Effect Of Cajanus Cajan On 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 leaf extract of *C. cajan* on cold stress induced Ulcer in albino rats. The effect was assessed by estimation of ulcer index, P^H of gastric juice, 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. *C. cajan* extract significantly ($p<0.001$) 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 and TNF-α significantly decrease and histopathology of stomach restore to normal compare to control, this obtained results revealed that the extract of *C. cajan* has significant anti ulcer activity in rats.

Keywords: *C. cajan* Cold stress,Ulcer, IL-6,IL-8andTNF-α

INTRODUCTION

Cajanus cajan of famaliy (Fabaceae), commonly known as "Pigeon pea" (English), is widely grown in the tropics and the subtropics. It's used as medicine and the health food in Asia and South America different parts of world. possess potent antioxidant, anti-inflammation, anticancer, hypoglycemic, anti-microbial, hypocholesterolemia, hepatoprotective and nephroprotective activities[1-2]
was reported anti-cancer activity.[3] Zhang *et al* demonstrated that the major active compounds responsible for CR were polyphenols, especially isoflavonoids. Duker-Eshun *et al*.[4-5] identified the major components, which included betulinic acid, cajanol, genistein, and a flavonoid with phytoestrogen activity, is abundant in soy foods and possesses antioxidant[6] anti-inflammatory[7] and anti-cancer activities[8].
Stress is a typical body's natural defense to a generic physical or psychic change, which involves a temporary alteration of organism homeostasis [9] . Based on observations on gastric ulcer patients, a higher incidence of ulcerative disease was found among subjects who underwent a greater tension-emotional state. Stress frequency became definitely more assiduous during strong psychological tension periods (war or economic crisis) on a large portion of the population [10]. Therefore, there seems to be a specific linking mechanism between ulcer onset and psycho-physical stress prolonged exposure. Thus, it is reasonable to affirm that psychosomatic factors, such as distress, play a pivotal role in the mechanisms involved in gastric ulcer induction. [11] The present work is cold stress induced anti ulcer activity of *C. cajan* in rats.

MATERIALS AND METHOD:

The leaf of *C. cajan* 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 leaves 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 [12].

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).

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 *C. cajan* 400mg/kg p.o,
Group IV cold stress subjected and extract *C. cajan* 600 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 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. stomach tissue was fixed in 10% formaldehyde for histopathological evaluation using haematoxylin and eosin (H & E) stain. [13-21]

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 *C. cajan* shows the presences of priminary and secondary metabolites such as Alkaloids, glycoside, sterol, flavonoids, terpenoids, protein and amino acids and carbohydrates.
Cold stress induced significantly increases the incidence and severity of gastric ulcers. In the present study *C. cajan* treatment showed ulcer protection by significant reduction in Ulcer incidence (%), increase in P^H of Gastric juice (figure 1).

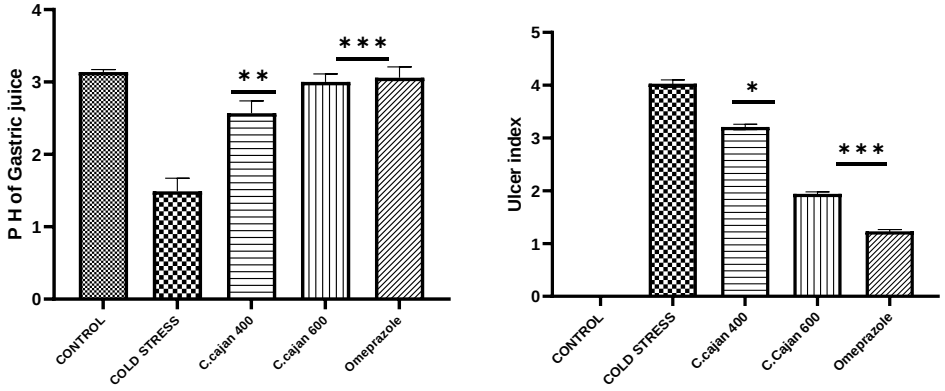
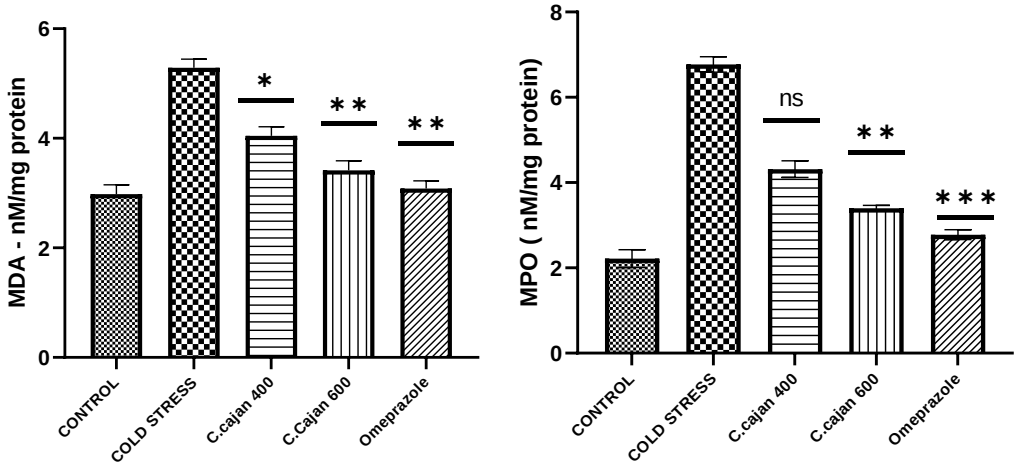


Figure 1: Effect of *C. cajan* on Cold Stress induced Ulcer parameter



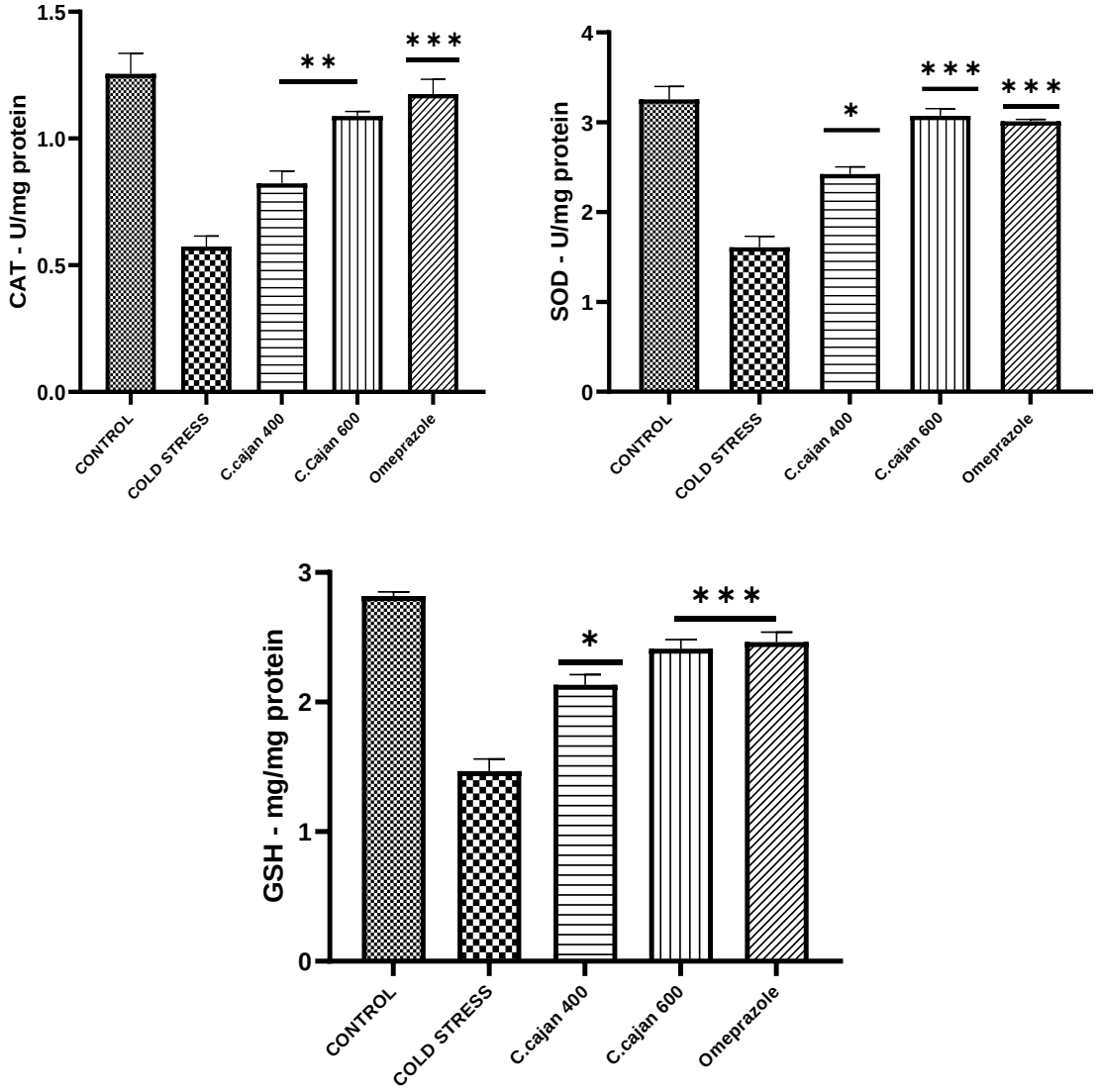


Figure 2: Effect of *C. cajan* on oxidative stress markers in Cold Stress induced Ulcer parameter

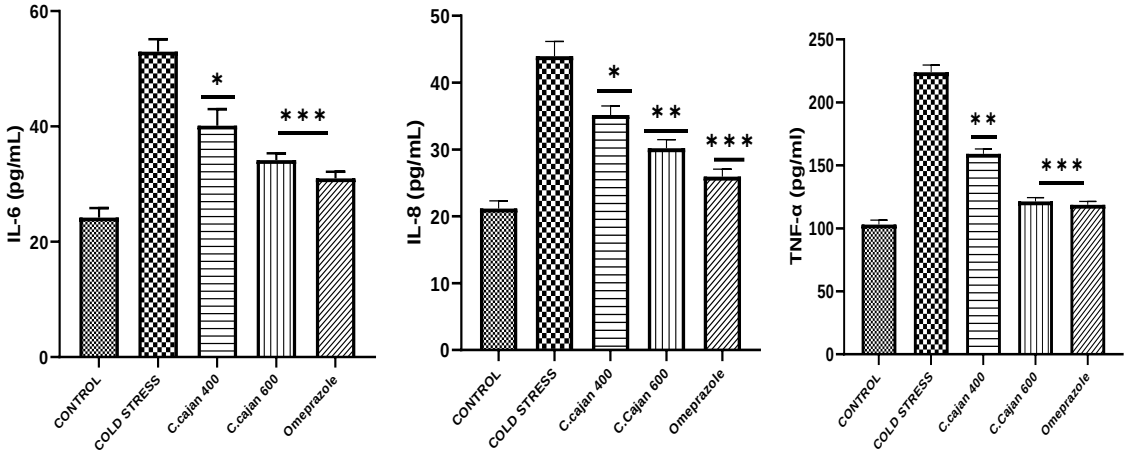
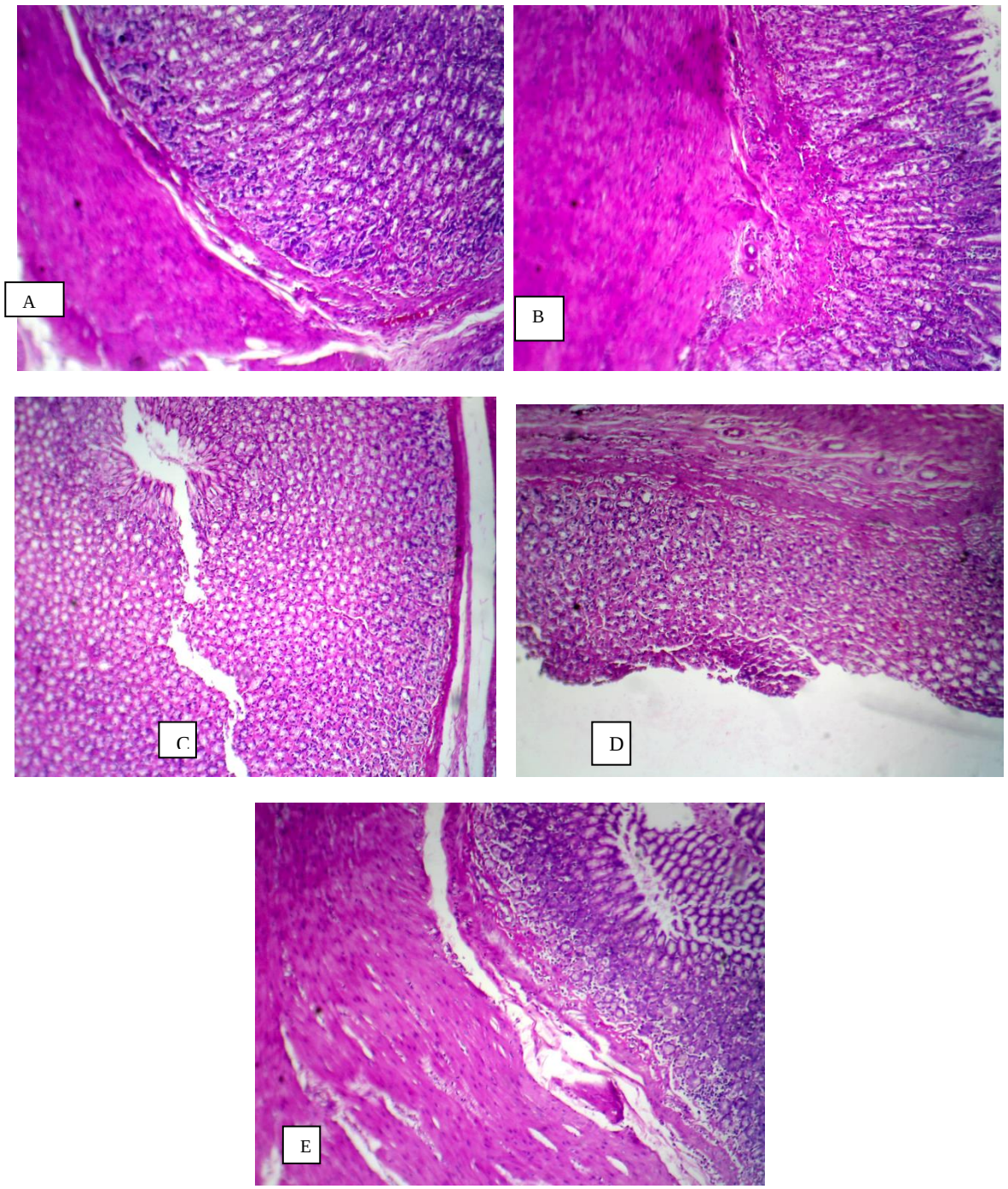


Figure 3: Effect of *C. cajan* on Pro inflammatory cytokines markers in Cold Stress induced Ulcer parameter

Histopathology of Stomach



Photograph 1 : Histological characteristics of stomach tissue section in different experimental groups. Hematoxylin-Eosin staning was performed in this purpose. A) Control group: shows intact epithelial layer , normal muscular arrangement, B) Cold stress induced group: shows extensive surface disruption, hemorrhage and necrosis in epithelial layer, loss of glandular tissue, decrease in muscular height, (C D and E) *C. cajan* 400,600 and omeprazole Pre-treated group: shows very minimum loss of glandular tissue, apparently normal mucosal height and almost intact epithelia layer.

DISCUSSION:

The connection between the severe psychobiological and physiological stress and gastric damage has long been recognized [22] .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. This correlation was also observed in our animal model. Indeed, mucosa of stress-induced gastric ulcer rats showed a large number of ulcerative lesions, which disappeared when treated with omeprazole.

Stress-induced ulcer is probably mediated by the release of histamine. It plays various roles for ulceration increases gastric secretion, causes disturbances of the gastric mucosal microcirculation, creates abnormal motility, and reduces mucus production [23]. The other mechanisms of induction of gastric lesions by CRS are arteriolar vasoconstriction through

activation of peripheral sympathetic nerves, excessive production of free radicals, decrease in SOD level and Haber-Weiss reaction [24].

In this study, oxidative stress markers like MDA, reduced glutathione GSH were measured to evaluate the effect of *C. cajan* on oxidative stress. MDA as the end product of lipid peroxidation was significantly. It was established that cold-restraint stress induces the generation of free radicals [25] which starts lipid peroxidation and increases MDA production as end product. Accumulation of MDA alters the redox homeostasis as well as tissue damage [26]. Several phenolic compounds catechins, of *C. cajan* act as anti-ulcer agents by exerting scavenging role against free radicals to prevent gastric ulceration.

The GSH content in the tissue of stomach. It was observed that GSH content significantly Reduce glutathione (GSH) non-enzymatic anti-oxidant, acts as electron donor and reduces disulfide bond. During reaction GSH is converted to its oxidized state glutathione disulfide (GSSG) in presence of enzyme GPx. Oxidized glutathione (GSSG) returns back to GSH (reduced form) by glutathione reductase (GR) and maintains the normal cellular GSH level [24,27].

CONCLUSION

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

ACKNOWLEDGEMENT

Authors would like to acknowledge and thanks to MEDICULE for scientific solution Hyderabad, providing chemicals and his valuable suggestion in research work.

FUNDING

No funding source for this project.

CONFLICT OF INTEREST

We have no conflict of interest to declare

REFERENCES:

1. Pal D, Mishra P, Sachan N, Ghosh AK. Review: Biological activities and medicinal properties of Cajanus cajan (L) Millsp. J Adv Pharm Tech Res 2018;2:207
2. Ashidi JS, Houghton PJ, Hylands PJ, Efferth T. Ethnobotanical survey and cytotoxicity testing of plants of South-western Nigeria used to treat cancer, with isolation of cytotoxic constituents from Cajanus cajanMillsp. leaves. J Ethnopharmacol.2010;128:501-12.
3. Duker-Eshun G, Jaroszewski JW, Asomaning WA, Oppong-Boachie F, Brøgger Christensen S. Antiplasmodial constituents of Cajanus cajan. Phytother Res 2004;18:128-30
4. Luo M, Liu X, Zu Y, Fu Y, Zhang S, Yao L, *et al*. Cajanol, a novel anticancer agent from Pigeonpea [*Cajanus cajan* (L.) Millsp] roots, induces apoptosis in human breast cancer cells through a ROS-mediated mitochondrial pathway. J ChemicoBiol Int 2010;188:151-60.
5. Chinta SJ, Ganesan A, Reis-Rodrigues P, Lithgow GJ, Andersen JK. Anti-inflammatory role of the isoflavone diadzein in lipopolysaccharide-stimulated microglia: Implications for Parkinson's disease. Neurotox Res 2013;23:145-53.
6. Jackman KA, Woodman OL, Chrissobolis S, Sobey CG. Vasorelaxant and anti-oxidant activity of the isoflavone metabolite equol in carotid and cerebral arteries. Brain Res 2007;1141:99-107.
7. Park JS, Woo MS, Kim DH, Hyun JW, Kim WK, Lee JC, *et al*. Anti-inflammatory mechanisms of isoflavone metabolites in lipopolysaccharide-stimulated microglial cells. J Pharmacol Exp Ther 2007;320:1237-45
8. Zhou Y, Lee AS. Mechanism for the suppression of the mammalian stress response by genistein, an anticancer phytoestrogen from soy. J Natl Cancer Inst 1998;90:381-8.
9. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: a review. EXCLI J. (2017) 16:1057–72.
10. Mucci N, Giorgi G, Roncaioli M, Fiz Perez J, Arcangeli G. The correlation between stress and economic crisis: a systematic review. Neuropsychiatr Dis Treat. (2016) 12:983–93.
11. Sgambato D, Capuano A, Sullo MG, Miranda A, Federico A, Romano M. Gut-brain axis in gastric mucosal damage and protection. Curr Neuropharmacol. (2016) 14:959–66.
12. Roshan, S., A. Khan, B. Tazneem and S. Ali, To study the effect of Nigella sativa on various biochemical parameters on stress induced in albino rats. Int J Pharm Pharm Sci; 2010;Vol 2; Suppl 4: 185-189
13. Mohd Zubairali Anssari, Mohammed Fasiuddin, Syed Salman, Syed nazer Mohammed Imran, Mohd Toufeeq, Roshan S and N L Mohammed Pharmacological screening of polyherbal formulation for anti stress activity on albino rats. International Journal Of Pharmacological Research;2015;May; 5;5: 125-128.
14. Ali, S., S. Roshan and A. Khan. To study the effect of *Allium sativum* on swimming endurance, anoxia tolerance and cold stress. J. Global Pharma Technol., 2010.2: 27-32.

15. Roshan, S., R.V. Savadi, B. Tazneem, S. Ali and A. Khan,. Phytochemical investigation and effect of *Abutilon indicum* on various biochemical parameters on stress induced albino rats. Int. J. Curr. Pharmaceut. Rev. Res. 2010, 1: 17-26.
16. Roshan, S., B. Tazneem, A. Khan and S. Ali, To study the effect of *Allium sativum* on various biochemical parameters on stress induced in albino rats. Res. J. Pharmacol. Pharmacodyn. 2010., 2: 335-339.
17. Roshan, S, A. Khan, B. Tazneem and S. Ali,. The effect of *Echinacea angustifolia* on various biochemical parameters in stress induced rats. Pharmacologyonline, 2010. 2:542-550.
18. Hussain, S.M. and S. Roshan. Anti stress activity of *Cissus quandanguluris*. Int. J. Pharm. Biosci., 201910: 2:132-139.
19. Roshan S, Hussain SM. Anti stress activity of *olea europaea*. *Indian J Pharm Pharmacol* 2021;8(2):161-167.
20. Senay EC, Levine RJ. Synergism between cold and restrain for rapid production of stress ulcers in rats. Exp Biol. 1967.124:1221.
21. Subarnas A, Tadano T, Nakahata N, Arai Y, Kinemuchi H, Oshima Y, Kisara K and Ohizumi Y. A Possible mechanism of antidepressant activity of beta-amyrin palmitate isolated from *Lobelia inflata* leaves in the forced swimming test. Life Sciences. 1993.52:289-296.
22. Gareau MG, Silva MA, Perdue MH. Pathophysiological mechanisms of stress-induced intestinal damage. *Curr Mol Med*. 2008. 8:274–81.
23. Michael NP, Charles TR. Stressful life events, acid hypersecretion and ulcer disease. Gastroenterol 1983; 84: 114-119.
24. Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. Involvement of reactive oxygen species in gastric ulceration: Protection by melatonin. Indian J Exp Biol 2002; 40: 693-705.
25. Demir S, Yilmaz M, Köseoğlu M, Akalin N, Aslan D, Aydin A. Role of free radicals in peptic ulcer and gastritis. Turk J Gastroenterol 2003;14(1):39-43.
26. Sahin E, Gümüşlü S. Stress-dependent induction of protein oxidation, lipid peroxidation and anti-oxidants in peripheral tissues of rats: comparison of three stress models (immobilization, cold and immobilization-cold). Clin Exp PharmacolPhysiol 2007; 34(5-6):425-431.
27. Panda V, Sonkamble M. Anti-ulcer activity of Ipomoea batatas tubers (sweet potato). FunctFoodn Health Dis 2012; 2(3): 48-61.