59 **(n=4)**	10.38** ±1.79 **(n=4)**	8.68** ±0.89 **(n=4)**	7.1 ±0.0 **(n=1)**
Group II	26.68 ±2.61 **(n=4)**	18.9* ±0.55 **(n=4)**	14.58** ±1.72 **(n=4)**	11.25** ±1.29 **(n=4)**	9.73** ±0.96 **(n=4)**	
Group III	28.4 ±3.27 **(n=4)**	17.73** ±3.01 **(n=4)**	12.4** ±1.83 **(n=4)**	10.75** ±1.49 **(n=4)**	8.98** ±1.13 **(n=4)**	
Group IV	29.48 ±5.16 **(n=4)**	23.48 ±3.57 **(n=4)**	14.7* ±2.79 **(n=4)**	11.18** ±1.57 **(n=3)**	9.01* ±1.11 **(n=4)**	7.3** ±1.2 **(n=2)**
Group V	29.7 ±2.39 **(n=4)**	16.15** ±3.69 **(n=4)**	12.53 ±2.53 **(n=4)**	10.12 ±0.92 **(n=4)**		

$p<0.01$ (**), $p<0.05$ (*)

Dexamethasone itself may induce the characteristic blood leukocyte profile (neutrophilia, lymphopenia, monocytosis) known as the 'stress leukogram' as observed in this study. Further, gastric mucosal injury also is responsible of such haematological changes because of natural response of body to such injuries. Hence, the, withdrawal of dexamethasone and subsequent healing process of gastric lesions gradually reversed the adverse effects on haematological parameters in the present study. These finding are in consonance with those of Tyagi (2006) and Dogra (2011) who also reported a gradual restoration of Hb, PCV and TEC levels towards normal following administration of seabuckthorn seed oil in dexamethasone-induced GUE in dogs.

Table 16: Granulocytes, lymphocytes and in dogs at various intervals during phase II (Mean±S.E)						
Granulocytes (%)						
Days / Groups	**0**	**3**	**6**	**9**	**12**	**15**
Group I	83.7 ±1.75 **(n=4)**	80.1 ±1.16 **(n=4)**	79.98 ±3.62 **(n=4)**	77.78 ±0.31 **(n=4)**	77.35 ±2.31 **(n=4)**	81.6 ±0.0 **(n=1)**
Group II	85.78 ±1.50 **(n=4)**	82.18 ±2.08 **(n=4)**	79.23 ±2.26 **(n=4)**	76.5 ±3.18 **(n=4)**	76.23 ±3.15 **(n=4)**	
Group III	84.18 ±2.94 **(n=4)**	77.1 ±2.90 **(n=4)**	76.53 ±2.29 **(n=4)**	74.18* ±2.19 **(n=4)**	73.85* ±2.51 **(n=4)**	
Group IV	86.50 ±0.69 **(n=4)**	82.00 ±1.57 **(n=4)**	77.80* ±2.67 **(n=4)**	77.48* ±1.89 **(n=4)**	76.43** ±1.72 **(n=4)**	73.85** ±3.25 **(n=2)**
Group V	87.83 ±0.40 **(n=4)**	81.78 ±3.37 **(n=4)**	81.28 ±1.43 **(n=4)**	82.18 ±1.11 **(n=4)**		
Lymphocytes (%)						
Group I	11.93 ±1.22 **(n=4)**	16.1 ±1.62 **(n=4)**	16.18 ±3.38 **(n=4)**	17.43 ±1.79 **(n=4)**	17.98 ±1.49 **(n=4)**	15.2 ±0.0 **(n=1)**
Group II	11.08 ±1.30 **(n=4)**	14.05 ±2.32 **(n=4)**	16.35 ±2.14 **(n=4)**	19.15 ±2.78 **(n=4)**	20.65* ±1.88 **(n=4)**	
Group III	12.43 ±2.14 **(n=4)**	17.28 ±2.78 **(n=4)**	19.95 ±2.44 **(n=4)**	21.95* ±1.99 **(n=4)**	19.73 ±2.18 **(n=4)**	
Group IV	10.85 ±0.79 **(n=4)**	14.15 ±1.21 **(n=4)**	17.90 * ±2.49 **(n=4)**	17.15* ±1.40 **(n=4)**	18.05* ±0.93 **(n=4)**	19.5 * ±0.40 **(n=2)**
Group V	11.75 ±2.16 **(n=4)**	14.73 ±3.30 **(n=4)**	14.25 ±1.49 **(n=4)**	13.9 ±1.1 **(n=4)**		

$p<0.01$ (**), $p<0.05$ (*)

Biochemical parameters

Serum aspartate amino transferase (AST) and serum alanine amino transferase (ALT) levels did not change much and remained within normal physiological range in all the groups. Blood urea nitrogen (BUN) and serum creatinine (Cr) levels also did not vary much and remained within normal physiological limits throughout the period of study.

Based upon the above observations and comparison with previous studies, following conclusions were drawn-

1. The seabuckthorn seed oil has a dose-dependent therapeutic effect in the healing of dexamethasone-induced gastric ulcerations and erosions in dogs.
2. Seabuckthorn seed oil @ 1 ml/ dog PO *b.i.d.* is not effective in treating the GUE in dogs.
3. Seabuckthorn seed oil @ 2.5 ml/ dog PO *b.i.d* though hastens the healing of GUE lesions in dogs, the faster healing occurs with the dose rate of 5 ml/dog.
4. The otherwise ineffective dose of seabuckthorn seed oil *i.e.* 1ml/dog when combined with famotidine @ 1 mg/Kg BW PO *b.i.d.* shows good synergistic effect in treating GUE in dogs and results in fastest healing of lesions when compared with any other drug used in the different trials of the sub-project so far.

4. Studies on therapeutic efficacy of Seabuckthorn seed oil in combination with sucralfate and misoprostol for GUE in dogs

16 dogs divided in to 4 equal groups were utilized in this study. Frequency of administration of drugs/treatment combinations was increased from two times (as used in previous trials) to three times a day to see any improvement in their therapeutic efficacy.

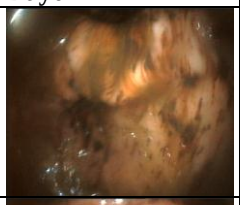
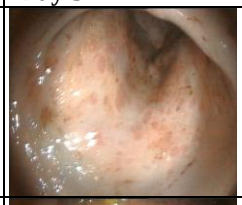
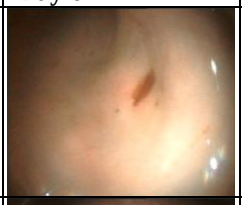
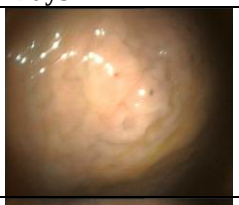
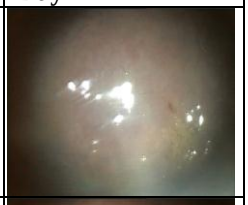
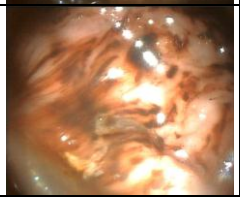
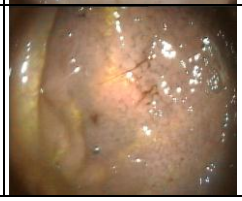
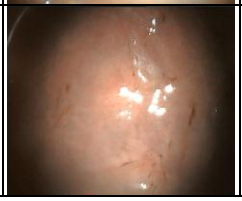
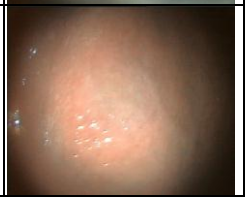
Table 17: Details of treatment in different groups

Group I	Misoprostol(Misoprost-200, Cipla, India) @ 10µg /kg PO *t.i.d.* (thrice a day)
Group II	Misoprostol (Misoprost-200, Cipla, India) @ 10µg/kg + Seabuckthorn oil @ 1ml PO *t.i.d.*
Group III	Sucralfate (Sparacid, Dr. Reddy's Laboratories, India) @ 1g PO *t.i.d.*
Group IV	Sucralfate (Sparacid, Dr. Reddy's Laboratories, India) @ 1g+ Seabuckthorn oil @ 1ml PO *t.i.d.*

Gastro-endoscopic observations:

The average number of days taken for complete healing of GUE lesions was determined to be 8.25 days for group IV, 9.75 days each for group I and II and 10.5 days for group III. Though no statistical intergroup difference was observed in GUE indices, the subjective assessment revealed better and faster healing in group IV followed by group I and II. The healing days in misoprostol treated group varied markedly and ranged from 6-15 days whereas, in group IV (Sucralfate plus SBT oil) the healing time was largely uniform. Dogra (2011) reported average healing time of 10.5 days with misoprostol treatment and 13.5 days with sucralfate treatment of the similarly induced GUE in dogs with a lesser dose frequency of only twice a day as against thrice a day in the present study. This indicated that the healing period of GUE can be shortened by more frequent administration of these two drugs. The average healing time of GUE reduced from 13.5 days to 10.5 days in case of Sucralfate and from 10.5 days to marginally lower 9.75 days in case of misoprostol treatment. Kumar (2013) compared different doses of seabuckthorn seed oil *i.e.* 1 ml, 2.5 ml and 5 ml per animal for the treatment of similarly produced GUE in dogs and reported dose-dependent effect of SBT oil on the healing of GUE lesions; the average healing time reported was 10.5 days for 1 ml dose, 9 day for 2.5 ml and 7.5 days for 5 ml dose. The otherwise considered ineffective dose of seabuckthorn seed oil (*i.e.* 1ml/dog) and Sucralfate when combined together resulted in earlier healing of gastric lesions. This indicated that SBT oil has synergistic therapeutic effect with sucralfate even in lower doses just like its combination with famotidine as seen in previous trials. However, the misoprostol either alone or in combination with SBT seed oil proved ineffective in treatment of GUE in dogs.

Plate 7: Endoscopic view of gastric mucosal surface of dogs in different groups at various observation intervals

Groups	Day0	Day 3	Day 6	Day9	Day 12
G I (Miso)					
G II (Miso + SBT oil)					

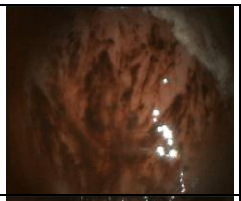
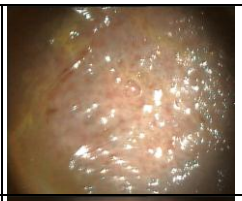
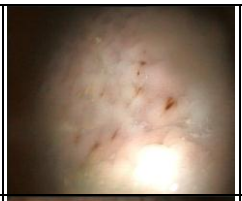
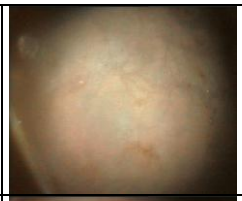
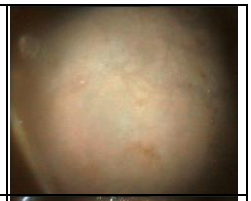
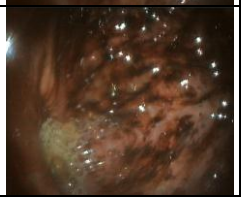
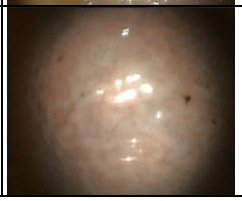
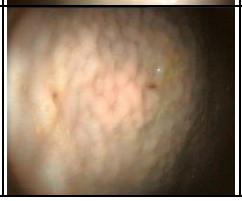
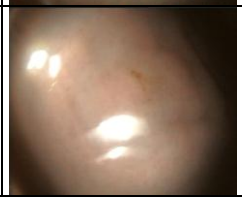
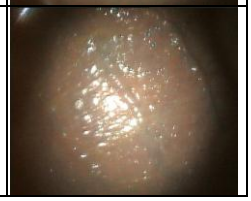
G III (Sucral)						
G IV (Sucral + SBT oil)						

Table 19: GUE indices of dogs at various intervals (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Group I (Miso)	7.75±0.25 N=4	5.00±1.22 N=4	1.5**±0.86 N=4	1.5**±0.50 N=2	0.5**±0.50 N=2	0.0±0.00 N=1
Group II (Miso + SBT)	8.0±0.00 N=4	3.25**±0.85 N=4	2.25**±0.75 N=4	1.33**±0.66 N=3	0.0**±0.00 N=2	
Group III (Sucral)	7.25±0.25 N=4	4.5**±0.64 N=4	2.5**±0.64 N=4	0.5**±0.50 N=4	1.0±0.00 N=1	0.0±0.00 N=1
Group IV (Sucral + SBT)	7.5±0.28 N=4	3.25**±1.03 N=4	1.25**±0.75 N=4	1.0**±1.0 N=2	0.0±0.00 N=1	

p<0.01 (**), p<0.05 (*) intragroup comparison

Clinical observations:

Insignificant variation with no particular trend was observed in heart rate, respiration rate and rectal temperature in different groups at different intervals. These values remained within the normal physiological limits in all the animals. These findings are similar to those observed by Tyagi (2006), Dogra (2011) and Gupta (2012). A marked improvement in appetite was observed in all the animals during treatment. Most of the animals started showing improvement 3 days after the start of treatment but four dogs continued with decreased appetite till 6th day. Towards the end of the study all the animals had regained their normal appetite. During this phase of the study two dogs showed vomiting in group 3 at day 0 and 3. Melena was observed till day 6 in group I, 3 in group II, 6 in group III and 3 in group IV. The severity of melena gradually decreased towards the end of study in all the groups.

A non-significant increase in body weight of animals was observed. Regaining lost body weights during convalescent period of GUE is naturally expected because of improved appetite and digestion. Further, rapid regaining of weight is indirectly indicative of better treatment efficacy.

Faecal Occult blood test (FOBT)

The faecal occult blood test was strongly positive in all the groups at day 0. Thereafter, the strength of FOBT reactions gradually decreased. In general a direct correlation was observed between detection of blood clots or gastric lesions endoscopically and a corresponding FOBT reaction. However, a false negative FOBT reaction was observed in 1 dog each of groups I and III on day 12. In these animals the GUE lesions, though mild in nature, were still detectable endoscopically. Faecal occult blood test is considered proficient in diagnosing smaller quantities of blood in faeces in cases of subclinical GUE in dogs. However, occasional false positive or false negative reactions have also been reported by some other works (Gupta 2012). However, Thakur (2011) and Dogra (2011) reported 100% accuracy of FOBT in similar kind of studies.

Haematological observations:

In general, gradual rise in Hb, PCV was observed from day 0 till the end of the study in all the groups. Hb PCV and TEC level increased, but it remained statistically insignificant at all intervals when compared within and in-between the groups.

Table 21: Haemoglobin, packed cell volume and total erythrocyte count of different groups at various intervals (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Hb (g/dl)						
Group 1 (Miso)	10.55 ±0.55 N=4	10.62 ±0.21 N=4	11 ±0.33 N=4	11.05 ±0.45 N=2	11.15 ±0.55 N=2	12 ±0.00 N=1
Group 2 (Miso + SBT)	9.25 ±0.56 N=4	10.17 ±0.78 N=4	10.95 ±0.83 N=4	10.13 ±0.75 N=3	10.5 ±1.50 N=2	
Group 3 (Sucral)	9.72 ±0.39 N=4	9.27 ±0.52 N=4	9.77 ±0.59 N=4	10.1 ±0.70 N=4	11 ±0.00 N=1	11.2 ±0.00 N=1
Group 4 (Sucral + SBT)	10.55 ±0.11 N=4	9.8 ±0.25 N=4	10.55 ±0.25 N=4	10.95 ±0.55 N=2	10.6 ±0.00 N=1	
PCV(%)						
Group 1 (Miso)	29.37 ±2.55 N=4	30.9 ±0.96 N=4	31.5 ±1.37 N=4	32.8 ±1.90 N=2	32.25 ±2.55 N=2	35.7 ±0.00 N=1
Group 2 (Miso + SBT)	28.27 ±2.30 N=4	30.55 ±2.41 N=4	32.82 ±2.12 N=4	30.4 ±2.37 N=3	30.15 ±5.75 N=2	
Group 3 (Sucral)	29.4 ±0.38 N=4	29.25 ±0.61 N=4	30.4 ±1.08 N=4	31.32 ±1.34 N=4	35.2 ±0.00 N=1	36.4 ±0.00 N=1
Group 4 (Sucral + SBT)	31.5 ±0.61 N=4	29.4 ±1.45 N=4	31.8 ±1.59 N=4	32.3 ±1.05 N=2	34.5 ±0.00 N=1	
TEC($X10^{12}$/L)						
Group 1 (Miso)	4.13 ±0.40 N=4	4.42 ±0.3=4	4.53 ±0.32 N=4	4.93 ±0.36 N=2	4.98 ±0.51 N=2	5.74 ±0.00 N=1
Group 2 (Miso + SBT)	4.02 ±0.15 N=4	4.55 ±0.31 N=4	4.95 ±0.30 N=4	4.82 ±0.57 N=3	4.49 ±0.71 N=2	
Group 3 (Sucral)	4.53 ±0.08 N=4	4.42 ±0.12 N=4	4.61 ±0.24 N=4	4.96 ±0.35 N=4	6.16 ±0.00 N=1	6.2 ±0.00 N=1
Group 4 (Sucral + SBT)	4.61 ±0.08 N=4	4.24 ±0.19 N=4	4.47 ±0.18 N=4	4.9 ±0.15 N=2	5.29 ±0.00 N=1	

$p<0.01$ (**), $p<0.05$ (*) intra group comparison

TLC and granulocytes levels gradually decreased in all the groups to reach near its normal levels by the end of last observation interval. Granulocytes were statistically lower in group IV at day 9^{th} when compared with group I, group II and group III, when comparison was done in-between the groups i.e. inter-group comparison. However, there is no intra-group significance in these levels were found at any observation intervals. A corresponding gradual increase in lymphocytes levels were also observed in all the groups which reached to normal base levels by the end of this phase. Increase in lymphocyte level was

statistically significant in group IV at day 9th when compared with group II. However, there is no intra-group significance in these levels were found at any observation intervals. Monocytes levels varied with and in between the groups, but these variations were statistically insignificant and were within normal physiological range.

Table 22: Total leukocyte count TLC (X 10^9/L) of different groups at various intervals (Mean± S.E.)

Days / Groups	0	3	6	9	12	15
Group 1 (Miso)	28.3 ±5.61 N=4	20.25 ±3.47 N=4	14.87 ±2.89 N=4	10.9 ±1.90 N=2	8.45 ±0.35 N=2	8.4 ±0.00 N=1
Group 2 (Miso + SBT)	25.12 ±2.04 N=4	18.25 ±1.69 N=4	13.02** ±1.87 N=4	12.63* ±3.02 N=3	10.7* ±0.90 N=2	
Group 3 (Sucral)	28.12 ±3.78 N=4	22.3 ±1.29 N=4	17.02* ±2.26 N=4	14.55** ±1.33 N=4	10.2 ±0.00 N=1	9.8 ±0.00 N=1
Group 4 (Sucral + SBT)	28.15 ±5.01 N=4	18.85 ±7.67 N=4	17.37 ±5.40 N=4	11.55 ±1.55 N=2	11.2 ±0.00 N=1	

p<0.01 (**), p<0.05 (*) intra group comparison

Table 23: Differential leucocyte count of different groups at various intervals (Mean±S.E)

Days / Groups	0	3	6	9	12	15
Granulocytes (%)						
Group I (Miso)	88.07 ±3.03 N=4	86.3 ±2.03 N=4	87.82 ±1.34 N=4	87.05[a] ±1.65 N=2	84.15 ±2.65 N=2	87.2 ±0.00 N=1
Group II (Miso + SBT)	89.27 ±0.54 N=4	84.1 ±0.64 N=4	85.67 ±2.63 N=4	87.56[a] ±1.12 N=3	87.3 ±0.90 N=2	
Group III (Sucral)	87.72 ±1.40 N=4	86.72 ±0.78 N=4	87.67 ±2.63 N=4	87.55[a] ±0.49 N=4	87.2 ±0.00 N=1	88.2 ±0.00 N=1
Group IV (Sucral + SBT)	87.4 ±2.16 N=4	84.17 ±0.63 N=4	81.82 ±1.11 N=4	81.65[b] ±1.75 N=2	74.2 ±0.00 N=1	
Lymphocytes						
Group I (Miso)	10.05 ±3.12 N=4	10.67 ±1.79 N=4	10.17 ±1.18 N=4	9.75[ab] ±1.45 N=2	12.1 ±2.00 N=2	9.8 ±0.00 N=1
Group II (Miso + SBT)	8.75 ±0.65 N=4	13.22 ±0.67 N=4	12.35 ±2.26 N=4	6.4[a] ±2.10 N=3	11.85 ±1.05 N=2	
Group III (Sucral)	9.8 ±1.20 N=4	11.05 ±0.59 N=4	9.92 ±0.64 N=4	10.62[ab] ±0.29 N=4	9.6 ±0.00 N=1	8.8 ±0.00 N=1
Group IV (Sucral + SBT)	9.9 ±1.89 N=4	12.82 ±0.31 N=4	14.5 ±0.98 N=4	14.85[b] ±0.45 N=2	20.2 ±0.00 N=1	

[a,b](p<0.01)significance in between groups

Biochemical parameters

AST and ALT levels did not change much and remained within normal physiological range in all the groups. Similarly, blood urea nitrogen (BUN) and serum creatinine (CRTN) levels also did not vary much with the base values and remained within normal physiological limits. Similar results were reported by Tyagi (2006), Dogra (2011) and Gupta (2012). This indicates that the different therapeutic agents used in this study do not have any untoward side effects on liver and kidney functioning of dogs.

Based upon the above observations and previous trials, following conclusions were drawn:

1. The combinations of seabuckthorn seed oil with Sucralfate or famotidine has synergistic therapeutic effect for the healing of GUE in dogs but no such effect was observed by using the combination of SBT seed oil and misoprostol.
2. The healing of GUE lesions in dogs occur fastest with the combination of famotidine and SBT seed oil followed by the combination of sucralfate and SBT seed oil when compared with any of them alone or the combination of misoprostol with SBT oil.
3. The healing period of GUE in dogs can be reduced by more frequent administration of sucralfate or misoprostol though the reduction in healing time in case of misoprostol is insignificant.

5. Studies on comparative evaluation of prophylactic efficacy of Seabuckthorn seed oil *vis-e-vis* routinely used allopathic drugs for GUE in dogs

This study was carried out in 24 dogs divided in to six equal groups. Inj. dexamethasone @ 1mg/kg I/V bid was used to create non-fatal GUE in dogs. Simultaneously, these animals were treated with different drugs to evaluate their gastro-protective actions. The dexamethasone was continued until endoscopic GUE index reached to 7/8 as explained previously.

Table 24: Details of treatment in different groups

Group I	Lansoprazole (Lanzol-30, Cipla, India) @ 1.5mg/kg PO bid
Group II	Sucralfate (Sparacid, Dr. Reddy's Laboratories, India) @ 1g/10kg PO bid
Group III	Misoprostol (Misoprost-200, Cipla, India) @ 10µg/kg PO bid
Group IV	Famotidine (Famtac, Piramal Healthcare, India) @ 1mg/kg PO bid
Group V	Seabuckthorn oil @ 5ml PO bid
Group VI	No treatment

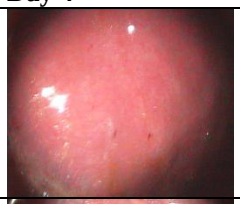
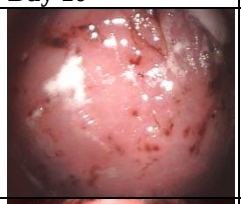
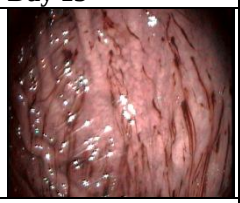
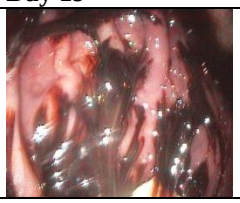
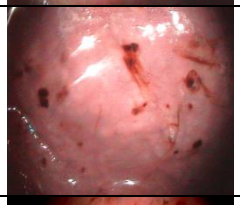
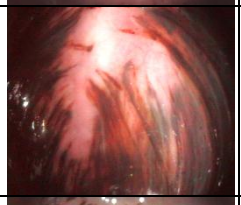
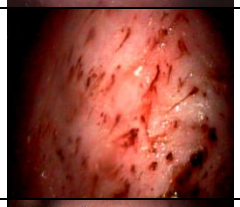
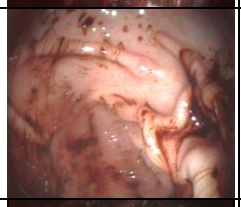
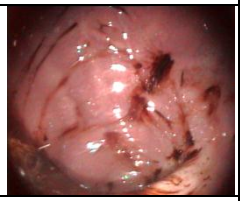
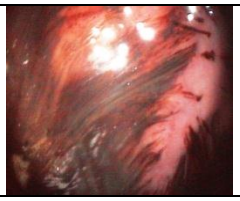
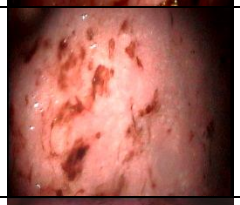
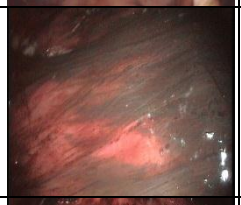
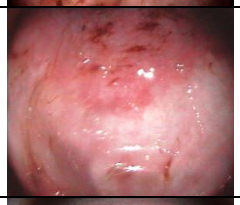
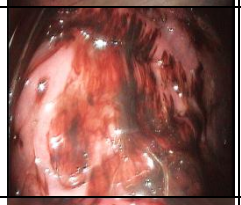
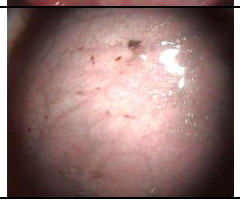
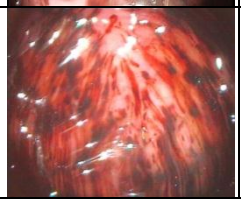
Results and Discussion:

Gastro-endoscopic observations:

The endoscopic examination of the stomach on day 0 revealed absence of gastric lesion in all the dogs but subsequently GUE lesions appeared in all the groups and observed as early as on 4th day. Thereafter, their severity and number increased gradually in all the groups as reflected by a corresponding increase in GUE indices. By the 4th day of study, the severity of GUE lesions was comparatively lesser in all the treatment groups i.e. I, II, III, IV and V when compared to negative control i.e. group VI and the GUE indices were considerably lesser in group I and III. However, thereafter except Lansoprazole and Misoprostol treated groups *i.e.* group I and III, the rate of progression of GUE lesions and their severity was comparable in all other groups i.e. II, IV, V and VI. By the 10th day, except all the 4 dogs of group 1 and 2 dogs of group 3, all animals achieved the GUE indices of 7/8. This indicated complete absence of gastroprotection of Sucralfate, famotidine and SBT seed oil by the 10th day. In groups I and III, the progression rate of GUE lesions from mild to moderate to severe remained slower. Statistically the GUE index of group I was significantly lesser than other groups on days 7 and 10. In case of misoprostol

treated group III, 2 dogs reached to GUE index of 7/8 on 10th day, 1 dog on 13th day and the last one on 19th day, whereas, all the 4 dogs of lansoprazole group showed uniform and prolonged gastroprotection and developed GUE index of 7/8 together on 19th day.

Plate 8 : Endoscopic view of gastric mucosal surface of dogs in different groups at various observation intervals

Groups	Day 4	Day 10	Day 13	Day 19
Group I Lansoprazole				
Group II Sucralfate			-	-
Group III Misoprotol				
Group IV Famotidine			-	-
Group V SBT oil			-	-
Group VI Negative Control			-	-

Thus the mean numbers of days taken to develop the predetermined level of GUE index (7/8) in dogs were 19±0, 10±0, 13.0±2.12, 8.5±0.86, 10±0 and 9.25±0.54 in groups I, II, III, IV, V and VI respectively. This duration was significantly longer in group I when compared with other groups.

Long term administration of steroidal drugs in dogs has been reported to result in development of gastric ulcerations and erosions (Rohrer et al., 1999; Boston et al., 2003; Tyagi, 2006). Same trend was observed in the present study in which GUE lesions of various degrees developed in all animals after dexamethasone administration. In the present study, although various drugs commonly used for the treatment of GUE, were administered simultaneously with dexamethasone, but gastric lesions still developed in all the groups. It proved that no drug does have an effective long term gastro-protective capability in the face of continuing ulcerogenic insult in dogs. However, some of the drugs like

lansoprazole, misoprostol, sucralfate and the seabuckthorn oil showed some limited gastro-protective activity and delayed the development of GUE lesions in dogs when subjected to long term exposure to gastric ulcerogen. Among them, the lansoprazole provided better and more consistent gastroprotection for a considerably longer duration up to 19 days. The findings of present study are in line with the findings of Fumihiko *et al*., (2005) who reported that lansoprazole shows gastro-protective effects by potently inhibiting gastric acid secretion, causing a decrease in total gastric activity and increasing mucosal blood flow via capsaicin-sensitive afferent nerves. Morini et al. (1995) also reported dose-dependent activity of the lansoprazole as gastro-protective agent in indomethacin-induced gastric lesions in rats.

Famotidine is a common drug used to treat GUE in human and its therapeutic efficacy in dexamethasone-induced GUE in dogs has also been documented (Dogra *et al.* 2013). However, in the present study, the famotidine did not show any gastroprotective activity.

Table 25: Gastric ulcerations-erosions (GUE) indices at different intervals (Mean± S.E)

Days / Groups	0	4	7	10	13	16	19
I. Lansoprazole (n=4)	0	1.50 ±0.50	3.00[a] ±0.70	3.50[a] ±0.56	5.75 ±0.47	5.50 ±0.50	7.50 ±0.28
II. Sucralfate (n=4)	0	2.75 ±1.10	6.50 ±0.28	8.00 ±0.00	------	------	------
III. Misoprostol (n=4)	0	4.00 ±0.40	5.00 ±0.70	6.75 ±0.75	6.50 ±1.50 (n=2)	5.00 ±0.00 (n=1)	8.00 ±0.00
IV. Famotidine (n=4)	0	3.75 ±0.85	7.50 ±0.28	7.50 ±0.50 (n=2)	------	------	------
V. Seabuckthorn (n=4)	0	3.25 ±0.75	6.75 ±0.47	8.00 ±0.00	------	------	------
VI. Negative control (n=4)	0	5.25 ±0.85	7.00 ±0.40	7.33 ±0.33 (n=3)	------	------	------

[a]p<0.001 intergroup comparison.

Clinical observations:

A general decline in body condition as evidenced by steady decrease in body weight was observed in all the dogs of various groups. The percentage decrease of body weight was least in seabuckthorn oil treated group by 7th day and was still markedly lesser than lansoprazole and sucralfate treated groups on 10th day. The sucralfate treated group showed strikingly greater degree of weight reduction compared to other groups. The percent weight reduction was also considerable in Lansoprazole-treated group (I) on 7th day and even more notable on 10th day on comparative basis.

Table 26: Body weight (Kgs) in different groups at different intervals (Mean±S.E)

Days / Groups	0	4	7	10	13	16	19
I. Lansoprazole (n=4)	19.12 ±2.06	18.82 ±2.47	18.02 ±2.57	16.82 ±2.16	16.57 ±2.02	16.35 ±1.83	15.87 ±1.94
II. Sucralfate (n=4)	20.10 ±0.84	19.37 ±1.17	17.97 ±0.86	16.37 ±0.98	------	------	------
III. Misoprostol (n=4)	18.60 ±0.72	18.25 ±0.62	17.85 ±0.68	17.15 ±0.92	16.70 ±0.70 (n=2)	17.80 ±0.00 (n=1)	17.00 ±0.00 (n=1)
IV. Famotidine (n=4)	21.37 ±0.47	20.75 ±0.66	20.15 0.95	18.50 ±1.50 (n=2)	------	------	------

V. Seabuckthorn (n=4)	21.25 ±3.15	21.12 ±3.22	20.52 ±2.99	19.50 ±2.88	------	------	------
VI. Negative control (n=4)	18.00 ±2.44	16.62 ±2.11	16.12 ±1.85	16.33 ±2.16 (n=3)	------	------	------

The decrease in body weight of dogs could be attributed to reduced consumption of food, reduced assimilation of consumed food and or metabolic disturbances caused by long term administration of high doses of corticosteroid. Chronic administration of dexamethasone for up to 21 days in dogs itself has been associated with anorexia, dehydration, weight loss and intermittent diarrhoea (Parent, 1982). Tyagi (2006) also reported gradual fall of body weight in dogs till dexamethasone is continued in dogs for 16 days. However, it was remarkable to note that the rate of reduction in body weight was considerably lesser in seabuckthorn oil treated group when compared to other groups. And though endoscopic GUE index of this group was almost similar to that of sucralfate treated group, the weight reduction rate in the latter was greatest among all the groups including negative control by as early as 7th day.

The appetite also reduced in all the animals; the reduction was more in severe cases of GUE. Instead of correlation with rate of weight-reduction, the appetite reduction was more associated with severe degree of GUE irrespective of groups. Self-limiting vomiting on 6th day was observed in 1 dog of group III and on 6th and 10th day in another dog of group IV. Self-limiting diarrhoea was observed only in one animal of group III on 13th day. Overall all animals showed a marked dullness and reluctance to exercise towards later phase of study.

Vomiting used to be considered as classical sign of GUE (Hall and Twedt, 1988). But many studies in human and animals has shown lack of vomiting as well as classical gastrointestinal clinical signs in GUE (Sweeney, 1992; Tyagi, 2009; Davis et al., 2003). In the present study also, no association was found between extent of gastric lesions and vomiting in dogs.

Faecal occult blood test:

Faecal occult blood test (FOBT) was negative in all the animals on day 0, but on 4th day, the test was positive in majority of the dogs (19/24) who exhibited gastric lesions upon gastro-endoscopy. By day 7th and onwards all the animals showed marked positive FOBT reaction till the end of the study. Tarry-coloured faeces or melena was observed in 6 dogs on day 4, in 19 dogs on day 7 and all the dogs thereafter. Detection of melena by naked eye was considered possible only when substantial blood loss occurs in gastrointestinal system and therefore, many laboratory tests have been developed to detect smaller or negligible (occult) quantities of blood in faeces. Gilson et al. (1990) reported that faecal occult blood tests could detect quantities of blood that were smaller than those required to cause melena.

Haematological observations:

Haemoglobin (g/dl), packed cell volume (%) and total erythrocyte count (10^{12}/L) levels dropped progressively in all groups whereas, the total leucocyte count (10^{9}/L) increased markedly. There was progressive increase in granulocytes with a corresponding decrease in total lymphocyte count. Total monocyte count decreased gradually over the period of time except in group I and group II, in which no definite pattern was observed.

Table 27: Haemoglobin, packed cell volume and total erythrocyte count in dogs of different groups at various observation intervals (Mean±S.E)

Days / Groups	0	4	7	10	13	16	19
Haemoglobin (g/dL)							
I. Lansoprazole (n=4)	14.35 ±1.00	13.9 ±1.25	12.35 ±1.01	11.9 ±0.86	11.77 ±0.92	11.22 ±1.17	10.03 ±1.13
II. Sucralfate (n=4)	14.57 ±0.52	13.47 ±1.05	12.87 ±0.88	11.77 ±0.78	------	------	------
III. Misoprostol (n=4)	13.87 ±0.80	12.6 ±0.80	11.77 ±0.51	10.12[a] ±0.51	10.15 ±0.35	10.2 ±0.0 (n=1)	10.0 ±0.0

					(n=2)		(n=1)
IV. Famotidine (n=4)	13.72 ±1.01	12.62 ±1.09	12.17 ±0.79	10.25 ±0.15 (n=2)	------	------	------
V. Seabuckthorn (n=4)	13.55 ±1.08	12.57 ±0.86	11.8 ±0.97	10.35 ±0.87	------	------	------
VI. Negative control (n=4)	12.35 ±0.60	11.92 ±0.63	11.05 ±0.49	10.63 ±0.24 (n=3)	------	------	------
Packed cell volume (%)							
I. Lansoprazole (n=4)	38.42 ±2.83	37.47 ±3.56	32.97 ±3.12	31.25 ±2.61	31.95 ±3.37	29.9 ±3.16	26.03 ±2.88
II. Sucralfate (n=4)	40.6 ±2.41	36.87 ±2.52	34.87 ±2.09	31.92 ±2.76	------	------	------
III. Misoprostol (n=4)	37.3 ±2.25	34 ±2.10	31.47 ±1.18	27.07 [aa] ±1.52	25.7 ±0.30 (n=2)	24.8 ±0.0 (n=1)	24.0 ±0.0 (n=1)
IV. Famotidine (n=4)	35.75 ±3.81	34.15 ±3.20	33.97 ±3.13	27.4 ±1.10 (n=2)	------	------	------
V. Seabuckthorn (n=4)	37.15 ±2.61	34.5 ±2.31	32.9 ±1.53	27.62[a] ±1.15	------	------	------
VI. Negative control (n=4)	36.5 ±1.58	35.1 ±1.71	32.87 ±1.32	31.53 ±0.87 (n=3)	------	------	------
Total erythrocyte count (10^{12}/L)							
I. Lansoprazole (n=4)	5.48 ±0.44	5.30 ±0.51	4.64 ±0.41	4.40 ±0.28	4.34 ±0.47	4.18 ±0.57	3.47 ±0.27
II. Sucralfate (n=4)	5.73 ±0.28	5.28 ±0.35	4.94 ±0.42	4.44 ±0.22	------	------	------
III. Misoprostol (n=4)	5.38 ±0.17	4.73 ±0.29	4.39 ±0.16	3.75 [aa] ±0.10	3.52 ±0.07 (n=2)	3.36 ±0.0 (n=1)	3.20 ±0.0 (n=1)
IV. Famotidine (n=4)	5.22 ±0.51	4.85 ±0.53	4.43 ±0.52	4.11 ±0.31 (n=2)	------	------	------
V. Seabuckthorn (n=4)	5.26 ±0.29	4.73 ±0.16	4.365 ±0.27	3.46[aa] ±0.36	------	------	------
VI. Negative control (n=4)	5.53 ±0.25	5.28 ±0.28	4.83 ±0.22	4.64 ±0.12 (n=3)	------	------	------

[a] $P < 0.05$ (Inter-group comparison)
[aa] $P < 0.01$ (-do-)

Table 28: Total and differential leucocyte count in dogs of different groups at various observation intervals (Mean±S.E).

Days / Groups	0	4	7	10	13	16	19
Total leucocyte count (10^9/L)							
I. Lansoprazole (n=4)	14.05 ±1.46	18.82 ±1.53	21.7 ±1.80	23.97 ±2.02	30.0 ±2.39	28.02 ±2.02	30.0 ±5.38
II. Sucralfate (n=4)	9.7 ±0.67	21.07 ±2.53	24.45 ±2.15	28.2[aa] ±2.36	------	------	------
III. Misoprostol (n=4)	12.57 ±1.12	18.12 ±0.93	22.0 ±2.13	25.6[aa] ±2.52	25.05 ±5.55 (n=2)	20.2 ±0.0 (n=1)	24.6 ±0.0

IV. Famotidine (n=4)	10.47 ±1.03	21.32 ±2.66	26.4[aa] ±3.33	25.4 ±5.2 (n=2)	------	------	------
V. Seabuckthorn (n=4)	13.85 ±1.95	21.1 ±2.85	25.12 ±2.60	29.1[aa] ±2.83	------	------	------
VI. Negative control (n=4)	10.17 ±1.78	24.62 ±9.13	31.67 ±6.56	30.23 ±6.44 (n=3)	------	------	------
Total granulocyte count (%)							
I. Lansoprazole (n=4)	74.37 ±2.82	82.1 ±2.26	84.35 ±2.54	85.37 ±2.39	86.9 ±2.15	88.1 ±1.28	87.9 ±1.01
II. Sucralfate (n=4)	74.92 ±2.43	81.72 ±3.45	84.25 ±2.00	89.72 [aa] ±0.53	------	------	------
III. Misoprostol (n=4)	76.05 ±4.96	84.95 ±3.44	86.57 ±3.04	88.82 [aa] ±3.38	92.85 ±1.25 (n=2)	91.6 ±0.0 (n=1)	92.6 ±0.0
IV. Famotidine (n=4)	70.12 ±8.30	82.02 ±4.19	84.02 3.87	84.55 ±7.95 (n=2)	------	------	------
V. Seabuckthorn (n=4)	74.1 ±1.63	85.47 ±3.42	86.62 ±2.91	87.05 [a] ±2.73	------	------	------
VI. Negative control (n=4)	83.52 ±1.18	87.65 ±2.18	88.55 ±1.49	87.23 ±3.05 (n=3)	------	------	------
Total lymphocyte count (%)							
I. Lansoprazole (n=4)	21.35 ±2.82	14.07 ±1.61	13.35 ±2.85	10.62 ±2.24	9.5 ±2.007	9.72 ±1.01	9.35 ±0.81
II. Sucralfate (n=4)	20.5 ±2.22	14.92 ±2.26	12.37 ±1.81	7.92 [aa] ±0.46	------	------	------
III. Misoprostol (n=4)	20.12 ±4.72	12.42 ±3.27	11.4 ±3.22	9.5 ±3.52	5.45 ±0.75 (n=2)	7.2 ±0.0 (n=1)	5.2 ±0.0
IV. Famotidine (n=4)	25.27 ±7.97	14.97 ±3.75	12.95 ±3.34	12.6 ±7.40 (n=2)	------	------	------
V. Seabuckthorn (n=4)	21.47 ±2.20	11.2 ±3.29	10.77 ±3.16	10.37 ±3.22	------	------	------
VI. Negative control (n=4)	11.62 ±0.40	9.42 ±1.36	9.0 ±1.13	8.66 ±1.67 (n=3)	------	------	------

[a] $P < 0.05$ (Inter-group comparison)
[aa] $P < 0.01$ (-do-)

Simultaneous reduction in Hb, PCV and TEC indicates substantial blood loss from the body (Rich and Coles, 1995). Stanton and Bright (1989) reported that non-regenerative anemia is a common (33/43 dogs) finding in dogs suffering from gastro-duodenal ulcerations. (Tyagi, 2006; Dogra, 2013) also reported a decrease in Hb, PCV and TEC when dexamethasone was administered for long periods in dogs. Similar trends were observed in the present study. (Lowe et al., 2008) also reported that administration of dexamethasone for long periods results in significant increase in white blood cell count, neutrophil count, monocyte count and significant decrease in mean lymphocyte count and eosinophil counts in cats.

Biochemical observations:

Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) levels remained elevated than base values in all the groups at all observation intervals though these remained within the

normal physiological limits. Serum blood urea nitrogen (BUN) and serum creatinine levels did not change much in different groups and also remained within the normal physiological limits throughout the period of study. A non-significant variation in the mean value of total proteins was observed during the study. Dillon *et al.*, 1983 observed increased ALT levels in healthy dogs, receiving long term high doses (2.2 mg/kg b.i.d. per day) of dexamethasone. (Rogers and Reubners, 1977) also reported that hepatopathy is induced in dogs and rabbits by single or multiple small doses (1 mg/kg) of corticosteroids. (Tyagi, 2006; Dogra, 2011) also reported similar kind of finding s in dexamethsone induced gastric GUE in dogs.

Based upon the above observations and previous trials, following conclusions were drawn –

1. Lansoprazole has both therapeutic and prophylactic efficacy in dexamethasone-induced GUE in dogs
2. Seabuckthorn oil and famotidine has therapeutic but not the prophylactic efficacy in dexamethasone-induced GUE in dogs
3. Misoprostol have limited therapeutic efficacy as well as prophylactic efficacy in dexamethasone-induced GUE in dogs
4. Sucralfate alone does not have the therapeutic or prophylactic efficacy in dexamethasone-induced GUE in dogs but in combination with SBT oil shows limited therapeutic efficacy.

6. Studies on therapeutic efficacies of herbal extracts alone and other combinations of seabuckthorn seed oil for GUE in dogs.

In the extended period of the project some more research studies were undertaken to evaluate the therapeutic efficacy of other herbal extracts alone and some other combinations of SBT seed oil in GUE in dogs. These trials are still underway are likely to be completed by end of February 2014.

Table 29:	**Details of treatment in different groups**
Group I:	Turmeric PO bid (N=2)
Group II:	Aloe Vera PO bid (N=2)
Group III:	Aloe Vera + 1ml SBT oil PO bid (N=2)
Group IV:	Lansoprazole @ 1.5mg/kg + 1ml SBT oil PO bid (N=2)

The preliminary trends as per gastroendoscopic observations are as follows-

Table 20: Average number of days for complete healing of GUE lesions in different groups		
Group I:	Turmeric (500 mg) PO bid (N=2)	12.0 days
Group II:	Aloe Vera (40% dehydrated) PO bid (N=3)	15.0 days
Group III:	Aloe Vera (40% dehydrated) + 1ml SBT oil PO bid (N=2)	9.0 days
Group IV	Lansoprazole @ 1.5mg/kg + 1ml SBT oil PO bid (N=2)	7.5 days

As of now the sample size is inadequate and data has not been analyzed. Therefore, no more results and discussion is presented regarding to this study.

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7) **Innovations**

	Innovation	Impact
1.	**Improvement of experimental model of non-fatal gastric ulcerations and erosions (GUE) in dogs-** There were only a few controlled studies on the GUE in dogs earlier. And therefore, there were great variability in the experimental models. The one of the earlier model of GUE in dogs developed by the PI in his previous studies, though gave more consistent results, yet it still took 16 days in most of the cases to produce the desirable severity of gastric lesions deemed fit for such studies. Also in cases of earlier model, the gastric ulcerogen was administered uniformly for 16 days in every case even if some animals developed a more severe GUE than others. Hence, in the pilot trials for this research project, a number of studies were undertaken to improve that experimental model of GUE in dogs with the objectives of reducing the time of development of gastric lesions and to restrict the administration of gastric ulcerogen only for a certain period till a uniform level of gastric lesion severity is achieved. Such studies entailed evaluation of a number of potential gastric ulcerogenic steroidal as well as non-steroidal drugs (NSAIDs) drugs like dexamethasone, prednisolone, meloxicam and ketoprofen. These drugs are commonly used in clinical cases of dogs and have been incriminated for gastric ulcerations. After initial rounds of trials, the drug dexamethasone was chosen for fine-tuning the model as it exhibited greater repeatability of results. And after a series of subsequent trials, the 1mg/kg dose of dexamethasone twice a day was finalized for dogs which resulted in development of GUE lesions of sufficient severity within 10 days (range 7-13days) in most cases. The drug was stopped when GUE endoscopic index reached to a certain degree so as to ensure that the treatment get started in every experimental dog from a uniform level of gastric ulcerations. Thus a new improved model of non-fatal gastric ulcerations and erosions was developed and adopted for GUE research in dogs.	Reduction in the duration for development of experimental GUE in dogs. More uniformity in controlled studies to evaluate therapeutic efficacy of different drugs in GUE in dogs.
2.	**Improvement of endoscopic gastric ulcerations and erosions (GUE) index in dogs-** The endoscopic gastric ulcer index developed and used by the PI in his previous studies was further modified and improved during pilot trials. The severity of gastric lesions is basically a subjective assessment; however, based upon experience separate scores were assigned to different types of lesions which seemed in accordance with their severity. This helped in making the subjective observation into a quantifiable objective one. The severity of GUE depends upon the extent of gastric mucosal involvement or the number of lesions as well as the type of lesions. The developed endoscopic GUE index gave equal weightage to both theses aspects separately. The number of lesions was assigned separate score ranging from 0-4. Whereas, for assessing the severity score, a number of gastric lesions appearing during the course of GUE were first identified and then categorized on a scale of 0-4 taking care that the scale reflect the seriousness of the condition as much as reasonably possible. The GUE index was determined by adding the GUE number score and severity score. The maximum level of attainable non-fatal GUE index was thus ascertained to be 8. However, as level 7 and 8 are nearby, it was also decided to stop administration of dexamethasone and start treatment in animals in which GUE index 7 is observed on two consecutive intervals or 8 on any one interval. This GUE index was able to represent the severity of gastric ulcerations in a reasonably more specific manner in repeated double blind studies based on observation of endoscopic view of stomach by different workers.	Improved assessment of the impact of various drugs being used for treatment and prevention of GUE in dogs in a more reliable manner. A humane approach of GUE research obviating the need of using and killing laboratory animals like rats just to determine GUE index.
3.	**Utilization of herbal and allopathic drug combination for improved therapeutic efficacy in GUE of dogs-** Earlier the gastric ulcer-related studies remained focused mainly on validation of one kind of drug or herbal preparation or at best two known allopathic drugs combinations for their effect on GUE. Whereas, in this sub-project, first we validated the therapeutic efficacy of SBT oil and various other drugs in dexamethasone-induced non-fatal gastric ulcerations and erosions (GUE) in dogs. Later we also undertook a series of trials to explore the additive/synergistic therapeutic efficacy of seabuckthorn oil and other allopathic drugs already proved useful for management of GUE in dogs. We were able to determine the synergistic therapeutic efficacy of SBT oil with famotidine or sucralfate for GUE in dogs and were successful in reducing the recovery period. Thus the best therapeutic combination for management of GUE in dogs was ascertained by this study.	Improved treatment regimen of gastric ulcerations and erosions in dogs.

8) **Process/ Product/Technology/ Value Chain/ Rural Industry Developed**
(List partner-wise major Process/ Product/Technology developed and their outcome in quantifiable terms)

S. No.	(Process/Product/Technology/ Value Chain/ Rural Industry Developed	Adoption/ Validation/ Commercialization, etc.	Responsible Partner
	Development of non-fatal gastric ulceration-erosions (GUE) model in dogs	Validation of the reliability of faecal occult blood test to be a sensitive method of detecting sub-clinical gastric ulceration and erosions in dogs.	Department of Surgery and Radiology, DGCNCOVAS, CSKHPKV, Palampur
	Development of the method of endoscopic evaluation of GUE progression	Validated no toxic effects of SBT seed oil on body system of dogs	
	More effective treatment regimen developed for management of GUE in dogs utilizing SBT seed oil.	Establishment of the dose-dependent therapeutic activity of Seabuckthorn seed oil for GUE in dogs.	
	More effective gastroprotective regimen ascertained for prevention of GUE in dogs utilizing lansoprazole.	Establishment of the dosing frequency-dependent therapeutic activity of misoprostol and Sucralfate for GUE in dogs.	
		Discovered the synergistic therapeutic effect of the combinations of Seabuckthorn seed oil with Famotidine or Sucralfate in GUE in dogs.	
		Found no synergistic activity of the combination of Seabuckthorn oil plus misoprostol.	

Note: Use pro-forma (1, 2, and 3) for details.

9) **Patents (Filed/Granted)**

S. No.	**Title of Patent**	**Inventor(s) (Name & Address)**	**Filed/Published/ Granted (No./Date)**	**Responsible Partner**
	NIL			

10) **Linkages and Collaborations**

S. No.	**Linkages developed (Name & Address of Organization)**	**Date/Period From-To**	**Responsible Partner**

11) **Status on Environmental and Social Safeguard Aspects**
(Please see NAIP website for clarity on the subject)

12) **Constraints, if any and Remedial Measures Taken**

13) **Publications** (As per format of citation in Indian Journal of Agricultural Sciences)

A. Research papers in peer reviewed journals. Details as per the guidelines for citation of publications (Annexure I)

S. No.	Authors, Title of the paper, Name of Journal, Year, Vol. & Page No.	NAAS Ratings	Responsible Partner
1	Richa Dogra, **S P Tyagi,** and Amit Kumar 2013. Efficacy of Seabuckthorn (*Hippophae rhamnoides*) oil *vis-à-vis* other standard drugs for management of gastric ulceration and erosions in dogs. *Vet. Med. Int.* (2013), Article ID 176848, 11 pages.	-	

B. Books/ Book chapters/ Abstracts/ Popular articles, Brochures, etc.

S. No.	Authors, Title of the papers Name of Book/ Seminar/ Proceedings/Journal, Publisher, Year, Page No.	Responsible Partner

	Tyagi SP, Varshney AC and Kumar Amit. Prophylactic efficacy of Seabuckthorn oil *vis-a-vis* other gastroprotective agents against gastric ulcerations and erosions. Presented in "34th Annual Congress of Indian Society for Veterinary Surgery" and International symposium on *"Newer concepts on surgical techniques for farm and companion animal practice"* held at College of Veterinary Sciences, Puducherry, India from Dec. 08-10, 2010.	
	Tyagi SP, Varshney AC and Kumar Amit. Prophylactic efficacy of Seabuckthorn oil and omeproazole in gastric erosions and ulcerations in dogs. Presented in 5th International Seabuckthorn Association Conference (ISA 2011) held at Xining, China from 3-8th August 2011.	
	Tyagi SP, Dogra R and Kumar Amit (2011). A comparative evaluation of Famotidine, lansoprazole and seabuckthorn seed oil for treatment of gastric ulcer and erosions in dogs. 35th Annual Congress of Indian Society for Veterinary Surgery and International symposium *on* "Nanobiomaterials in biomedical research; Their application in Veterinary Surgery" held at Department of Veterinary Surgery and Radiology of Veterinary College, West Bengal University of Animal Sciences and Fisheries, Kolkata from Nov. 11-13, 2011.	
	Dogra R, **Tyagi SP**, Kumar Amit, Kumar Adarsh, Varshney AC and Singh V (2011). Therapeutic evaluation of sucralfate, misoprostol and seabuckthorn oil for management of gastric ulcerations and erosions in dogs. "National Conference on Seabuckthorn: Emerging trends in R& D on Health Protection and Environmental Conservation" held at Department of Biology and Environmental Sciences, College of Basic Sciences, CSKHPKV, Palampur from Dec. 1-3, 2011.	
	Kumar Amit, **Tyagi SP, Dogra R** and Gupta S (2013). Dose-dependent effects of Seabuckthron seed oil on the healing of gastric ulcers and erosions in dogs. 6th International Seabuckthorn Association Conference (ISA 2013) held at Potsdam, Germany from 14-17 October 2013.	

14) Media Products Developed/Disseminated

S. No.	CD, Bulletins, Brochures, etc. (Year wise)	No. of Copies	Distribution	Responsible Partner

15) Meetings/Seminars/Trainings/Kisan Mela, etc. organized

S. No.	Details of Meetings/Seminars/ Trainings, etc.	Duration (From-To)	No. of Personnel Trained	Budget (`)	Organizer (Name & Address)

16) **Participation in Conference/ Meetings/Trainings/ Radio talks, etc.**

S. No.	Details of Meetings/Seminars/ Trainings/Radio talk, etc.(Name &Address)	Duration (From-To)	Budget (`)	Participant (Name & Address)
	"34th Annual Congress of Indian Society for Veterinary Surgery" and International symposium on *"Newer concepts on surgical techniques for farm and companion animal practice"* held at College of Veterinary Sciences, Puducherry, India.	Dec. 08-10, 2010.		Dr SP Tyagi Dr Adarsh Kumar Dr Amit Kumar
	5th International Seabuckthorn Association Conference (ISA 2011) held at Xining, China.	3-8th August 2011.		Dr SP Tyagi
	35th Annual Congress of Indian Society for Veterinary Surgery and International symposium *on* "Nanobiomaterials in biomedical research; Their application in Veterinary Surgery" held at Department of Veterinary Surgery and Radiology of Veterinary College, West Bengal University of Animal Sciences and Fisheries, Kolkata.	Nov. 11-13, 2011.		Dr SP Tyagi Dr Adarsh Kumar Dr Amit Kumar
	"National Conference on Seabuckthorn: Emerging trends in R& D on Health Protection and Environmental Conservation" held at Department of Biology and Environmental Sciences, College of Basic Sciences, CSKHPKV, Palampur.	Dec. 1-3, 2011.		Dr SP Tyagi Dr Amit Kumar
	6th International Seabuckthorn Association Conference (ISA 2013) held at Potsdam, Germany.	14-17 October 2013		Dr Amit Kumar

17) **Foreign Trainings/ Undertaken (National/ International)**

S. No.	Name, Designation and Address of the Person	Place of Training	Area of Training	Time and Duration	Total Cost (`)

18) **Performance Indicators (from inception to completion)**

S. No.	Indicator	Total No.	
1.	No. of production technologies released and/or adopted	-	
2.	No. of processing technologies released and/or adopted	-	
3.	Number of technologies/products commercialized based on NAIP research	-	
4.	No. of new rural industries/enterprises established/ upgraded	-	
5.	No. of product groups for which quality grades developed and agreed	-	
6.	Total no. of private sector organizations (including NGOs) participating in consortium	-	
7.	No. of farmers involved in consortia activities	-	
8.	Total number of farmers' group developed for marketing and processing	-	
9.	Number of patent/intellectual property protection applications filed based on NAIP research	-	
10.	Number of patents/intellectual property protections granted/published based on NAIP research	-	
11.	Number of scientists trained overseas in the frontier areas of science	-	
12.	Number of scientists trained overseas in consortium-based subject areas	-	
13.	No. of scientists participated in conference/seminar etc. abroad	-	
14.	No. of training organized/ farmers trained	Training No.	Farmers No.
15.	Success stories		
16.	Incremental employment generated (person days/year/HH)	Baseline	Final
17.	Increase in income of participating households (` per annum)	Baseline	Final
18.	Number of novel tools/protocols/methodologies developed	10	
19.	**Publications**		
	Articles in NAAS rated journals	-	

	Articles in other journals	1
	Book(s)	-
	Book chapter(s)	-
	Thesis	4
	Popular article(s) (English)	-
	Newspaper article(s)	
	Seminar/Symposium/Conference/Workshop Proceedings	5
	Technical bulletin(s)	-
	Manual(s)	-
	CDs/Videos	-
	Popular article(s) in other language	-
	Folder/Leaflet/Handout	-
	Report(s)	-

19) **Employment Generation (man-days/year)**

S. No.	Type of Employment Generation	Employment Generation up to End of Sub-project	Responsible Partner

20) **Assets Generated**

(Details to be given on equipments and works undertaken in the sub-project, costing more than ` 10,000/- in each case)

(i) Equipment/ Vehicles/ Research Facilities

S. No.	Name of the Equipment with Manufacturers Name, Model and Sr. No.	Year of Purchase	Quantity (Nos.)	Total cost (`)	Responsible Partner
1.	Video-gastro-duodenoscope, Karl Storz, Germany	2009	1	9.98,539	
2.	Automatic blood cell counter, BC Vet 2800, Mindray, China	2009	1	4.576	
3.	Electricity Inverter 5KVA/96V, Sukam-Exide	2009	1	1.27	
4.	Air and water bacterial filtration and temperature control units, Kent, Voltas, India	2009	2	1.0362	
5.	Digital weighing scale	2009	1	0.135	
6.	Desktop computer	2009	1	0.48	

(ii) Works

S. No.	Particulars of the Work, Name and Address of Agency Awarded the Work	Year of Work Done	Quantity (Nos.)	Total Cost (`)	Responsible Partner
7.	Renovation civil works	2009	-	2.97027	
8.	Furniture	2009	-	0.49711	

(iii)Livestock

(Details of livestock procured/produced in the sub-project)

S. No.	Details of Livestock (Breed, etc.)	Year of Procurement/Production	Nos.	Total Cost (`)	Responsible Partner

(iv) Revenue Generated

(Details may be given on revenue generated in the sub-project viz., sale of seeds, farm produce, products, patents, commercialization, training, etc.)

S. No.	Source of Revenue	Year	Total amount (`)	Responsible Partner

21) **Awards and Recognitions**

S. No.	Name, Designation, Address of the Person	Award/ Recognition (with Date)	Institution/ Society Facilitating (Name & Address)	Responsible Partner

22) **Steps Undertaken for Post NAIP Sustainability**

Research project have been submitted to undertake follow up work with the objectives of developing more efficient GUE treatment and preventive formulations involving other subspecies of seabuckthorn as well.

23) **Possible Future Line of Work**

All research on the subject remained focused on only one subspecies of seabuckthorn i.e. *Hippophae rhamnoides turkestanica*. There are many other subspecies of seabuckthorn already existing in India and many others are likely to be introduced shortly which need to be studied for their different therapeutic efficacies. Secondly so far no herbal combination involving seabuckthorn had been studied for its utility in gastric ulcerations and erosions which needs to be studied for better efficacies.

24) **Personnel**

(Staff of Lead Centre & Partner-wise, their Name, Designation, Discipline and Duration)

	From – To (DD/MM/YYYY)
Research Management (CL)	
1.	
2.	
3.	
Scientific (CPI, CCPI, others)	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	
12.	
Technical (CPI, CCPI, others)	
13.	
14.	
15.	
16.	
17.	
18.	
19.	
20.	
21.	
Contractual (CPI, CCPI, others)	
22. Ms. Omeshwari	29/08/2008 to 31/05/2009
23. Mr. Shahid Hussain	05/06/2009 to 12/05/2010
24. Mr. Bhanu Pratap Thakur	08/09/2010 to 30/09/2012
25.	
26.	
27.	
28.	

29.	
30.	
31.	

25) **Governance, Management, Implementation and Coordination**

A. Composition of the various committees (CIC, CAC, CMU, etc.)

S. No.	Committee Name	Chairman (From-To)	Members (From-To)
1.	**CIC**		
2.	**CAC**		
3.	**CMU**		

B. List of Meetings organized (CIC, CAC, CMU, etc.)

S. No.	**Details of the meeting**	**Date**	**Place & Address** (Where meeting was organized)
1.	**CIC**		
2.	**CAC**		
3.	**CMU**		

Part-III: Budget and its Utilization

STATEMENT OF EXPENDITURE (Final)

(Period from _____to_____)

(Date of start) (Date of completion)

Sanction Letter No. _____
Total Sub-project Cost `_____
Sanctioned/Revised Sub-project cost *(if applicable)* **`_____**
Date of Commencement of Sub-project _____
Duration: From _____to _____ (DD/MM/YYYY)
Funds Received in each year
I Year `_____
II Year `_____
III Year ` _____
Bank Interest received on fund ***(****if any)* **` _____**
Total amount received ` _____
Total expenditure ` _____
Expenditure Head-wise:

Sanctioned Heads	**Funds Allocated (*)**	**Funds Released**			**Expenditure Incurred**						**Total Expenditure**	**Balance as on date**	**Requirement of additional funds**	**Remarks**
		1st Year	2nd Year	3rd Year	1st Year	2nd Year	3rd Year	4th year	5th year	6th year				
A. Recurring Contingencies														
(1) TA					0.07356		0.14692	0.08092	0.01513	0.05				
(2) Workshops					-		-	-	-	-				
(3) Contractual Services/RA/SRF					0.99115		1.218	2.08552	1.014	-				
(4) Operations expenses					1.59772		2.15	2.60	1.99997	1.0				
Sub-Total of A (1-4)					2.66243		3.51492	4.76644	3.0291	1.05				
B. HRD Component								-	-	-				
(5) Training					-		-	-	-	-				
(6) Consultancy					-		-	-	-	-				
Sub-Total of B (5-6)					-		-	-	-	-				
C. Non-Recurring							-	-	-	-				

(7) Equipment					17.48259		-	-	-	-				
(8) Furniture					0.49711		-	-	-	-				
(9) Works (new renovation)					2.97027		-	-	-	-				
(10) Others (Animals, Books, etc.)					0.09931		0.04945	0.05	-	-				
Sub-Total of C (7-10)					21.04928		0.04945	0.05	-	-				
D. Institutional Charges*														
Grand Total (A+B+C+D)					23.71171	3.86614	3.56437	4.81644	3.0291	1.05	**40.03776**			

* Institutional charges will be 10% of the recurring contingencies for the Lead Consortium and 5% for Consortia Partners.

Name & Signature of CPI :

Date:_____

Name & Signature of Competent Financial authority:

Date:_____

Date:_____

Signature, name and designation of Consortia Leader

PART-IV: DECLARATION

This is to certify that the final report of the Sub-project has been submitted in full consultation with the consortium partners in accordance with the approved objectives and technical programme and the relevant records, note books; materials are available for the same.

Place:_____

Date:_____ Signature of Consortium Principal Investigator

Signature & Date
Consortium Co-Principal Investigator

Signature & Date
Consortium Co-Principal Investigator

Signature & Date
Consortium Co-Principal Investigator

Signature & Date
Consortium Co-Principal Investigator

Comments & Signature of Consortium Leader

Date:

Pro-forma 1

Details of Technologies Developed/ Validated/ Adopted
(Page limit: 3 pages/ technology)

1) Title of the sub-project:

2) Name of CPI/ CCPI:

3) Title of the technology:

4) Information on existing farming systems, practices, productivity levels and income in the target area:

5) Key Intervention(s) introduced:

6) Results
   Status of dissemination/ commercialization; and, extent of adoption and success, if applicable; with supporting data (with tables and photographs as annexure):

7) Brief description of technology for release:

8) Expected Outcome/ Impact of the technology:
   8.1. Expected increase in area, production and net income
   8.2. Others

9) Whether findings have been published? If so, give the citation and enclose copy of the publication.
10) Any other information.

Note: Use separate pro-forma for each technology
Attach photograph(s) relevant to the technology

Pro-forma 2

Details of Technologies/ Innovations Commercialized
(Page limit: 3 pages/ technology)

1) Title of the sub-project:

2) Name of CPI/ CCPI:

3) Title of the technology:

4) Commercialization status with date of licensing/ MOU:

5) Brief description of intervention/ innovation:

6) Name and address of the firm(s) which has commercialized it:

7) Area (state(s)/ district(s)) covered:

8) Volume/ quantity of Annual production and approximate sale value:

9) Benchmark (existing similar product) and Consumer acceptance, particularly in case of food products:

10) Status of patenting, if patentable, trademark or any other IPR title, if applicable:

11) Status of publication and publicity:

Note: Use separate pro-forma for each technology
Attach photograph(s) relevant to the technology

Pro-forma 3

Details on Rural Entrepreneurships/ Rural Industries Developed
(Page limit: 3 pages/ rural industry)

1) Title of the sub-project:

2) Name of CPI:

3) Name of rural industry with address:

4) Contact: Phone and E-mail of rural industry:

5) Investment (Rs): NAIP Funds
   Industry/ Entrepreneur

6) Product(s) produced and marked:

7) Annual Production (kg or litre):

8) Raw Material(s) and Quantity used/ year (kg or litre):

9) Cost of raw material (per kg or litre):

10) Price of Product: In Whole Sale
    In Retail

11) Type of Beneficiaries:

12) Number of Beneficiaries:

13) How the Industry is beneficial to primary producers:

14) Estimate Employment Generation/ Year (person days):

15) CPI to explain whether the industry is approved by FPO/BIS or any other statutory body and how the food safety and quality assurance of end product are being ensured?

Note: Use separate pro-forma for each entrepreneurship/ rural industry
Attach photograph(s) relevant to the industry/ entrepreneurship

EXECUTIVE SUMMARY

The executive summary is an important part of the project report that gives an overview and summarizes the entire project. The executive summary (3-4 pages) should reflect important accomplishments under the project. Contents of the summary should include:-

Background Information about the Project (quarter page): ……

Baseline Information on the Pre-Project Situation in the Project Area (half page): ……

Work Proposed and its Execution Plan (half page): ……

Achievements: Achievements both in absolute and relative terms as running material (1-2 pages). Significant achievements should be given in numerical term as bullets.

- Production technologies developed and adopted:
- Process technologies developed, adopted and commercialized:
- Rural industries established/commercialized:
- Patents (filed/granted):
- Publications:
    i. Research papers published:
    ii. Popular articles published:
    iii. Books/book chapters published:
    iv. Bulletins/brochures/leaflets published:
    v. Training manuals published:
    vi. Film/ CD developed:
    vii. Coverage in press, TV, media:
- Trainings undertaken and scientists/other staff trained (national/international):
- Trainings organized and farmers/other stake holders trained:
- Field demonstrations organized:
- Field day/farmer day/'mela' organized:
- Success stories:
- Self help groups/farmer groups developed:
- Employment generation (man days/year):
- Assets generation (equipments/implements procured, civil work done and revenue generated):
- Awards/honors:

Socio-Economic Impact (Economic Rate of Return): Socio economic impact per household in the project area (half page):

Environmental Impact: Environmental impact due to project interventions in respect of reduction in use of chemicals, improvement in water availability, reduction in air pollution and improvement in biodiversity (quarter page).

Sustainability Plan: steps taken to sustain the gains due to project interventions in the project area and for horizontal expansion after the project is closed should be indicated (quarter page):

Annexure 1

Guidelines for Citation of Publications from NAIP sub-projects

(Note: Give only those publications (under different categories) which are published during the project term)

1. **Research Article:**

   Dubey P K, Selvakumar M, Kathiravan P, Yadav N, Mishra B P and Kataria R S. 2010. Detection of Polymorphism in Exon 2 of Toll-like Receptor 4 Gene of Indian Buffaloes using PCR –SSCP. *Journal of Applied Animal Research* **37**: 265-268. **(NAAS rating 6.6)**

2. **Book**

   Kathiresan R M. 2010. Components Integration in Small Holder Farms, p 119 Lambert Academic Publishing AG & Co. Koln, Germany.

3. **Book Chapter**

   Bhargava A, Jain N and Panwar J. 2011. Synthesis and Application of Magnetic Nanoparticles: A Biological Perspective. (*in*) *Current Topics in Biotechnology and Microbiology*, p 117-155, Dhingra H K, Jha P N and Bajpai P. (*Eds*), LAP Lambert Academic Publishing AG & Co. KG, Dudweller Landstr, Germany.

4. **Thesis:**

   Kumbar Shivanand. 2010. 'Rural Community Organization for Strengthening the Livelihood Security of Buffalo Rearers through Strategic Supplementation of Mineral Mixture and Urea Molasses Mineral Blocks.' M Sc thesis, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar Karnataka, p 110.

5. **Popular Article:**

   Pandey M M and Tiwari P S. 2009. Precision Farming-A New Concept for Present and Future Agriculture. *Indian Farming* **59**(6): 3-9.

6. **Newspaper Article:**

   Dharajothi B and Gopalakrishnan N. 2009. Mealybug - A New Threat to Cotton Cultivation. *The Hindu*, 1.1.09.

7. **Seminar/ Symposium/Conference/Workshop Proceedings**

   Chattopadhyay S K, Dey S K and Sreenivasan S. 2009. Composite Yarns from Natural Fibres for Production of Technical Textiles. *(in) Proceedings of International Conference on Emerging Trends in Production, Processing and Utilization of Natural Fibres,* held during 16-18 April 2009 at Worli, Mumbai, India, pp. 338-346.

8. **Technical Bulletin:**

Singh M and Sharma A. 2011. Precision Farming and its Potential in Punjab Agriculture. *Tech. Bull. No. 04,* 42 p Department of Farm Machinery and Power Engineering, Punjab Agricultural University, Ludhiana.

9. Manual

Balachandar D, Karthikeyan S and Kumar K. 2011. Current Perspectives in Molecular Microbial Diversity. Tamil Nadu Agricultural University, Coimbatore, p. 107.

10. Seminar/ Symposium/Conference/Workshop Presentation

Adhikari T, Goswami A, Biswas A K, Kundu S, Tarafdar J C and Subba Rao A. 2010. Synthesis of Rock Phosphate Nano Particle and Its Effect on Seed Germination of Selected Crops. (in) International Conference on Nanoscience and Nanotechnology (ICONN 2010), held during 24-24 February 2010 at SRM University, Kattankulathur, Chennai, Tamil Nadu, India.

11. CDs/Videos:

Atreja S K. 2011. *In vitro* Capacitation of Cryopreserved Buffalo Spermatozoa in Egg Yolk Extender. National Dairy Research Institute, Karnal.

12. Popular article in other Language

Behera B. 2008. *Odissare Gramina Jibika O Khadya Nirapatta* (Oriya) (Rural Livelihood and Food Security in Orissa). *Krishi Sambada*, Directorate of Agriculture and Food Production, Govt. of Orissa, October-December, 2008, p 21-24.

13. Folder/Leaflet/Handout:

Adhya T K and Bhattacharyya P. 2008. Soil Organic Carbon Dynamics vis a vis Anticipatory Climatic Changes and Crop Adaptation Strategies. CRRI, Cuttack.

14. Report:

Murthy G R K, Reddy K M, Nanda S K, Rao Rama D and Rao Bhasker E. 2009. *Identification and Integration of Decision Support Tools through Content Management Models for Effective Knowledge Transfer*. Research Report, National Academy of Agricultural Research Management, Hyderabad, p. 33.

……

Note:

Name of CPIs and CCPIs to be given in *italics*

Journal name to be given in full

Annexure 2

General Guidelines for Developing Final Reports

1) The CPI will send the consolidated report to PIU-NAIP after compiling the progress reports received from all the consortium partners. The report should also list the constraints (if any) being faced by consortia partners.
2) The Final Report should not be a mere repetition of Annual Reports. The purpose of the final report is to link all findings from the sub-project so that the overall achievements are discussed in terms of scientific accomplishments, contributions to scientific, human capital development, the relevance of findings to development, and how the technology is to be disseminated.
3) The Executive Summary should review and summarize the entire Sub-project. The Executive Summary should clearly place sub-project accomplishments in the overall context of agricultural development.
4) Steps undertaken for post project sustainability. Plan should be developed in respect of 1) packaging of location specific technologies, 2) conservation of natural resources – water, soil, forest and bio-diversity, 3) formation of SHGs and VLCs, 4) creation of rural technology center/ community center, 5) access to market and credit, 6) establishment of rural industries and farm fresh outlets, 7) generation of sustainability funds and development of an institutional mechanism to internalize and sustain the gains once the project closes.
5) Summary in Hindi must be included.
6) Final Report should be of A-4 size and the total number of pages must not exceed 50-60 in any case.
7) The text of the Final Report should be in the following format:
   - MS Word document
   - Line spacing: 1.15
   - Font: Times New Roman
   - Main headings: 12 point bold
   - Running text: 12 point normal
8) Light pink #FF99CC color should be used for cover page (front & back) of the report.
9) Ten hard bound printed copies of Final Report should be submitted. Also, soft copy of the Final Report in MS Word document (2003) should be sent in the CD in duplicate.
10) The details of performance indicators claimed in the listing should be submitted as soft copy in CD in MS Word Format. A copy of each publication, film, knowledge products, patent application to be attached in a separate folder.
11) CPIs must strictly follow the guidelines while composing and printing the sub-project Final Report.
12) The draft of Final Report in soft copy be sent 15 days before sub-project closing date to concerned National Coordinator. Final printing be done after getting comments/suggestions on draft report.