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# EFFECT OF SCAPHIUM AFFINE ON FORCED SWIMMING TEST AND COLD STRESS INDUCED ULCER IN RATS

**Lakshmana V Bendre [1*], Roshan. S[2]**

[1*]Research Scholar, Department of Pharmacy, Bir Tikendrajit University, Imphal, Manipur
[2]Research Supervisor, Department of Pharmacy, Bir Tikendrajit University, Imphal, Manipur

***Corresponding Author:** Lakshmana V Bendre
*Research Scholar, Department of Pharmacy, Bir Tikendrajit University, Imphal, Manipur
E-mail- ivb508@gmail.com Contact number :+91- 9008603508

## Abstract
The present study was carried out to evaluate the effect of ethanolic fruit extract of *Scaphium affine* on swimming endurance test and cold stress induced Ulcer in albino rats. The effect was assessed by swimming survival time, estimation of ulcer index, $P^H$ of gastric juice, biochemical parameters like Glucose, Cholesterol, Triglycerides, Plasma cortisol and Blood urea nitrogen (BUN) oxidative marker MDA, MPO, serum nitrate content, SOD and GSH content and pro inflammatory cytokines IL-6, IL-8 and TNF -α and histopathology of stomach at a dose of 300 and 500 mg/kg (per oral) were determined. *S. affine* extract significantly ($p<0.001$) increases swimming time, decrease in blood glucose, cholesterol, triglyceride, cortisol and BUN. Significant ($p<0.01$) decrease in ulcer index and increase $P^H$ of gastric juice was observed compared to control group, Increased in SOD, GSH, CAT and NO decrease in MPO and MDA contents, pro inflammatory cytokines IL-6, IL-8 andTNF-α significantly decrease and histopathology of stomach restore to normal compare to control, this obtained results revealed that the extract of *S. affine* has significant anti stress and anti ulcer activity in rats.

**Keywords:** *S. affine* Cold stress,Ulcer, IL-6,IL-8andTNF-α

## INTRODUCTION
Stress basically is a biological response to aversive conditions that tend to threaten the homeostasis of the organisms. Stress has been shown to induce a marked rise in the brain levels of biogenic amines such as adrenaline and nor-adrenaline. These chemical substances are released in response to stress signals and are meant to assist the organisms to manage with the stressful situation. However, increased utilization of these amines results in their depletion in case of prolonged severe stress resulting in fatigue, reduced stamina, lowered mood (hopelessness) or despair seen in individuals under intense stress. It has been reported that drugs with anti-stress properties induce a state of non-specific resistance against stressful conditions. However, the incidence of toxicity and dependence has limited the therapeutic usefulness of these drugs in the control of stressful events. The potential utilities of safer and cheaper herbal medicines as anti-stress agents have been reported in literature [1-3].
*Scaphium affine* (*S. affine*) the fruits belonging to the family Sterculiaceae. *S. affine* has been used as both traditional medicine and health food in many Asian countries. In China, it is one of the herbs listed as both edible and medicinal resources (Medicine Food Homology) The *S. affine* decoctions

have also been traditionally used for the treatment of tussis, sore throat, toothache, constipation, laryngitis, cough, menorrhagia, and pain [4-6].
Pharmacologically, both *in vitro* and *in vivo* studies have indicated that *S. affine* exhibits a variety of pharmacological activities, including analgesic, anti-pyretic, antimicrobial, anti-hypertensive, anti-inflammatory, weight-losing, laxative, and calcium oxalate inhibition effects [7-11]. Phytochemically, several bioactive compounds have been isolated from *S. affine*, including polysaccharides, alkaloids, flavonoids, organic acids and cerebrosides. The major constituents of *S. affine* are polysaccharides and lipids [7, 12-14] which are the mostly studied constituents. The present work is stress induced anti ulcer activity of *S. affine* in rats.

## MATERIALS AND METHOD:

The fruits of *S affine* were collected in the month of August-September from the local areas of Hyderabad and make herbarium. The plants were identified, confirmed and authenticated by Dr.Vijaya Bhasker Reddy, Assistant Professor, Department of Botany , Osmania university, Hyderabad. A voucher specimen (No.OUAS-151).
The fresh fruits around 2kg shade dried for 15 days; fruit material was powdered using mixer grinder and passed through sieve no 85. Weight About 150gm of dried fruit powder was subjected to soxhlet's apparatus extraction using ethanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator

**Preliminary phytochemical analysis:** the extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents [15].

### Experimental animals

Adult wistar rats of male 9 to 11 week age, weighing 150-170gm were procured from Mahaveera enterprises, Hyderabad. Animals were housed in standard laboratory conditions at 25°c with 12 hr light-dark cycle with free access to chow and water *ad libitum*. The research protocol was approved by (HKES/COP/MTRIPS/IAEC/105/2022).

### SWIMMING ENDURANCE TEST

Wistar rats of male and female weighing between 150-170gm were randomly cauterized into four groups of six rats in each group.
Group I- swimming test [ST] control,
Group II –Swimming + *S affine* 300 mg/kg (p.o) for 3weeks.
Group III- Swimming + *S affine* 500 mg/kg (p.o) for 3weeks ,
Group IV - Swimming + Omerrazole 20mg/kg (p.o) respectively for 3weeks.
Swimming endurance test methods was described is modified in our laboratory [16]. Above Group II-IV treatment was given to rats for 3 weeks. On 1$^{st}$ week, 2$^{nd}$ week and 3$^{rd}$ week 1 hr. after treatment, all the rats (except normal control) were subjected to swimming endurance test. The mice were allowed to swim individually in swimming tank (30 cm height with 20 cm diameter) containing water of 25 cm height maintained at 25 ± 1°C temperature. The rats were allowed to swim till exhausted and moment when animal drowned is considered as the endpoint. The mean swimming time for each group was calculated .[16-17].

### Cold stress induced ulcer

Albino rats 150-180gm of either sex were divided in to 5 groups of 6 animals each.
Group -I served as control,
Group-II served as cold stress control,
Group-III served as cold stress induced and *S affine* 300mg/kg p.o,
Group IV cold stress subjected and extract *S affine* 500 mg/kg and
Group - V cold stress induced and Omerrazole 20mg/kg (p.o).

Cold Stress was induced in 2nd, 3rd, 4th and 5th groups in albino rats by exposing temperature 4 ±1°C daily for 2 hrs for10 days[16]. On 11th day all the animals were sacrificed blood are collected for estimation of serum [18]. The animals were dissected and the stomach carefully keeping the esophagus closed opened along the greater curvature the gastric contents were collected in a tube and centrifuged at 3000 rpm for 5 min, the volume of supernatant was expressed as ml/100g body weight. The mucosa was flushed with saline finally the ulcers were observed macroscopically. The observation was made for any ulceration or inflammation in the stomach. The stomachs were opened along the greater curvature and the mucosa was exposed for evaluation. Ulcer index, percentage protection and the $P^H$ of gastric juice was determined statistically stomach tissue was fixed in 10% formaldehyde for histopathological evaluation using haematoxylin and eosin (H & E) stain, stomach tissue the used for measurement of oxidative stress markers like Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide dismutase (SOD) and Myeloperoxidase (MPO). Estimation of pro inflammatory cytokines are IL-6,IL-8, IL-1β and TNF- α level in homogenized stomach and kidney supernatant analysed with ELISA kit. [18-25].

**Statistical analysis**

The results were expressed as mean ± SEM. The data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A value of $P < 0.05$ was considered as statistically significant.

**RESULTS:**

The phytochemical studies of *S. affine* shows the presences of priminary and secondary metabolites such as Alkaloids, glycoside, sterol, flavonoids, terpenoids, protein and amino acids and carbohydrates.

In Swimming endurance test ethanolic extract of *S. affine* at a dose of 500mg/kg b.w has shown significantly ($p<0.001$) increase in swimming time compared to control (figure1).

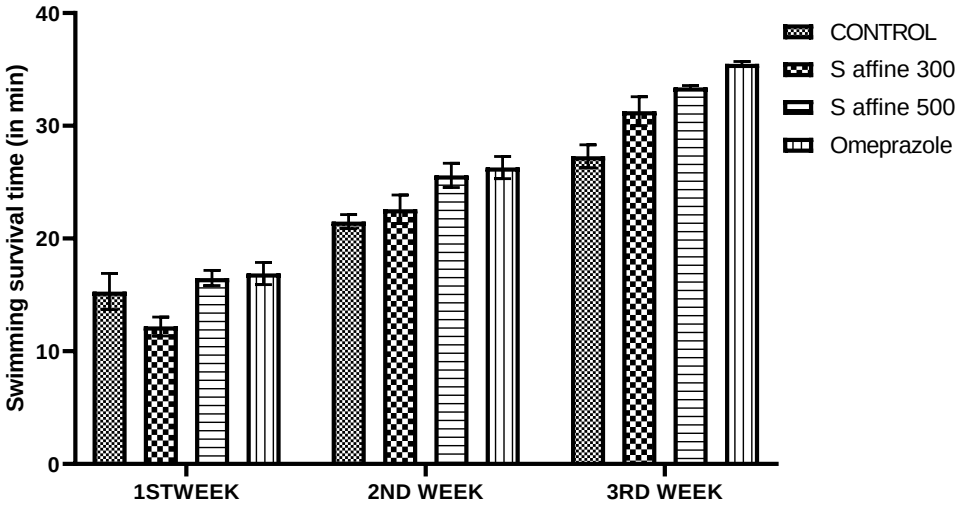



**Figure 1:** effect of *S. affine* on Swimming endurance test

In cold stress induced animals significantly increased serum glucose, cholesterol, triglyceride, BUN and plasma cortisol parameters levels, ethanolic extract of *S. affine* has significantly ($p<0.001$) reduced the elevated levels of biochemical parameters like glucose, cholesterol, triglyceride, BUN and plasma cortisol levels compared with stress control group (figure2).

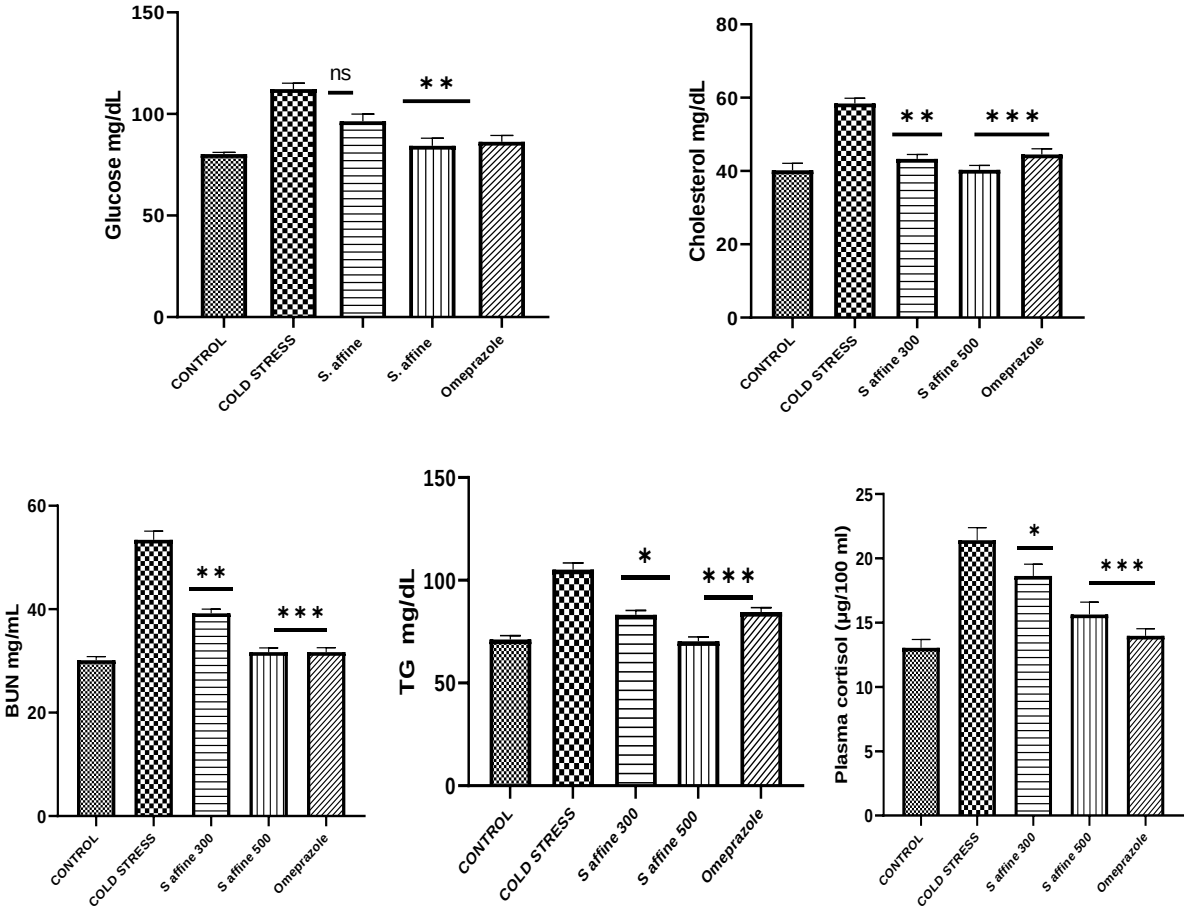



**Figure 2:** Effect of *S. affine* on biochemical parameters in cold stress

Cold stress increases the incidence and severity of gastric ulcers. In the present study *S. affine* showed ulcer protection by significant reduction in Ulcer incidence (%), increase in $P^H$ of Gastric juice (figure 3).

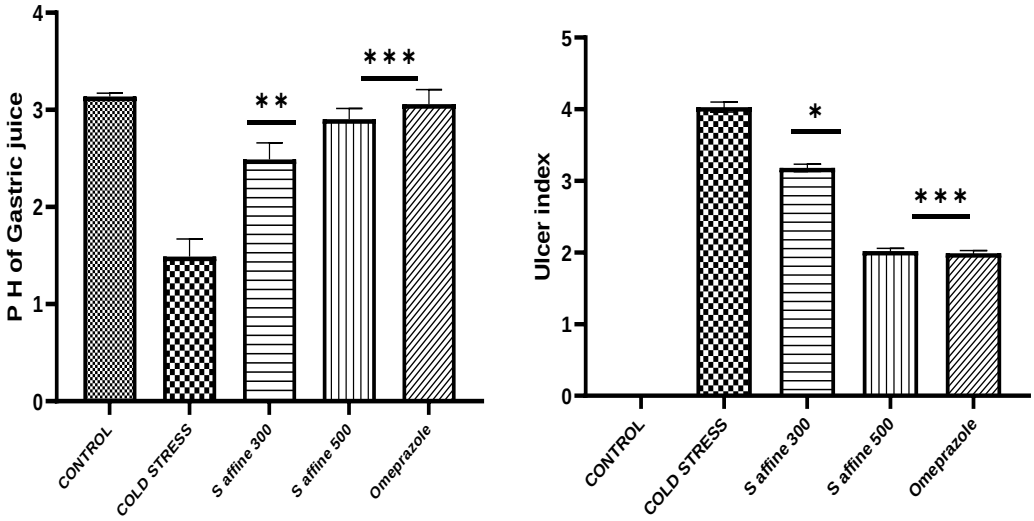



**Figure 3:** Effect of *S. affine* on Cold Stress induced Ulcer parameter

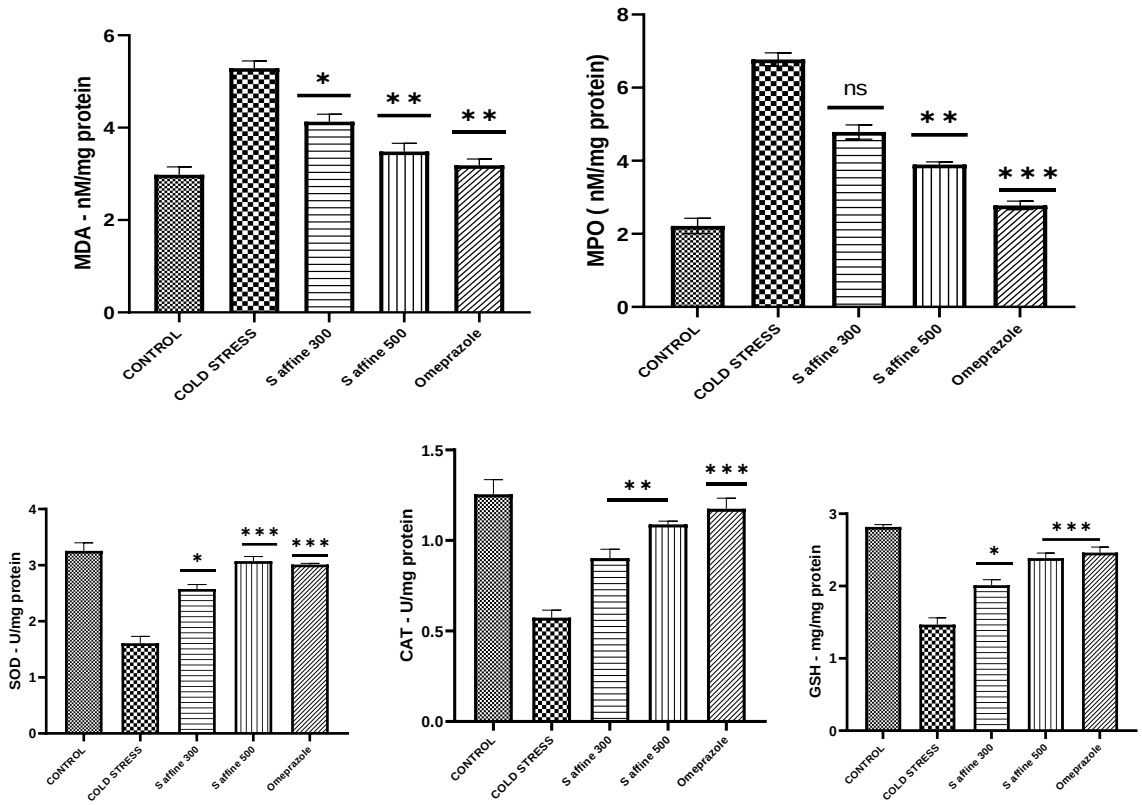



**Figure 4:** Effect of *S. affine* on oxidative stress markers in Cold Stress induced Ulcer parameter

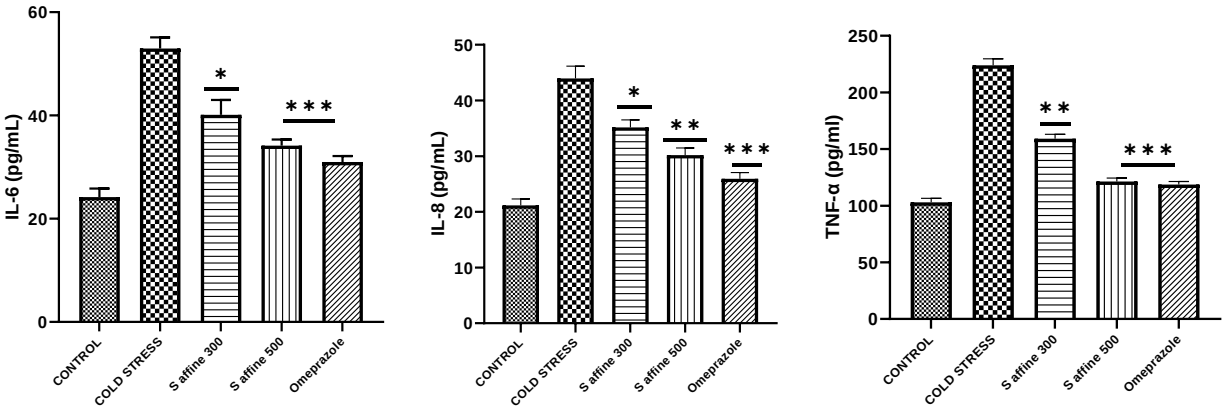
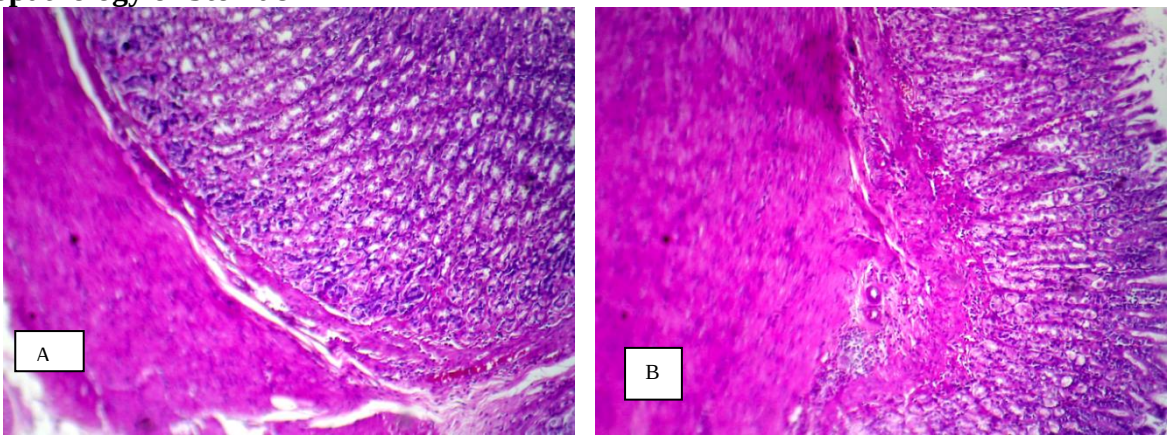
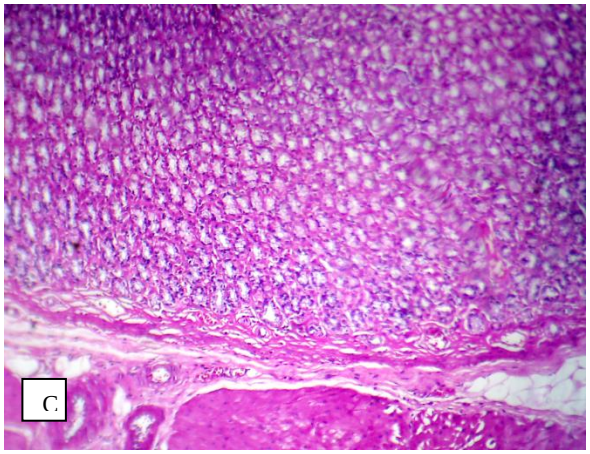
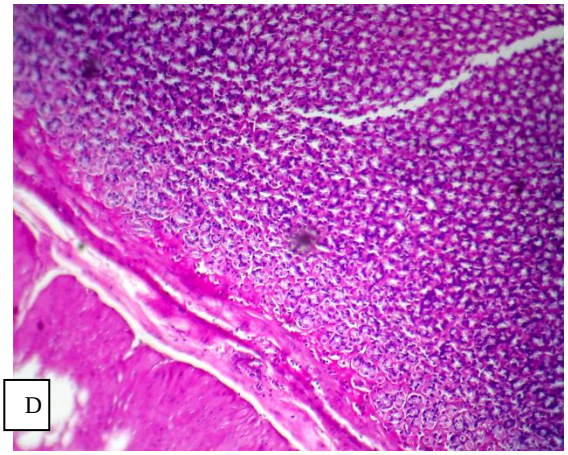


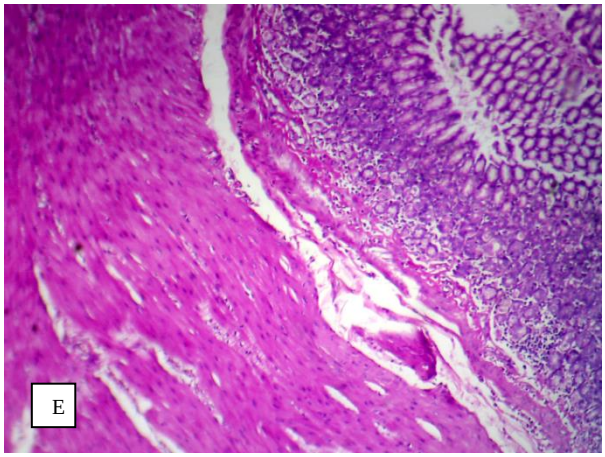

**Figure 5:** Effect of *S. affine* on Pro inflammatory cytokines markers in Cold Stress induced Ulcer parameter

**Histopathology of Stomach**






Photograph 1 : Stomach histopathology stain with stained by H&E , Histopathological study had revealed the normal mucosal height of the stomach wall, continuous surface epithelial lining, no surface erosion, perfect alignment of glandular tissues in control group (A). Marked changes in the normal pattern of the gastric tissue along with mucosal damages, epithelial cell layer disruption, focal surface erosion, disruption and disarrangement of connective tissue, glandular tissue had been observed in cold-restraint stress treated group (B). A distinct improvement had been seen in pre- S. affine 300,500 and omeprazole treated (C,D and E) which indicates the preventive action on gastric ulcer along with recovery of linear alignment of epithelial cell layer, less surface erosion, no vacant spaces and normal arrangement of glandular tissue .

**DISCUSSION:**

Forced swimming test is most widely used paradigm for the evaluation of anti-stress and antidepressant property. This paradigm is based on the observation that animals forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity[26]. The appearance of immobility therefore, reflects a state of tiredness, fatigue, reduced stamina or a lowered mood (hopelessness)[1,26]. These signs represent the core symptoms observed in depressed patients and in individuals under severe stress. It is well known that drugs with anti-stress properties reduce the duration of immobility in animals[1,27].

The present study showed that the ethanolic extract of *Nigella sativa* could reduce the duration of immobility in the forced swimming test by significant improvement in the swimming time suggesting anti-stress activity.

Animals when subjected to a period of stress produce characteristic changes in several hormones and parameters associated with central nervous system and hypothalamic-pituitary-adrenal axis (HPA). HPA changes include an increase in cortisol, a reduced sensitivity of the HPA to feedback down-regulation, and a disruption in the circadian rhythm of cortisol secretion. Central nervous system

changes include the stress-induced depletion of catecholamine neuro transmitters such as nor epinephrine and dopamine. An acute increase in beta-endorphin levels is also observed under stressful conditions [28].

ACTH is released during stress which acts on adrenal cortex where cortisol and corticosterone is secreted. Increased plasma cortisol influences the mobilization of stored fat and carbohydrate reserves, which in turn increases blood glucose level. The increased cortisol levels and increased blood glucose level are reversed by anti-stress agents [28-29].

An increase in Blood glucose level in response to stress is due to release of Glucocorticoids as a result of HPA axis stimulation to compensate the initial demand of energy. The acute demand of glucose is fulfilled by increase in glucogenolysis from liver during stress[30]. Increase in Blood glucose level was significantly reduced by *S. affine* and it also significantly reduced stress induced plasma cortisol level exhibiting anti-stress activity which is comparable to reference standard.

The mechanism by which stress raises serum cholesterol, triglycerides and BUN levels in stress induced animals is due to the enhanced activity of hypothalamo-hypophyseal axis resulting in increased liberation of catecholamines and corticosteroids[31]. *S. affine*as well as the standard significantly reduced the elevated serum cholesterol, triglyceride and BUN levels which might be due to the inhibition of stimulation of sympathetic nervous system.

The connection between the severe psychobiological and physiological stress and gastric damage has long been recognized [32]. It has been widely accepted that stomach wall secretion and motility alteration may manifest in stressful situations causing more or less severe ulcerations of gastrointestinal mucosa. Increase in gastric motility, vagal overactivity, mast cell degranulation, decreased mucosal blood flow, and decreased prostaglandin synthesis are reported to be involved in the genesis of stress-induced ulcers[33]This correlation was also observed in our animal model. Indeed, mucosa of cold stress-induced gastric ulcer rats showed a large number of ulcerative lesions, which disappeared when treated with ethanolic extract of *S. affine* in preventing gastric ulceration in cold stress induced rats suggesting its anti-ulcer activity. This study enlightened the ulcer healing effect of *S.affine* which appears to be related to the free radical scavenging property. The significant restoration of SOD, CAT and GPx activities after administration of *S.affine* indicates that it has the ability to reinstate these enzymes along with inhibition of lipid peroxidation and GSH depletion. Furthermore, *S.affine* also ameliorates the gastric mucosal damage by exerting antioxidant mediated cytoprotective activity. Thus, the present study indicates the health benefits of *S.affine*.

## CONCLUSION

In This study cold stress ulcer healing effect of *S.affine* which appears to be related to the free radical scavenging property. The significant restoration of SOD, CAT and GPx activities after administration of *S.affine* indicates that it has the ability to reinstate these enzymes along with inhibition of lipid peroxidation and GSH depletion. Furthermore, *S.affine* also ameliorates the gastric mucosal damage by exerting antioxidant mediated cytoprotective activity. Thus, the present study indicates the health benefits of *S.affine*.

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## CONFLICT OF INTEREST

We have no conflict of interest to declare.

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