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Hepatoprotective and Antioxidant Effect of *Dolichousnea longissima* (*Ach.*) Articus Extract against Cisplatin Induced Liver Damage in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PV and CVR designed the study. Author SS performed the statistical analysis. Author SKP wrote the protocol and wrote the first draft of the manuscript. Authors AR and CR managed the analyses of the study. Author SKP managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Although it is being an extremely potent cytotoxic drug, cisplatin has vital poisonous adverse effects limiting its use like nephrotoxicity, neurotoxicity and ototoxicity hepatotoxicity. It is thought that cisplatin-induced hepatotoxicity is caused by oxidative stress ensuing from raised reactive oxygen species (ROS). This study was designed to scrutinize the hepatoprotective effect of plant extract from a lichen species, *Dolichousnea longissima* (*DUL*) at doses, 100 and 200 mg/kg, against cisplatin induced hepatic damage. 30 wistar albino rats were divided into 5 groups, including 2 experimental, 1 control and 1 standard, 5 mg/kg/day of cisplatin was injected intraperitoneally (I.P)

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daily for 7 days. Following liver damage, *DUL* was applied at doses of 100 and 200 mg/kg for 7 days. The group given normal diet and water was given a control group. Alanine aminotransferase, Aspartate aminotransferase, gamma-glutamyl transpeptidase, creatinine, urea and bilirubin were evaluated in the serum samples obtained from the rat groups. Liver tissues were removed and were assessed for antioxidant parameters and histological examination. The treatment with *DUL* along with cisplatin showed noticeably increased hepatic level in SOD, CAT, GPX and reduced malondialdehyde level. The results also showed that 200 mg/kg daily dose of *DUL* might be considered to have hepatoprotective effect by battening the studied biochemical and antioxidant parameters and also tissue histological structures. Thus, *DUL* could be potentially used as a hepatoprotective agent at a dosage of 200 mg/kg b.w against liver toxicity induced by cisplatin.

Keywords: Dolichousnea longissima; chitosan; cisplatin; antioxidant.

1. INTRODUCTION

Lichens turn out a good variety of secondary metabolites that take part in ecological interactions and respond to environmental changes. They are fashioned through interdependency amid fungi and algae and/or cyanobacteria. They're used for numerous regions in pollution observance, medicinal, perfumery, beauty and coloring clothes [1]. Lichen materials might play a more than one organic role, moreover in response to absolutely unique ecological elements. Anti-viral, anti-protozoal, anti- proliferative, analgesic, anti-inflammatory and anti-pyretic moves of usnic acid referred to as secondary metabolites are reviewed. Among the various lichen compounds, usnic acid is that the well-known and studied metabolite. This metabolite formerly rumored as antibiotic, anti-fungal, anti-herbivorous, phytotoxic, and photobiont-regulating and as a UV-filter [2-4].

Dolichousnea longissima (Ach.) Articus (syn. *Usnea longissima* Ach.) consists of a placing plant structure and lives on bushes (epiphytic). *DU. longissima* is one in each of the species most sensitive to pollutants. In a few European countries, its fitness is conventional as an indicator of pollution. The presence of *DU. longissima* indicates clear air, whereas its absence shows impure air. It's been accustomed strengthen hair and inside the manufacturing of hygienically products for ladies. In the human's medicine of various nations of the planet *DU. longissima* has additionally been used significant as an expectorant, for wound dressing and to unwavering nostril hemorrhage, none the less as inside the treatment of ulcers. It's more over been carried out in the remedy of injuries to the legs and loins, bone fractures, and pores and skin eruptions [5].

Moreover currently uses of lichen species is antipyretic, anti-tumor, cholesterol and nematocidal residences etc. [6,7]. The isolation of two new phenolic resin compounds longissiminonea (1) and longissiminone b (2). Glutinol (three) become remoted for the number one time from this lichen and that turned into antecedently stated from various flowers to own anti-fungal and analgesic activities. The structures of those compounds had been deduced through spectroscopical strategies [8].

As a result, the goal of the present study become designed to test the hepatoprotective activity of the plant *DU. longissima*, of lichens species with the lively constituent like usnic acid etc. which show hepatoprotective activity towards cisplatin triggered liver damage in Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Chitosan secluded from the cuttlebone of *S. kobiensis* with the DDA 85.55% and molecular weight of 322.04 kDa [9]. All other chemicals used in the study were of high analytical grade, product of Sigma Company (USA).

2.2 Animals

Thirty male Wistar albino rats weighing 160-190 g were used. The rats were obtained from Animal House-holding NBRI, Lucknow. Animals have been kept in special cages, and maintained on a constant 12-h light/12-h dark cycle with air conditioning and temperature ranging 20-22ºC and humidity (60%). Rats were fed with standard rat pellet chow with free access to distilled water ad libitum for one week before the experiment for acclimatization. The protocol for this study has been approved by the Institutional Animal Ethics

Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals-CPCSEA, New Delhi, with number (IAEC CPCSEA/07/2014).

2.3 Lichen Sample

Dolichousnea longissima (Ach.) Articus (syn. *Usnea longissima* Ach.) (*DUL*) was taken and identified by a lichenologist, Department of botany, NBRI, Lucknow.

2.4 Extraction of Lichen Sample (*DUL*)

The lichen samples was dried in the shade in an airy place and then stored in paper bags and kept at natural room temperature. Then the lichen material was milled by an electrical mill. The dried ground thallic of the investigated lichens DU. longissima (50 gm) was extracted using ethanol in a Soxhlet extractor. Then extract was filtered and concentrated under reduced pressure in a rotary evaporator (Buchi R-200 USA) at 45°C and then freeze-dried in lyophilizer (Labconco, USA) to obtain solid residue (ASE, yield 20.0% w/w). The extract was dissolved in 5% dimethyl sulphoxide (DMSO) for the experiments [10].

2.5 Experimental Protocol

Subsequent to 1 week acclimation, the rats were reserved fasting during the night prior to treatment and randomly divided according to dose. The protocol was lasts for 7 days.

Group I: Normal healthy animals

Group II: Intoxicated animals with cisplatin single dose (Pretreated) (5 mg/kg/day, i.p, cisplatin inj., 50 mg cipla Co., India) [11]

Group III: Received orally chitosan as standard drug at dose (200 mg/kg/day) [9]

Group IV & V: *DUL* (100 and 200 mg/kg) (post-treated) were given orally in 1% carboxy methylcellulose (CMC) respectively [10]

The groups II–V received cisplatin for 7 days to induce hepatotoxicity

Blood was collected from retro orbital plexus from the overnight fasted animals, after anaesthized with 100 mg/kg ketamine, i.p. The blood samples was taken with 20 µl EDTA (5%) in each eppendroff and centrifuged at 5000 rpm for 15 min (Sigma 3K30, UK). Supernatant (serum) was separated with the help of micropipette and placed it in new eppendroff with well labeled and stored in -80°c for further analysis. (Biochemical and antioxidant estimation).

After blood collection all rats were euthanized and liver tissue specimens were isolated and washed with normal saline (0.9%) for cleaning the blood part in liver and kept it on filter paper for dry, after dry it were preserved in the 10% buffered-neutral formalin for histopathological examination.

Liver tissue was positioned in liquid nitrogen and stored at -700C until evaluation for malondialdehyde (MDA) by measuring the existence of thiobarbituric acid reactive substances (TBARS) [12], Lipid peroxidation (LPO) product, Superoxide dismutase (SOD) by the technique of Sun et al [13], Catalase (CAT) according to Aebi's way [14], Glutathione peroxidase (GPx) was deliberate by the process of Paglia and Valentine [15]. The other segment of the liver was placed in formaldehyde solution for usual histopathological assessment by light microscopy.

2.6 Biochemical Evaluation

The collected blood samples after centrifugation were stored at -80°C for the biochemical evaluation of urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), and Bilirubin.

Frozen liver tissue about 150 mg was sliced into pieces on dry ice and homogenized in 10 volume of ice-cold Tris-HCl buffer respecting tissue weight (50 mmol/L, pH 7.4) using a homogenizer (LHG-15ADaihanLabtech, Korea) for 3 min at 6000 rpm. The supernatant solution was extracted with an equivalent volume of an ethanol/chloroform mixture (3/5, volume per volume [v/v]).

2.7 Histopathology of Liver

10% formalin was freshly planned and the right liver lobe of treated and control were fixed in 10% formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin and eosin.

2.8 Statistical Analysis

All the statistical comparison between the groups were made by means of One Way Analysis of Variance (ANOVA) and followed by Student-Newman-Keuls test. The p =0.05 regarded as significant using, Graph Pad Prism 5.03 Software (CA, USA). The data expressed are Mean ± standard error of mean (S.E.M).

3. RESULTS AND DISCUSSION

3.1 Body and Liver Weights

There were no significant differences in the body and liver weights obtained before and after the experiments amid the groups.

3.2 Biochemical Test Results

Cisplatin is one of the strong anti-neoplastic agents usually used in cancer remedy [16]. Even if there are various experimental studies on widespread toxic effects allied with cisplatin when used in elevated doses, the number of studies about hepatotoxicity is still deficient [17]. ALT is a hepato-specific enzyme explicitly normally positioned in the cytoplasm of hepatocytes [18]. The serum ALT and AST levels are responsive to the deed of hepatotoxic agents and give out as markers of liver damage, which is accompanied by the discharge of such amino transferase from hepatocytes into the blood stream [18,19].

The outcome from the biochemical assessment of the premeditated rat blood samples are recapitulated in (Fig. 2). The values of the biochemical parameters indicated that liver damage was certainly induced in the intoxicated group in our experimental setting (i.e. the group given cisplatin only). An enhancement in the level of serum urea was observed in the cisplatin group compared to that in the control group (Fig. 1). As compared to the levels in the intoxicated group, the urea level decreased in the group given 200 mg/kg of *DUL* and in standard group, whereas it increased in group, especially in 100 mg/kg of *DUL* (p =0.05) (Fig. 1). The statistical analysis showed significant changes among the creatinine levels in the studied groups (Fig. 1).

Bilirubin levels can be increased due to hepatocellular injury and cholestasis liver diseases [20]. However, the obtained results indicated that the administration of cisplatin significantly boost up the bilirubin level causing hepatocellular injury (Fig. 1). The bilirubin level was significantly reduced by all groups as compared to that in the cisplatin group (p =0.05).

There was a sizeable rise in the serum AST, ALT and GGT values as compared to those in the healthy rats (Fig. 2). These changes are notion to be a pinpointing factor of rigorous liver damage, seeing as these enzymes are well recognized to be released from hepatocytes into the blood at the spot of hepato necrosis. In disparity to cisplatin treatment only, the administration of 200 mg/kg of led to a decline in these parameters to the normal levels. Low dose of *DUL* signifies a less protective effect. As exposed in (Fig. 2), the AST, ALT and GGT levels in these two groups showed significant changes mainly in the blood samples from animals given the dose (200 mg/kg) of *DUL* as compared to that of intoxicated and healthy groups (p =0.05).

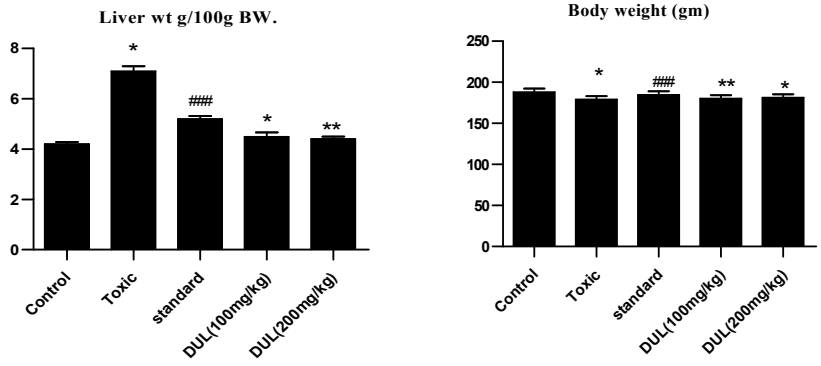

Fig. 1. Body weight and liver weight in cisplatin-treated rats

*All values are expressed as mean ± SEM. ### p =0.05 compared with normal; *** p =0.05, ** p =0.05, * p =0.05 compared with intoxicated group, using one way ANOVA followed by Student-Newman-Keuls test as a post-ANOVA test*

MDA is a lipid peroxidation artifact that is released explicitly because of the toxic effects of active ROS. ROS is created as an outcome of oxidation of unsaturated fatty acids in cell membranes [21]. The detection of amplified MDA production in cisplatin-induced hepatotoxicity was in sequence with our study. MDA levels were detected as significantly elevated in the cisplatin group when compared with healthy group (p =0.05) while treatment with *DUL* in both doses and also with standard, MDA levels were originated as lesser in group III-V which is statistically significant (p =0.05) (Fig. 3).

SOD, CAT and GPx, endogenous enzymatic antioxidants guard the organism and cells against cytotoxic free oxygen radicals. Oxygen radicals are transformed to H_2O_2 via SOD. Afterwards, CAT and GPx alter H_2O_2 into H_2O and O_2. Furthermore, GPx acts through the decline of the GSH. Glutathione reductase after that recycles the GSH. The levels of these enzymes inside the host boost to guard the tissues during oxidative damage [22]. Additionally, GPx production is perked up as superior defense mechanism when SOD and CAT are inadequate [21]. The levels in SOD, CAT and GPx were observed significantly decline in cisplatin group on comparison with healthy group (p =0.05) but on treatment with *DUL* in both doses and also with standard, the levels were elevated in group III-V which is rendered statistically significant (p =0.05) (Fig. 2).

3.3 Histopathology

10% formalin solution was freshly prepared and the right liver lobe of the treated and control group was fixed in the solution for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin and eosin.

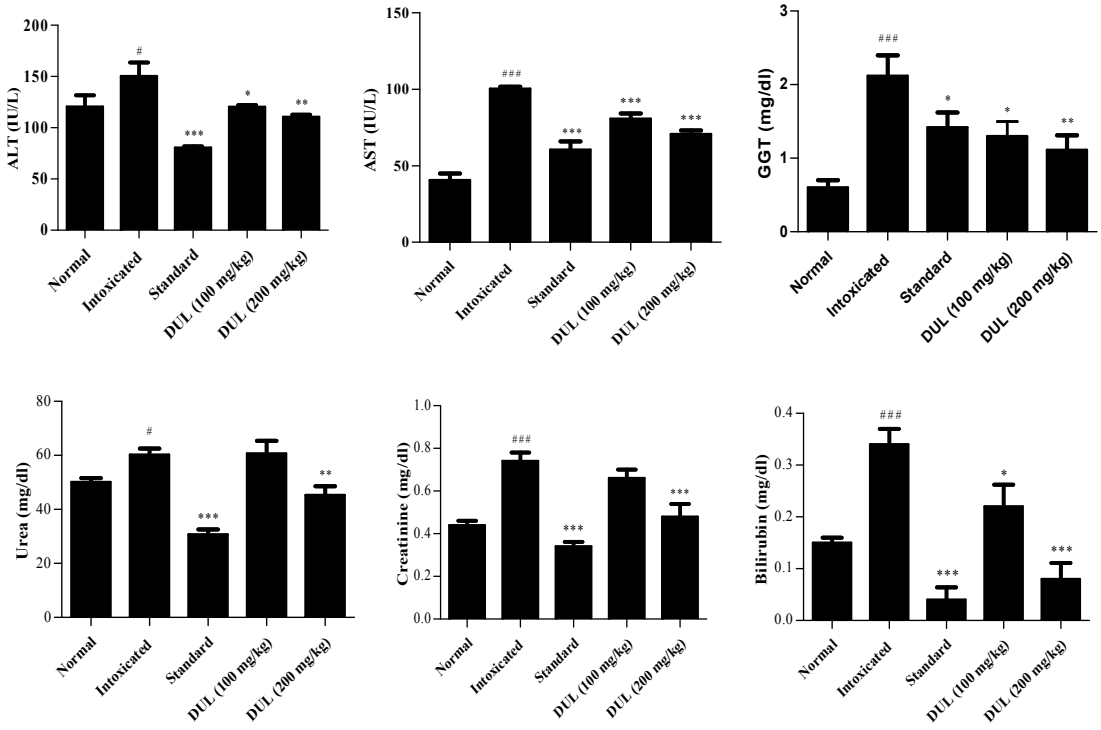

Fig. 2. Biochemical parameters in cisplatin-treated rats

*All values are expressed as mean ± SEM. ### p =0.05 compared with normal; *** p =0.05, ** p =0.05, * p =0.05 compared with intoxicated group, using one way ANOVA followed by Student-Newman-Keuls test as a post-ANOVA test*

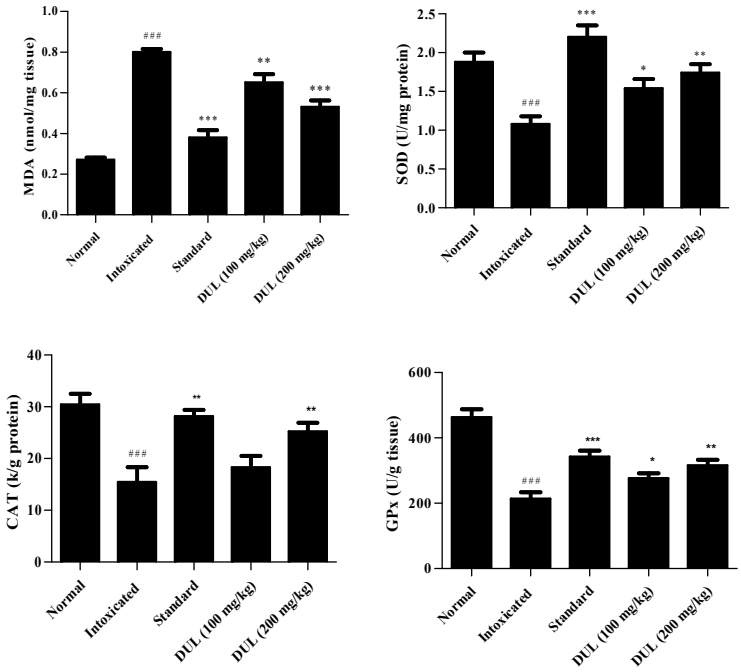

Fig. 3. Comparison of antioxidant biochemical parameters among the studied groups

*All values are expressed as mean ± SEM. ### p =0.05 compared with normal; *** p =0.05, ** p =0.05, * p =0.05 compared with intoxicated group, using one way ANOVA followed by Student-Newman-Keuls test as a post-ANOVA test*

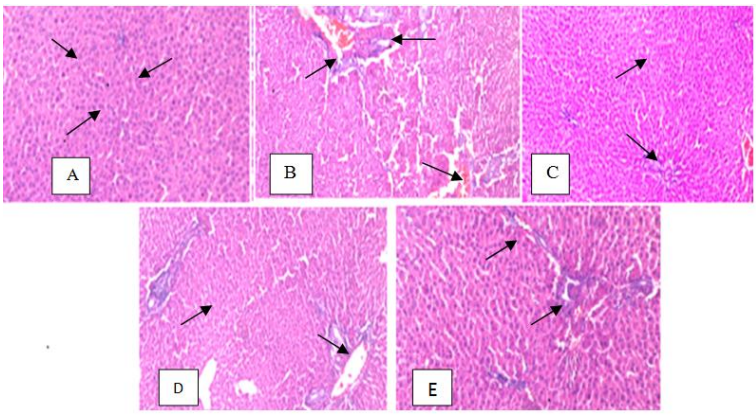

Fig. 4. A) Liver section of normal control rats showing normal hepatic cells.
B) Liver sections of cisplatin (5 mg/kg) treated rats showing, massive fatty changes, necrosis, balloon formation, central vein congestion, loss of cellular boundaries and mononuclear inflammation.(Indicated by arrow)
C) Liver section of rats treated with cisplatin (5 mg/kg/day, i.p) + chitosan as standard drug at dose (200 mg/kg/day), showing, and well brought out central vein, hepatic cell with well preserved cytoplasm, prominent nucleus & nearby normal liver architecture. (Indicated by arrow)
D) Liver sections of rats treated with cisplatin (5 mg/kg/day, i.p) + *DUL* (100mg/kg). Showing hepatic cells with well preserved cytoplasm, prominent nucleus, some of central veins and sinusoids exhibited congestion. (Indicated by arrow)
E) Liver sections of rats treated with cisplatin (5 mg/kg/day, i.p) + *DUL* (200mg/kg) showing well brought out central vein, hepatic cells with well preserved cytoplasm & prominent nucleus. (Indicated by arrow)

4. CONCLUSION

In this study is that the investigation to indicate that the presence of constituents from *DU. longissima*, biochemical and antioxidant activity against Cisplatin damage in wistar rats. This was disclosed by changes in level of LPO and GSH concentration in liver, additionally to the elevation of SOD, CAT and GPx activity. The *DU. longissima* evidenced its activity either through stabilization of cellular membrane or through anti-peroxidase activity. The outcome of this study reveals that, there is a powerful antioxidant and hepatoprotective activity of *DU. longissima*.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rao Zahid Abbas, et al. Integrated strategies for the control and prevention of dengue vectors. Pak Vet J. 2014;34(1):1-10.
2. Seyhan Oran, Saliha Sahin, Pinar Sahinturk, Sule Ozturk, Cevdet Demir. Antioxidant and antimicrobial potential and HPLC analysis of stictic and usnic acids of three *Usnea* species from uludag mountain (Bursa, Turkey). Iran J Pharm Res. 2016;15(2):527–535.
3. Antonella Piozziet, et al. Water soluble usnic acid-polyacrylamide complexes with enhanced antimicrobial activity against *Staphylococcus epidermidis*. Int J Mol Sci. 2013;14(4):7356–7369.
4. Sahib K, Kularatne NS, Kumar S, Karunaratne V. Effect of (+)-usnic acid on the shot-hole borer (xyleborus fornicatus eichh.) of tea. J. Nat. Sci. Sri Lanka. 2008;36:335-336.
5. Halici M, Odabasoglu F, Suleyman H, Cakir A, Aslan A, Bayir Y. Effects of water extract of *Dolichousnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine. 2005;12:656-662.
6. Idamokoro, et al. *In-vitro* antibacterial sensitivity of Usnea barbata lichen against selected *Staphylococcus* species. Archiv Tierzucht. 2014;57(25):1-9.
7. Choudhary MI, et al. Bioactive phenolic compounds from a medicinal lichen, *Dolichousnea longissima*. Phytochemistry. 2005;66:2346–2350.
8. Maulidiyah, Imran, Watu Muntu, Muhammad Nurdin. Secondary metabolites identification from lichen *Dolichousnea longissima* Ach. Bioactivity test of antibacterial. International Journal of Applied Chemistry. 2016;12(3):347-357.
9. Khakshoor, et al. Screening of antimicrobial potential of chitosan and polysaccharide extracted from cuttlebone of *Sepia pharaonis* (Ehrenberg, 1831). IJAPBS. 2015;5:2278-0246.
10. Brahma NS. The genus Usnea: A potent phytomedicine with multifarious ethanobotany, phytochemistry and pharmacology. RSC Adv. 2016;6:21672.
11. Chi-Hao Wu, An-Zhi Chen, Gow-Chin Yen. Protective effects of glycyrrhizic acid and 18β-glycyrrhetinic acid against cisplatin-induced nephrotoxicity in BALB/C Mice. J. Agric. Food Chem. 2015;63(4):1200–1209.
12. Batcioglu K, et al. Liver lipid peroxidation and antioxidant capacity in cerulein-induced acute pancreatitis. Braz J Med Biol Res. 2009;42(9):776-782.
13. Rukmini MS, Benedicta DS, et al. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. Indian Journal of Clinical Biochemistry. 2004;19(2):114-118.
14. Aebi H, Suter H. Catalase. Methods of enzymatic analysis. 1969;23:597-603.
15. Anandasadagopan SK, et al. S-Allyl cysteine alleviates inflammation by modulating the expression of NF-B during chromium (VI)-induced hepatotoxicity in rats. Human and Experimental Toxicology. 2016;1–15.
16. Cigremis Y, Akgoz M, Ozen H, Karaman M, Kart A, Gecer M, Atalan G. Resveratrol ameliorates cisplatin-induced oxidative

injury in new zealand rabbits. Can. J. Physiol. Pharmacol. 2015;93:727-735.
17. Prashant R Gore, et al. Protective effect of thymoquinone against cyclophosphamide-induced hemorrhagic cystitis through inhibiting DNA damage and upregulation of Nrf2 expression. Int J Biol Sci. 2016;12(8):944-953.
18. Vijaya Benerji G, Farid Babu M, Rekha Kumari, Aditi DS. Comparative study of ALT, AST, GGT & uric acid levels in liver diseases. Journal of Dental and Medical Sciences. 2013;7:72-75.
19. Dharmendra Singh, Arya PV, Neetu Koolwal, Singh V, Saxena R, Sharma MC, Gupta RS. Protective role of *Bacopa monniera* L. against hepatic oxidative stress in wistar albino rats. Journal of Pharmacognosy and Phytochemistry. 2015;4(4):233-241.
20. Akther N, Andrabi K, Nissar A, Ganaie S, Chandan B, Gupta A, Khuswant M, Sultana S, Shawl AS. Hepatoprotective activity of lc–esi-ms standardized iris spuria rhizome extract on its main bioactive constituents. Phytomedicine. 2014;21:1202-1207.
21. Mansour HH, Hafez HF, Fahmy NM. Silymarin modulates cisplatin-induced oxidative stress and hepatotoxicity in rats. J. Biochem. Mol. Biol. 2006;39:656.
22. Lu XY, et al. NADPH oxidase inhibition improves neurological outcome in experimental traumatic brain injury. Neurochemistry International. 2014;69: 14–19.

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