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Hydro-ethanolic extract of *Gloriosa superba* protecting acute splenic injury by modulating endogenous antioxidants pool in rats

Piyush Shukla, Naresh Kumar Sahu, Raj Kumar, Monika Bhadauria[1] and *Satendra Kumar Nirala

Department of Rural Technology and Social Development
[1]Department of Zoology
Guru Ghasidas University,
Koni - BILASPUR-495009 (C.G.) INDIA
*Corresponding Author
satendrak75@rediffmail.com

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ABSTRACT

Present investigation was designed to evaluate hydro-ethanolic extract of roots of *Gloriosa superba* against combined exposure to alcohol and LPS induced splenic injury in rats. Thirty female albino rats were divided into five groups having six animals in each. Splenic injury was induced by combined exposure to alcohol and lipopolysaccharide (LPS) and 50, 100 and 200 mg/kg dose of extract of *Gloriosa superba* was evaluated for its efficacy. Combined exposure to alcohol and LPS significantly decreased total proteins but significantly increased triglycerides, cholesterol and lipid peroxidation process in spleen tissues, indicating metabolic perturbance and peroxidative cellular damage. Co-exposure to alcohol and LPS significantly decreased activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidise, glucose-6-phosphate dehydrogenase as well as diminished reduced glutathione level in splenic tissue samples indicating exhaustion of endogenous antioxidant pool and development of oxidative stress. Conjoint administration of extract of *Gloriosa superba* at all the three doses offered significant protection in studied endpoints and maintained their values towards their respective control in dose dependent manner. It may be concluded that hydro-ethanolic extract of *Gloriosa superba* has potential to protect splenic injury by modulating endogenous antioxidants pool due to antioxidant phytochemicals present in extract.

Figures : 02 References : 27 Table : 00
KEY WORDS : Alcohol, Antioxidant pool, *Gloriosa superba*, Lipopolysaccharide (LPS), Oxidative stress.

Introduction

Co-exposure to alcohol and bacterial endotoxin, lipopolysaccharides (LPS) are very common in rural areas where societal alcoholism and unhygienic conditions prevail, which induce oxidative stress in population. Oxidative stress is defined as a status of an imbalance between cellular anti-oxidative capacity and reactive oxygen species (ROS) formation caused by deregulation of antioxidant system[10]. Alcohol-induced oxidative stress is linked to metabolism of ethanol involving both microsomal and mitochondrial systems. Ethanol metabolism is directly involved in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS)[6]. The LPS are amphiphilic molecules that are localized in the outer leaflet of the outer membranes of Gram-negative bacteria[9]. Combined exposure to alcohol and LPS produces multiple organ damage, including splenic injury, which consequently lead to immune dysfunction, hemopoetic disorder and many other health issues. Despite of important functions of several vital organs in an organism, the spleen plays multiple supporting role in body. It acts as a filter for blood as part of the immune system. Old red blood cells are recycled in the spleen and platelets and white blood cells are stored there. The spleen also helps to fight certain kinds of bacteria that cause pneumonia and meningitis. Bacterial infection triggers a response that involves production of inflammatory mediators such as cytokines[26]. In contrast to the general decrease in hypothalamic cytokine expression in LPS-pre-treated rats, an increase in most cytokines was observed in the spleen[20].

Nature has been a source of medicinal agents for thousands of years. Herbal medicine represents one of the most important fields of traditional medicine all over the world[11]. The *Gloriosa superba* Linn. has important place in the Indian medicine, which cures many

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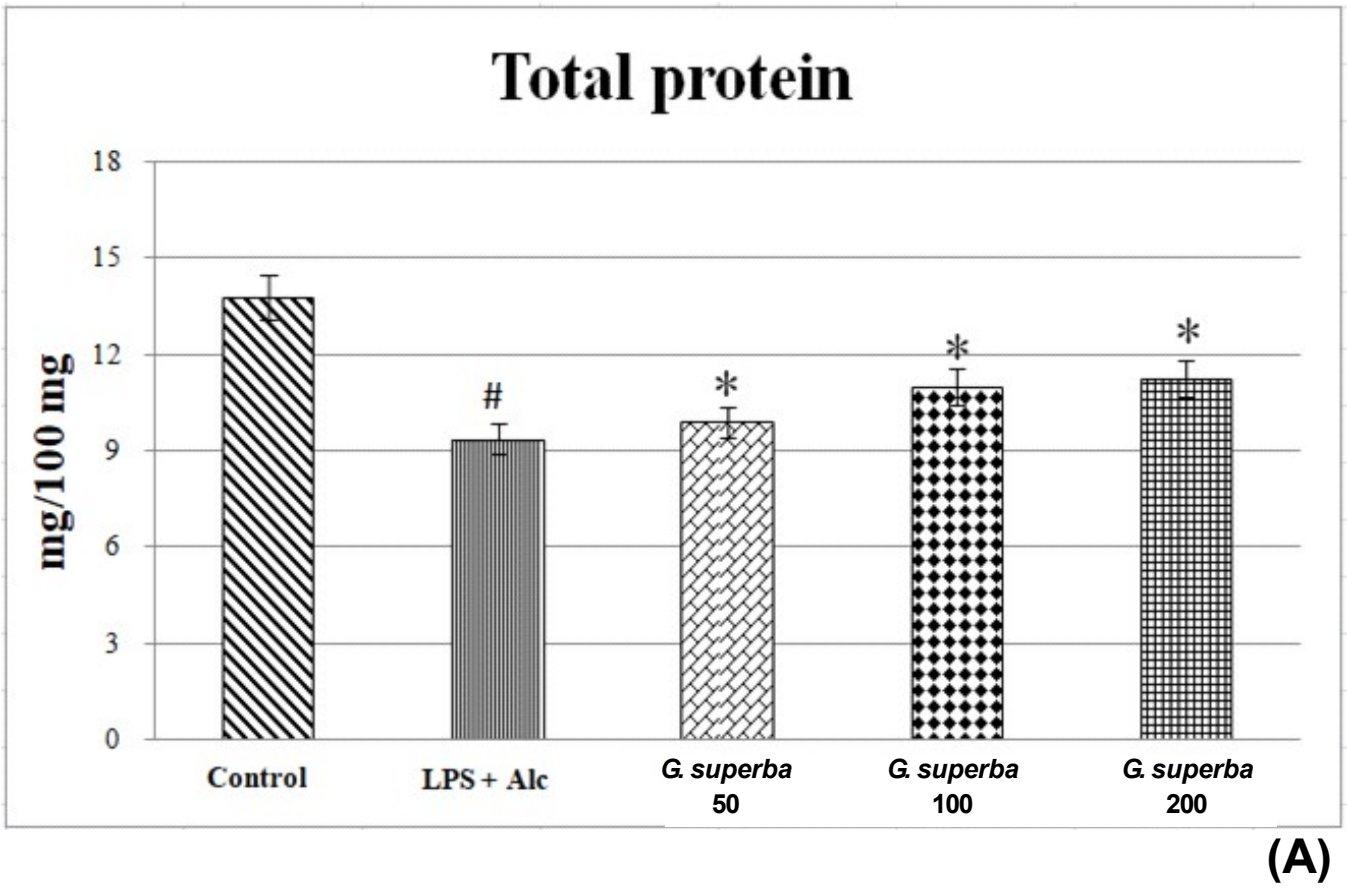

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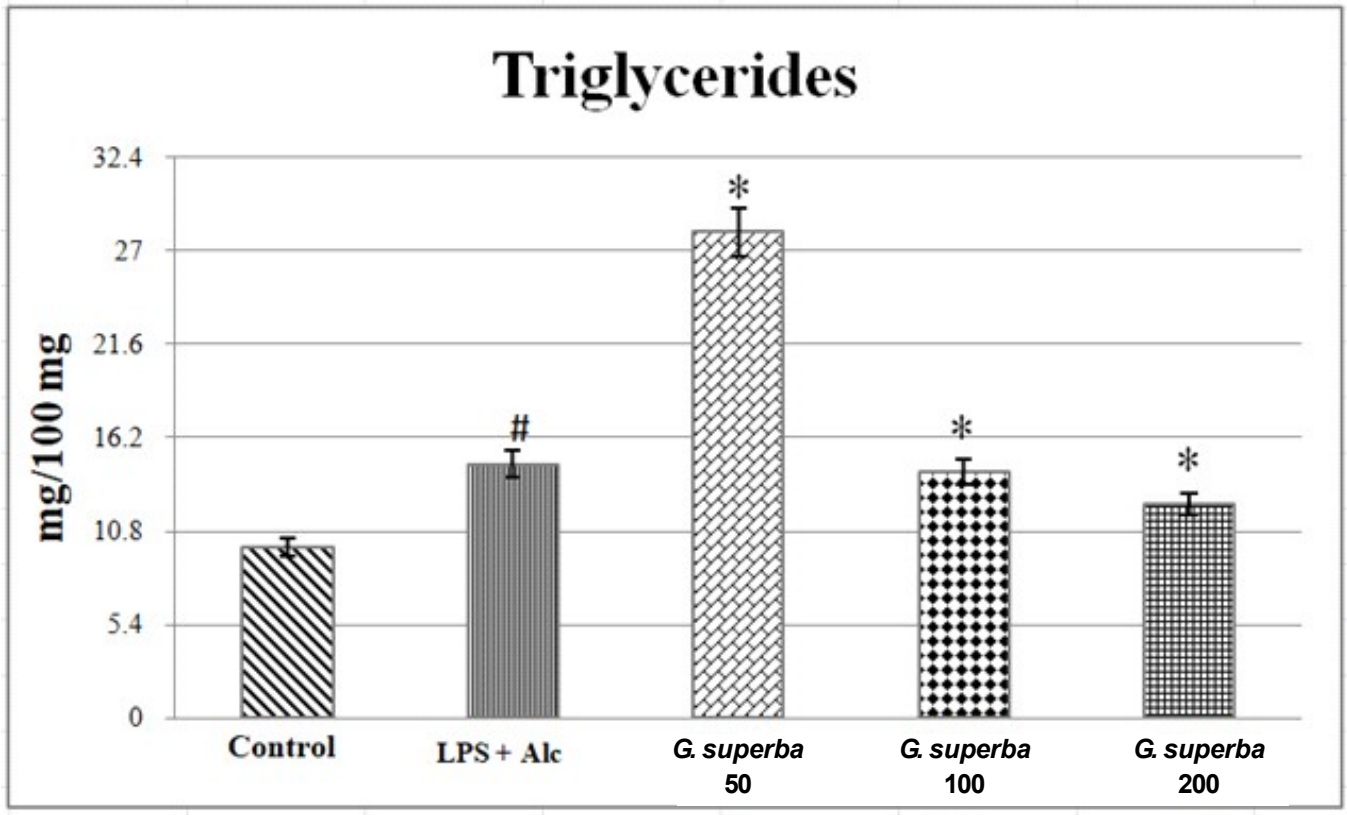

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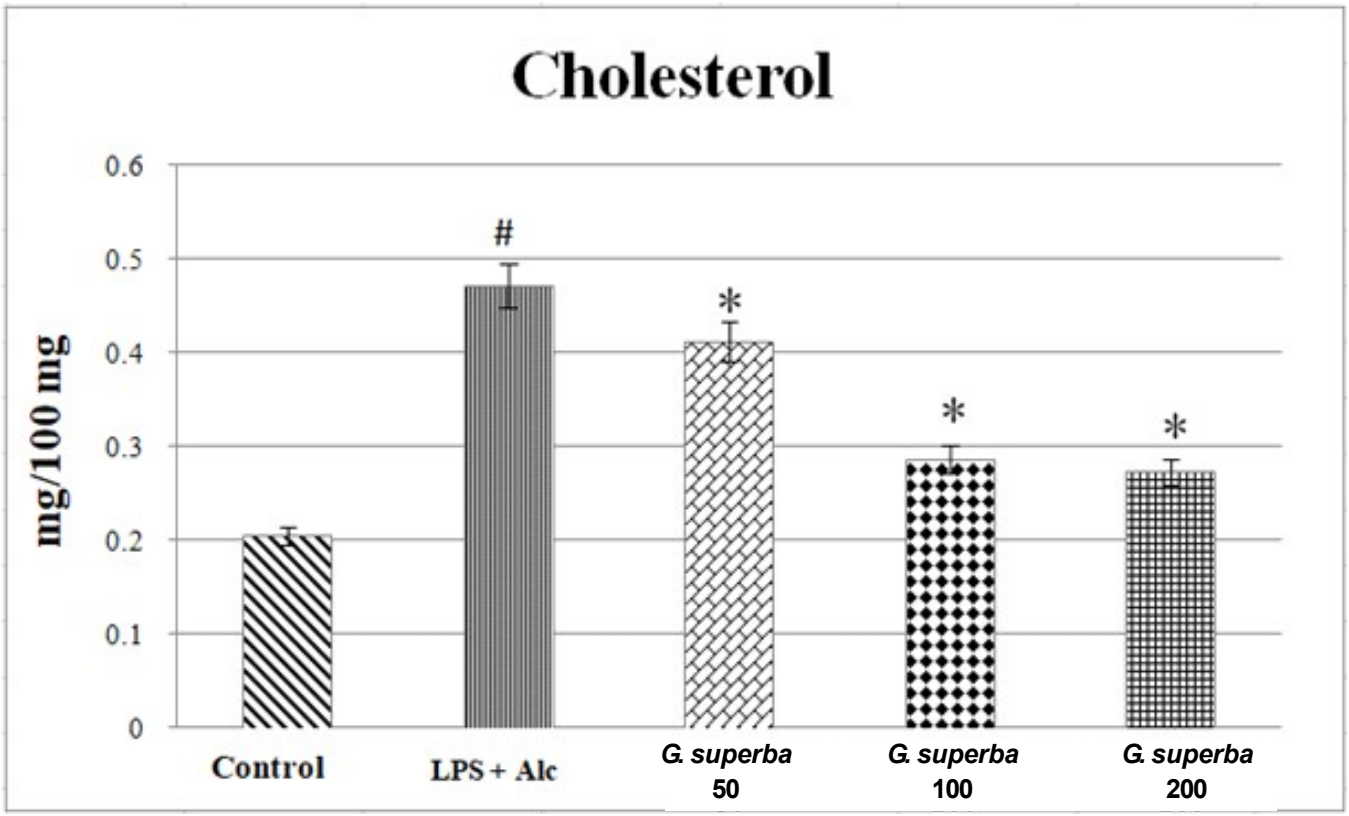
Fig. 1: Effect of *Gloriosa superba* on spleen tissue biochemistry



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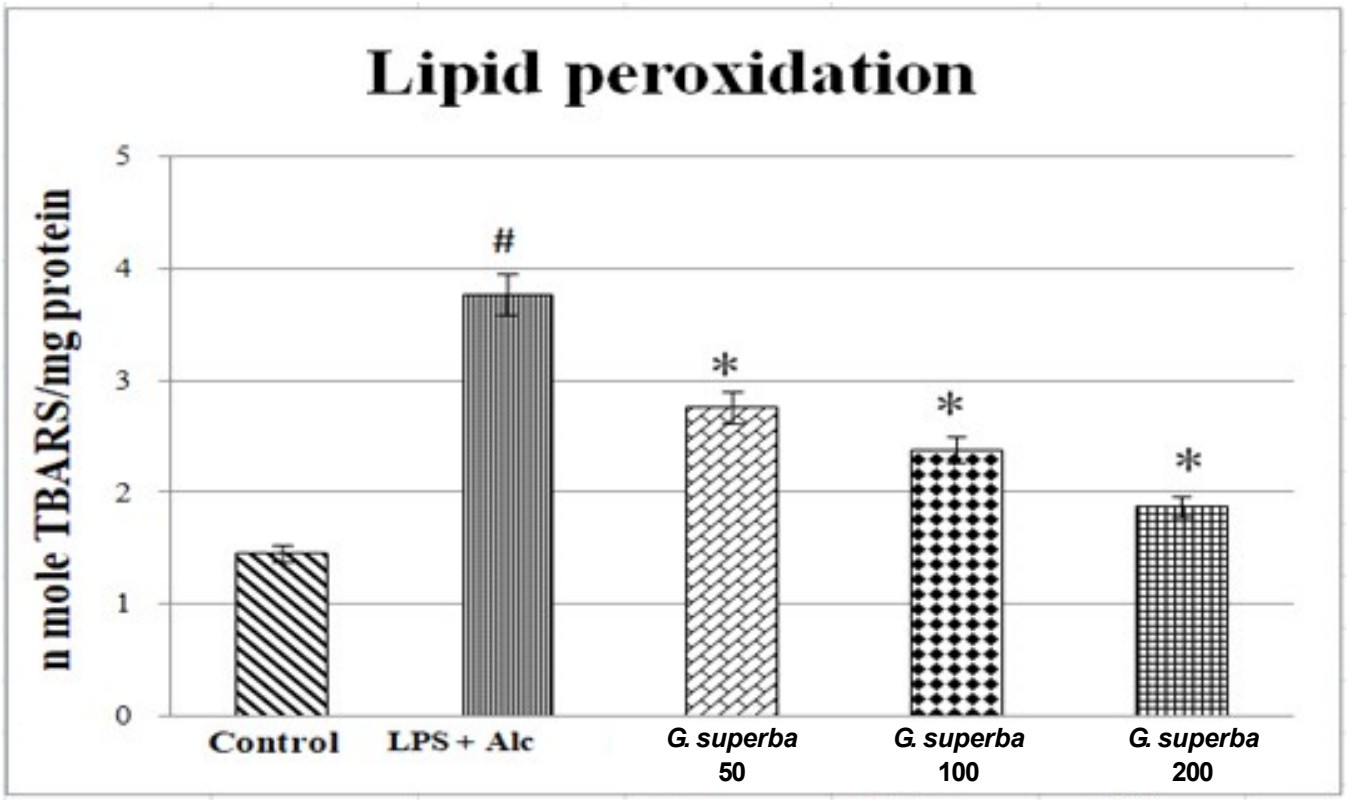

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Fig. 1: Effect of *Gloriosa superba* on spleen tissue biochemistry

ailments[23]. It is known as 'Malabar glory lily' in English, 'Kalihari' in Hindi, 'Agnisikha' in Sanskrit and its trade name is 'Glory lily'[19]. The medicinal values of plants lie in some chemical substances that produce a definite physiological action on human body[8].

Thus, this study was designed to evaluate therapeutic potential of *Gloriosa superba* Linn. against combined exposure to alcohol and LPS induced splenic injury in rats.

Materials and Methods

Animals and chemicals:

Female albino rats of Wistar strain (160 ± 10 g of body weight) were used in this study. They were housed under standard husbandry conditions (25 ± 2°C surrounding temperature, 60-70% of relative humidity and 12 hours of photoperiod) and had free access to standard rat feed and drinking water *ad libitum*. Chemicals were procured from Sigma Aldrich, SRL and Himedia Laboratiories, Ltd., India.

Preparation of plant extract:

Gloriosa superba was procured from standard dealer of medicinal plants and herbs and further identification of plant was made by senior botanist of the university. Roots of the plant were washed repeatedly with distilled water to remove dust, air dried in shade at room temperature, cut into small pieces and powdered for extraction. Hydro-ethanolic extraction was carried out in 70% ethanol using accelerated solvent extraction machine (DIONEX-ASE-150, at 20°C and 15 atm pressures). Obtained extract was further air dried at room temperature and stored at 4°C for further use. Doses of *Gloriosa superba* extract were prepared in 1% gum acacia and administered to animals at 50, 100, and 200 mg/kg.

Experimental design:

Animals were simultaneously randomized into 5 groups having 6 animals in each group and administered as follows:

Group I: Control (Vehicles only)

Group II: Alcohol (40%; oral, daily for 6 days) + LPS (10 µg/kg; *ip,* only on 6th day)

Group III: Alcohol+LPS (as in group II) + *G. superba* (50 mg/kg; oral, daily for 6 days)

Group IV: Alcohol+LPS (as in group II) + *G. superba* (100 mg/kg; oral, daily for 6 days)

Group V: Alcohol +LPS (as in group II) + *G. superba* (200 mg/kg; oral, daily for 6 days)

Animals of all the groups were euthanized under mild ether anaesthesia after 24 h of last treatment. Spleen was excised and stored at -20°C for further determination of various endpoints.

Splenic Tissue Biochemistry:

Pre-determined portion of spleen tissue from each animal was used for biochemical determination of total proteins[14], triglycerides[17] and cholesterol[27].

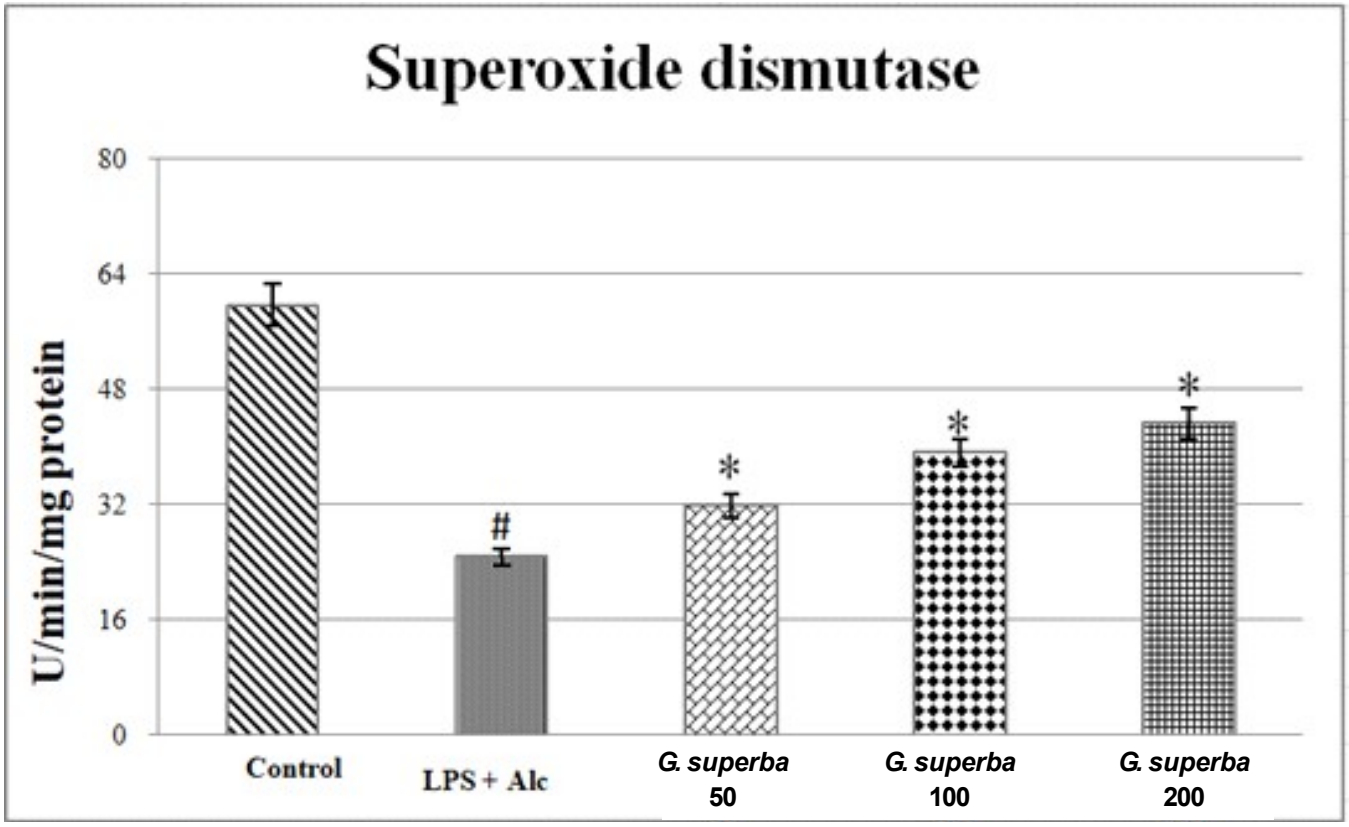
Splenic Antioxidant Status:

Spleen tissues were processed to determine lipid peroxidation[21], superoxide dismutase[16], catalase[1], reduced glutathione[5], glutathione reductase[24], glutathione peroxidase[18] and glucose-6-phosphate dehydrogenase[3].

Statistical Analysis: The results were expressed as the mean ± standard error (SE) of the six animals in each group. The data were subjected to statistical analysis using a one-way analysis of variance (ANOVA)[22] and statistical significance was set at P d" 0.05. The data were also subjected to Student's t-test with statistical significance set at P d" 0.05. In Figures 1 and 2, Symbol # indicates significant versus control group at P<0.05; symbol whereas, * indicates significant versus LPS+alcohol exposed group at P<0.05

Results and Discussion

The spleen is hematopoietic and secondary lymphoid organ, responsible for immune functions in body and is also necessary for homeostasis and iron recycling of red blood cells[15]. Any deviation from its regular physiology may not only disturb immune dysfunction but also perturb hematopoietic homeostasis that may lead to acute multiple organ damage. Oxidative stress and inflammation due to cytokine expressions play an important role in pathogenesis of LPS induced spleen injury[20]. Alcohol consumption represents an ever-increasing global health burden worldwide; however, exposure to nicotine or other agent alongwith alcohol exerts additive toxic effects[2]. Lipid peroxidation is responsible for free radical generation, which deteriorates cellular structures[13]. Total protein and lipid analyses are considered to be important biochemical assay for determining protein/lipid constituent aspects in body tissues/organ. In this study, combined exposure to alcohol and LPS significantly decreased total proteins but significantly increased triglycerides, cholesterol and lipid peroxidation process in spleen tissues, indicating metabolic perturbance and peroxidative cellular damage (Fig. 1; A-D). Hydro-ethanolic extract of roots of *Gloriosa superba* could reverse these endpoints towards their respective control significantly and showed its therapeutic potential in a dose dependent manner. All the three doses were found to be significantly effective; however, 200 mg/kg dose was found to be most potent. The pathological process of alcohol-induced organ injury has been well characterized by a broad spectrum of morphological changes ranging from hyperlipidemia with minimal injury to more advanced tissue damage. In the last two decades,



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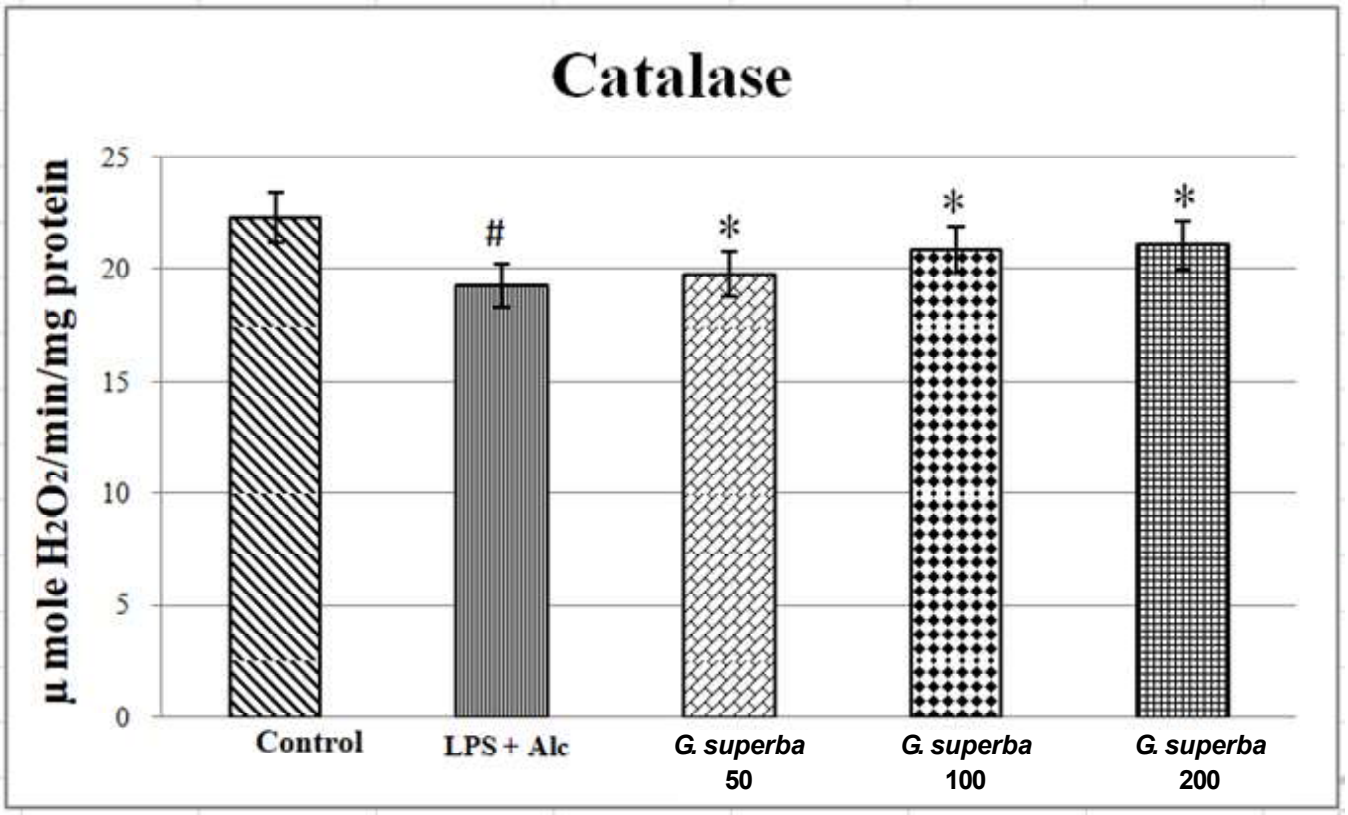

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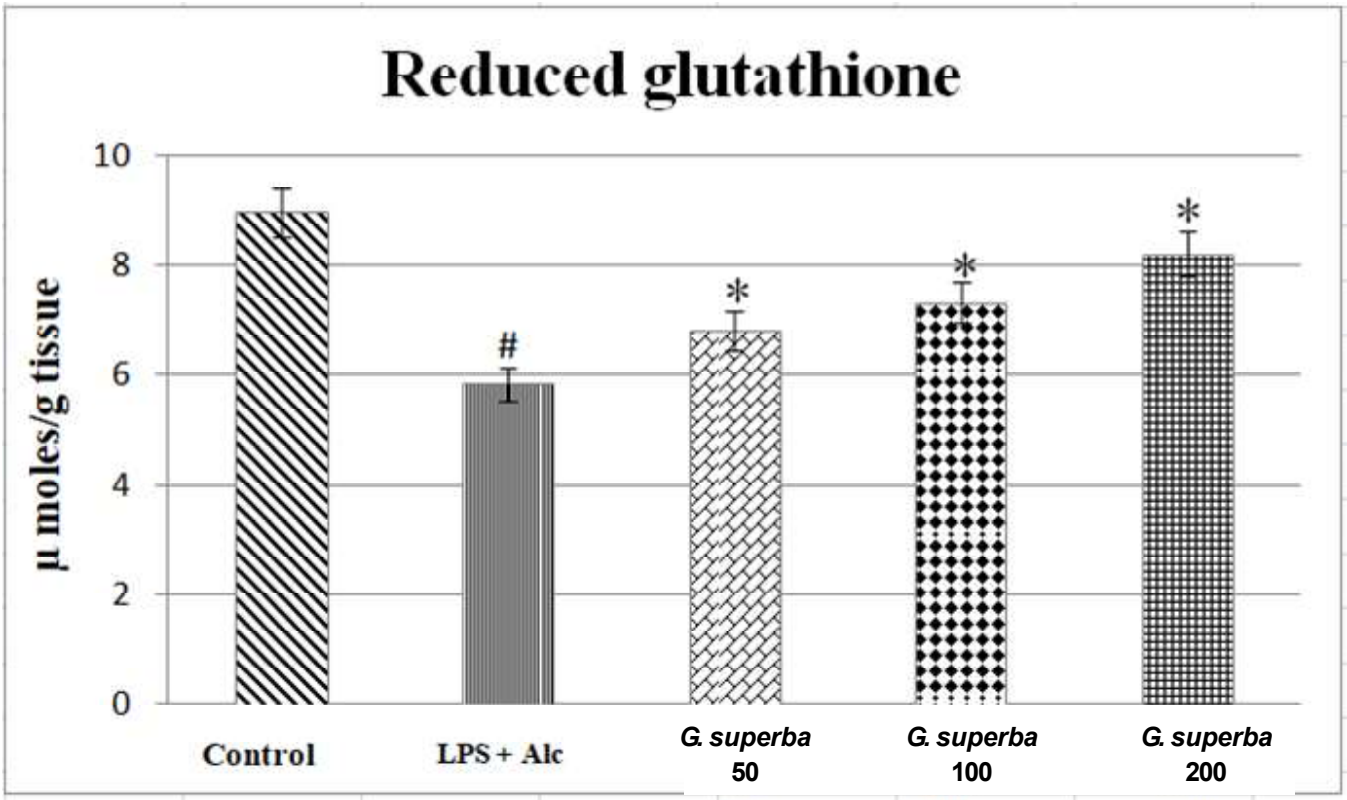
Fig. 2: Effect of *Gloriosa superba* on antioxidant pool in spleen tissues



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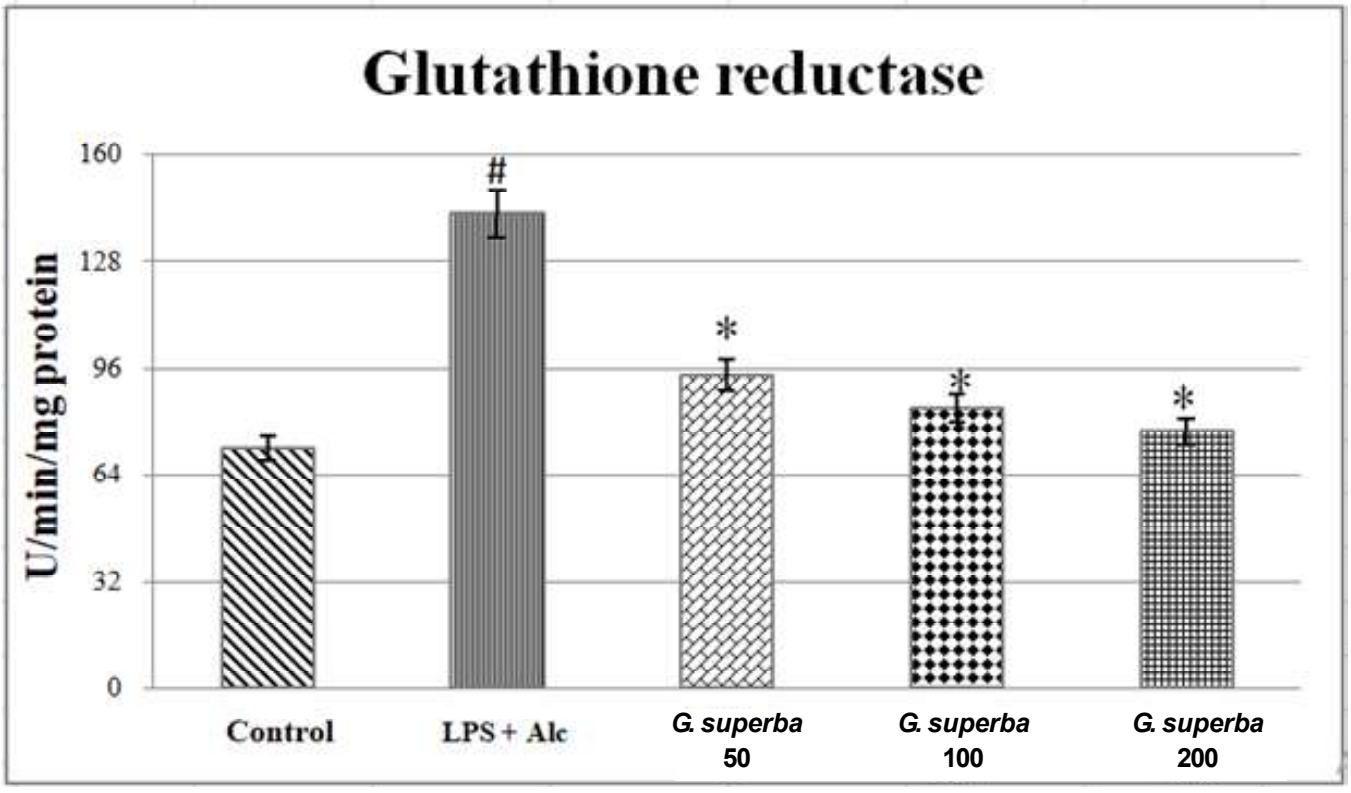

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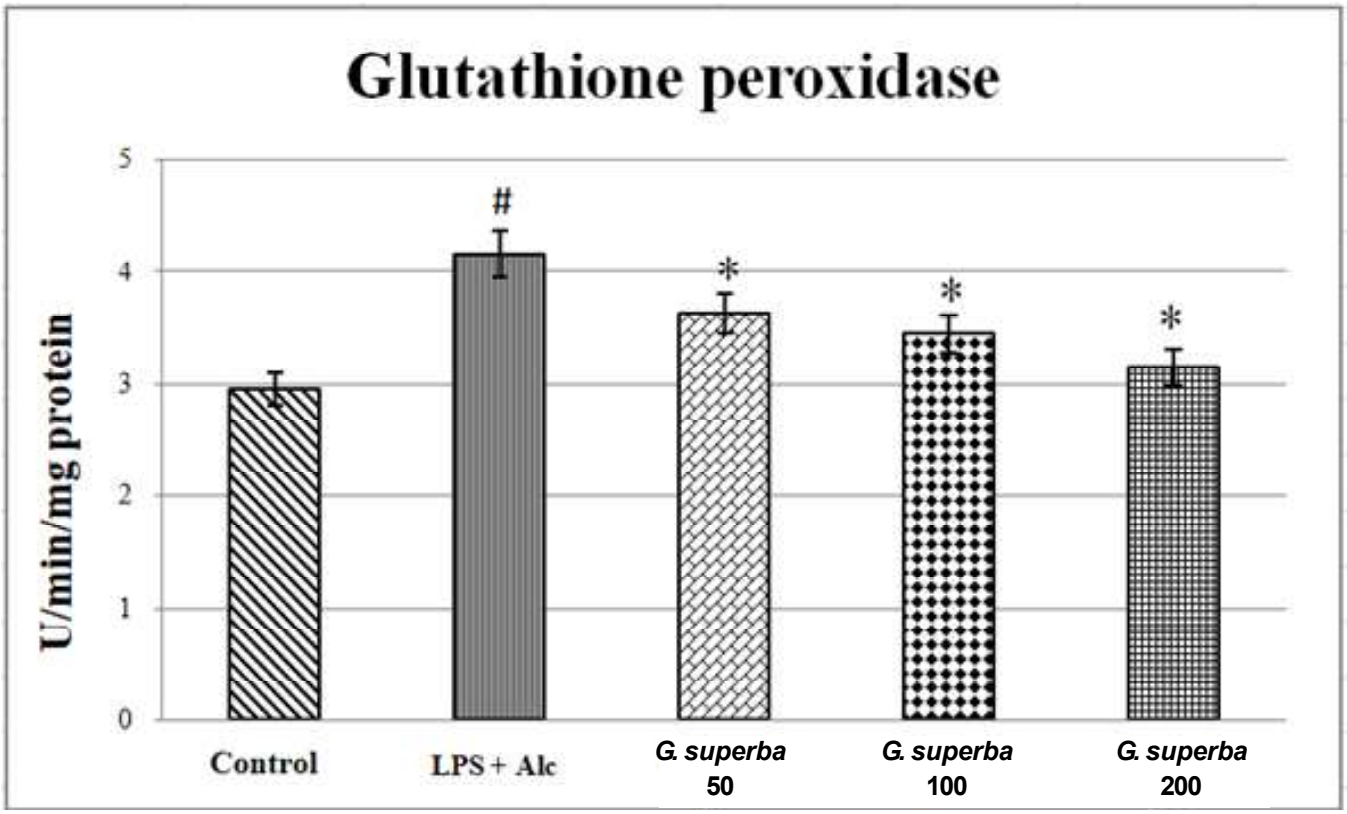
Fig. 2: Effect of *Gloriosa superba* on antioxidant pool in spleen tissues



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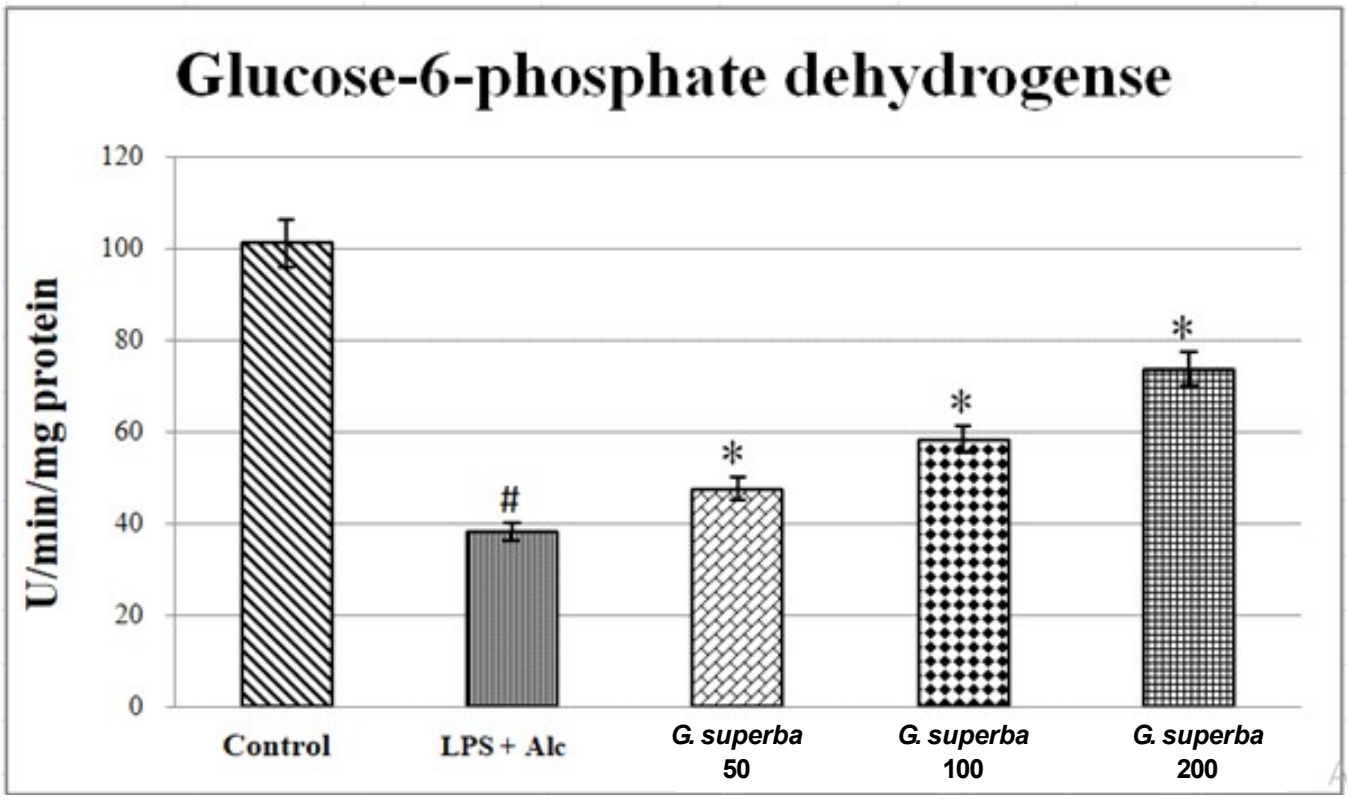

(F)

Fig. 2: Effect of *Gloriosa superba* on antioxidant pool in spleen tissues

there has been an explosive interest in the role of oxygen-free radicals, more generally known as reactive oxygen species, (ROS) and of reactive nitrogen species (RNS) in experimental and clinical medicine[4, 6]. The particular reaction of ROS with lipids is generally known as lipid peroxidation, which is an indicator of oxidative stress in patho-physiological conditions[4]. Effectiveness of extract *Gloriosa superba* might be due to its antioxidant potential to suppress lipid peroxidation processes and ability to modulate metabolic processes in the body.

Alcohol-induced oxidative stress is linked to the metabolism of ethanol involving both microsomal and mitochondrial systems. Ethanol metabolism is directly involved in the production of ROS and RNS. These form an environment favourable to oxidative stress that may be suppressed with the use of herbal products[7]. In the present investigation, co-exposure to alcohol and LPS significantly decreased activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidise, glucose-6-phosphate dehydrogenase as well as diminished reduced glutathione level in splenic tissue samples (Fig. 2; A-F). These variables form antioxidant pool in the tissues and are responsible to combat oxidative stress in the body. Hydro-ethanolic extract of roots of *Gloriosa superba* helped in reversing activity of this endogenous antioxidant pool towards their respective control significantly and determined its therapeutic effects against alcohol and LPS induced oxidative stress in spleen in a dose dependent manner. This effect might be due to various phytochemicals, including flavonoids and phenolics present in extract. The 50, 100 and 200 mg/kg doses were found to be significantly effective in this study; however, 200 mg/kg dose may be said to be most potent in bringing the values of studied parameters very near to control. The results of this investigation were also corroborated with previous studies where alcohol induced oxidative stress[6] was significantly reduced by treatment of phytochemicals[12, 25]. It was also reported that *Panax notoginseng* protect against alcoholic liver injury *via* inhibiting ethanol-induced oxidative stress and gut-derived endotoxin-mediated inûammation[7], which are in support of this investigation.

Conclusion

On the basis of the results evolved from this scientific investigation, it may be concluded that hydro-ethanolic extract of *Gloriosa superba* Linn. has potential to protect combined exposure to alcohol and LPS induced acute splenic injury by modulating endogenous antioxidants pool due to antioxidant phytochemicals present in extract.

References

1. Aebi HL. Catalase *in vitro*. Methods Enzymol. 1984; **105**: 121-126.
2. Ashakumary L, Vijayammal PL. Additive effect of alcohol and nicotine on lipid peroxidation and antioxidant defence mechanism in rats. *J Appl Toxicol.* 1996; **16**(4) 305-308.
3. Askar MA, Sumathy K, Baquer NZ. Regulation and properties of purified glucose-6-phosphate dehydrogenase from rat brain. *Indian J Biochem Biophys.* 1996; **33** (6): 512-518.
4. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Org J.* 2012; 9-19
5. Brehe JE, Burch HB. Enzymatic assay for glutathione. *Anal Biochem.* 1976; **74**: 189-197.
6. Das SK, Vasudevan DM. Alcohol-induced oxidative stress. *Life sciences.* 2007; **81** (3): 177-187.
7. Ding RB, Tian K, Huang LL, He CW, Jiang Y, Wang YT, Wan JB. Herbal medicines for the prevention of alcoholic liver disease: *a review. J Ethnopharmacol.* 2012; **144** (3): 457-465.
8. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotech.* 2005; **4**: 685-688.
9. Jessica V, Hankins JV, Madsen JA, Needham BD, Brodbelt JS, Trent MS. The outer membrane of Gram-negative bacteria: Lipid A isolation and characterization. *Methods Mol Biol.* 2013; **966**: 239–258.
10. Jiang W, Fen Luo F, Lu Q, Liu J, Li P, Wang X, Fu Y, Hao K, Yan T, Ding X. The protective effect of Trillin LPS-induced acute lung injury by the regulations of inflammation and oxidative state. *Chemico-biol interact.* 2016; **243**: 127-134.
11. Jose S, Thomas TD. Comparative phytochemical and antibacterial studies of two indigenous medicinal plants *Curcuma caesia* Roxb. and *Curcuma aeruginosa* Roxb. *Int J Green Pharm.*2014; **8** (1): 65-71.
12. Khan MS, Ali T, Kim MW, J MH, Jo MG, Badshah H, Kim MO. Anthocyanins protect against LPS-induced oxidative stress-mediated neuroinflammation and neurodegeneration in the adult mouse cortex." *Neurochem Int.*

2016; **100**: 1-10.

13. Khanum R, Thevanayagam H. Lipid Peroxidation: Its effects on the formulation and use of pharmaceutical emulsions. *Asian J Pharmaceut Sci.* 2017; **12**: 401-411.
14. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin's phenol reagent. *J Biol Chem.* 1951; **193**: 265-275.
15. McKenzie CV, Colonne CK, Yeo JH, Fraser ST. Splenomegaly: Pathophysiological bases and therapeutic options. *Int J Biochem Cell Biol.* 2018; **94**: 40–43.
16. Mishra ÇP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. *J Biol Chem.* 1972; **247**: 3170-75.
17. Neri BP, Frings CS. Improved method for determination of triglycerides in serum. *Clin Chem.* 1973; **19**: 1201-1202.
18. Paglia DE, Valentine WM. Studies on quantitative and qualitative charaterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 1967; **70**: 158-169.
19. Pulliah T. Medicinal plants in India, Vol. I. New Delhi: Regency Publications; 2002. pp. 269–70.
20. Rey AD, Randolf A, Wildmann J, Besedovsky HO, Jessop DS. Re-exposure to endotoxin induces differential cytokine gene expression in the rat hypothalamus and spleen. *Brain, Behav Immun.* 2009; **23**(6): 776-783.
21. Sharma SK, Krishna Murthy CR. Production of lipid peroxides by brain. *J Neurochem.* 1968; **15**: 147-149.
22. Snedecor GW, Cochran WG. Statistical Method, 8th Edition, Ames. Iowa, Iowa State University Press: 217-236; 1994.
23. Sundaraganapathy R, Niraimathi V, Thangadurai A, Kamalakannan D, Narasimhan B, Deep A. Anti-anxiety activity of *Gloriosa superba* Linn. *Hygeia J D Med.* 2013; **5**(1): 148-51.
24. Tayarani I, Cloez I, Clement M, Bourre JM. Antioxidant enzymes and related free elements in aging brain capillaries and chloroid plexus. *J Neurochem.* 1989; **53**: 817-824.
25. Wang M, Zhang XJ, Liu F, Hu Y, Chengwei He C, Li P, Su H, Wan JB. Saponins isolated from the leaves of *Panax notoginseng* protect against alcoholic liver injury via inhibiting ethanol-induced oxidative stress and gut-derived endotoxin-mediated inflammation. *J Funct Foods.* 2015; **19**: 214-224.
26. Xu M, Sulkowski ZL, Parekh P, Khan A, Chen T, Midha S, Iwasaki T, Shimokawa N, Koibuchi N, Zavacki AM, Sajdel-Sulkowska EM. Effects of perinatal lipopolysaccharide (LPS) exposure on the developing rat brain; modeling the effect of maternal infection on the developing human CNS. *The Cerebellum.* 2013; **12**(4): 572-586.
27. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med.* 1953; **41**: 486-492.