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Hepatoprotective activity of *Ipomoea staphylina* against d-glan/lps-induced acute hepatic failure in experimental rats

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Abstract: The goal of this study was to see ethanolic extract of *Ipomoea staphylina* leaves could protect rats from D-GalN/LPS-induced AHF. Five groups (n=6) of male Wistar rats were created. Group I was given a normal control (1ml/kg); Group II was given D-GalN/LPS; Group III was given D-GalN/LPS + silymarin (100 mg/kg; p.o.); Group IV was given D-GalN/LPS+ethanolic extract of *I. staphylina* (100mg/kg); and Group V was given D-GalN/LPS+ethanolic extract of *I. staphylina* (200mg/kg). All animals in groups II-V were given D-GalN/LPS (400mg/kg; and 30g/kg) on the 15th day after being treated with silymarin or *I. staphylina* extract for 15 days. Blood was collected from all groups of animals 24 hours after D-GalN/LPS administration to conduct biochemical analysis. The levels of SGOT, SGPT, ALP, GGT and total bilirubin in animals pretreated with the extract were all considerably lower. In addition, the total protein content was considerably greater in the extract-treated mice. The extract led to a considerable decrease in LPO levels as well as a notable increase in SOD, CAT and GSH levels in liver tissue. The extract dramatically lowered TNF-α, IL-6, iNOS, NO and MPO levels in the liver tissue.

Keywords: *Ipomoea staphylina*, D-GalN/LPS, acute hepatic failure, silymarin, biochemical.

INTRODUCTION

Acute hepatic failure (AHF) is a very uncommon clinical condition in which hepatic function rapidly deteriorates as a result of a devastating liver injury (Zhang *et al.,* 2014). AHF is frequently associated with no prior hepatic disease and lasts for only a few days (Bernal and Wendon, 2013). Viruses, drugs, alcohol, toxins, metabolic diseases and autoimmune hepatitis are all potential causes of AHF (Lee, 2013). AHF clinical features or signs, such as icterus, coagulopathy and defects in liver functionality, are common and if the patient is not treated, multiorgan failure and hepatic encephalopathy can occur, leading to death (Auzinger and Wendon, 2008).

Because there are no active clinical therapies for AHF other than liver transplantation, finding an effective medicine to prevent AHF is critical. Natural products from medicinal plants, such as phenols, flavonoids, alkaloids, glycosides, carotenoids, essential oils and others, have been reported to have mitigating hepatic effects in hepatic diseases (Muriel and Rivera-Espinoza, 2008; Bruck *et al.,* 1999; Emerit *et al.,* 2005).

In experimental animals, D-galactosamine (D-GalN) is used to cause AHF. A dosage of D-GalN causes hepatic damage that is extremely similar to viral hepatitis in people (Decker and Keppler, 1972). D-GalN causes liver injury by inhibiting RNA and protein synthesis via uridine nucleotide depletion caused by an excess of uridine diphosphate (UDP)-hexomine in the liver cells (Coen *et al.,* 2007; Czekaj *et al.,* 2022). In addition, the production of essential cellular membrane components and biomolecules catabolism in the cell is inhibited by D-GalN-induced RNA and protein formation, resulting in liver cell damage and death (Farber and El-Mofty, 1975). Furthermore, lipopolysaccharide (LPS) triggers hepatic inflammation by activating Kupffer cells and causing oxidative stress and production of nitric oxide (NO) plus tumor necrosis factor-alpha (TNF-α) (Su, 2002; Czekaj *et al.,* 2022). D-GalN/LPS overproduce oxidative radicals in the hepatocytes, resulting in severe hepatic damage (El-Agamy *et al.,* 2014). The D-GalN/LPS-induced AHF experimental process is a useful for AHF studies because of these above said facts (Inoue *et al.,* 2003).

This study looked at the preventive efficacy of an ethanolic extract of *Ipomoea staphylina* leaves over D-GalN/LPS-induced AHF on experimental animals. This

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plant belongs to convolvulaceae family and appears like a branching permanent herb. This plant is used in Asian traditional medicine as a medicinal plant. It's commonly used to treat liver problems, purgation, gastrointestinal issues, pain, inflammation and rheumatism.

This plant contains sitosteryl-3-O-D-glucoside as well as chiro deoxy inositol, according to experiments (Reddy *et al.,* 2013). Analgesic (Ghosh and Firdous, 2014), slowing down inflammation (Firdous and Koneri, 2012), mending of gastric ulcer (Banerjee and Firdous, 2015), liver protection (Bag and Mumtaz, 2013; Ramachandran *et al.,* i2019), renal protection (Bag and Mumtaz, 2013), blood glucose lowering (Firdous and Koneri, 2014; Firdous and Singh, 2016) and *in vitro* free radicals combating activities (Ramachandran *et al.,* 2019) have been reported for this plant's leaves extract. Nevertheless, in experimental animals, this plant has been shown to defend against carbon tetrachloride-induced liver injury (Bag and Mumtaz, 2013; Ramachandran *et al.,* i2019). As a result, in the current work, we looked at the preventive effect of *I. staphylina* leaves against D-GalN/LPS- challenged rats.

MATERIALS AND METHODS

Chemicals and drugs

Several chemicals and biochemical kits were used in this study. D-galactosamine (D-GalN) and lipopolysaccharide (LPS) were provided by Sigma Chemicals (USA). Kits for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein and total bilirubin were provided by Span Diagnostics in India. Kits for tumour necrosis factor alpha (TNF-α), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), nitric oxide (NO) and myeloperoxidase (MPO) were provided by Sigma Chemicals (USA). The other chemicals were all analytical reagent-grade and commercially available.

Plant materials

The leaves of *I. staphylina* were obtained in a forest near Bangalore, Karnataka. Dr. K. Karthigeyan of the Central National Herbarium, Botanic Garden, Howrah, taxonomically recognized and verified this plant, which again is archived with the order id-SMF-01.

Preparation of extract

The leaves of *I. staphylina* also were washed and thoroughly cleaned and then dried in the dark condition over many days prior getting pulverized. The powder was defatted with petroleum ether (60-80 GR) for 72 hours before being extracted with ethyl alcohol. The yield of 10.20 percent w/w dry extract was obtained after extraction with alcohol, which was kept at 4°C in a refrigerator (Firdous and Koneri, 2014).

Experimental animals

The institute's central animal house provided male Wistar rats weighing between 180 and 200g. For a 12 hour day/night cycle, the animals were maintained in polypropylene cages at a temperature of 221°C. In the departmental animal house, they were kept in sanitary polypropylene cages. This experimental protocol was reviewed and accepted by the "Research Ethics Committee of Taibah University, Saudi Arabia," which follows the Saudi National Regulation of the National Bioethics Committee U.S. CFR and the Principles of Laboratory Animal Care (protocol number- COPTU-REC-25-20211229).

Toxicity test (OECD 423)

Before receiving the ethanolic extract of *I. staphylina* leaves, rats were starved overnight. After that, a single oral dose of *I. staphylina* leaves ethanolic extract (2000mg/kg) was administered. First 3-4 hours, the animals were kept under special observation. After the first 30 minutes of dosing, they were monitored twice more and then every day for the next 24 hours (throughout the first four hours, with specific emphasis). Daily observation for 14 days was done to identify cardiovascular, respiratory, autonomic and central nervous system abnormalities (if any). If there was any mortality, it was noted (OECG, 2002).

Experimental design

For one week, all male Wistar rats were acclimatised and alienated into five groups (n=6).

The following treatment was given to each group:

Group I: Normal control animals received saline (1ml/kg;p.o.).

Group II: D-GalN/LPS control received merely D-GalN/LPS.

Group III: Received D-GalN/LPS+silymarin (100mg/kg; p.o.).

Group IV: Received D-GalN/LPS+ethanolic extract of *I. staphylina* (100mg/kg; p.o.).

Group V: Received D-GalN/LPS+ethanolic extract of *I. staphylina* (200mg/kg; p.o.).

Animals received an ethanolic extract of *I. staphylina* (100 or 200mg/kg; p.o.) for 15 days. The animals in groups I and II were given saline. On the 15th day, all animals in groups II-V were given D-GalN/LPS (D-GalN 400mg/kg; i.p. and LPS 30mg/kg; i.p.) (Wang *et al.,* 2015; Zhao *et al.,* 2021). For biochemical analysis, blood was collected via cardiac puncture from the animals 24 hours after D-GalN/LPS administration.

Biochemical analysis

SGOT, SGPT, ALP, total protein and total bilirubin were approximated utilizing commercialized kits (Span Diagnostics, India) in accordance with the manufacturer's instructions.

Measurement of antioxidants and inflammatory markers

In a Tris phosphate buffer, liver tissue homogenates (10% w/v) were prepared (50mM, pH 7.4). At 4°C, the homogenate was spun for 10 min at 3000 rev/min. The isupernatant was tested for ilipid peroxidation (LPO), isuperoxide dismutase (SOD), ireduced glutathione (GSH) and icatalase (CAT) (Ohkawa *et al.,* 1979; Roturck *et al.,* 1973; Marklund and Marklunf, 1974; Sinha, 1972). Following the manufacturer's instructions, pro-inflammatory cytokines (TNF-α and IL-6), iNOS and NO levels were determined using available for purchase or marketed ELISA kits. In addition, the level of myeloperoxidase (MPO) in the blood was determined using a commercially available kit and hydrogen peroxide dependent o-dianizidine oxidation, as directed by the manufacturer.

STATISTICAL ANALYSIS

Graph Pad Prism version 8.1 has been employed to perform a one-way analysis of variance (ANOVA) following Tukey Multiple Comparison Test to assess overall statistically importance of the results. The significance level was set at P less than 0.05. Means± SEM are used to represent the results.

RESULTS

Acute oral toxicity study

In the preliminary LD50 study extract showed no sign of toxicity up to a maximum dose of 2000mg/kg. Therefore, $1/20^{th}$ that is 100mg/kg as a lower dose and $1/10^{th}$ that is 200mg/kg as a higher dose were taken in consideration for the study (Firdous and Koneri, 2012; Firdous and Koneri, 2014; Firdous and Singh, 2016).

Assessment of biochemical parameters

In the animals given D-GalN/LPS, serum levels of SGOT, SGPT, ALP, GGT and total bilirubin all increased, indicating liver injury. Pretreatment with an ethanolic extract of *I. staphylina* at doses of 100 and 200mg/kg for 15 days noticeably reduced the levels of the above biochemical parameters. Animals given silymarin (100mg/kg) had significantly lower levels of SGOT, SGPT, ALP, GGT and total bilirubin. The effects of the extract on those parameters are comparable to that of silymarin. Furthermore, total protein levels were markedly higher in the extract and silymarin-treated animals (table 1).

Assessment of endogenous antioxidants and pro-inflammatory cytokines

A notable enhance in LPO levels in the liver tissue of D-GalN/LPS challenged rats was observed. SOD, CAT and GSH levels in the same group's liver tissue were significantly lower, indicating an increase in protein metabolism. After 15 days of pretreatment with an ethanolic extract of *I. staphylina* (100 and 200mg/kg), the LPO level was significantly reduced. Furthermore, the extract (100 and 200mg/kg) appreciably increased the levels of SOD, CAT and GSH in the liver tissue (table 2). Furthermore, MPO levels in D-GalN/LPS-challenged rats were established to be notably higher. The MPO level was appreciably reduced following pretreatment with the extract (100 and 200mg/kg).

TNF-α, IL-6, iNOS and NO levels all increased dramatically in the rats exposed to D-GalN/LPS. Increases in pro-inflammatory cytokines (TNF-α, IL-6), iNOS and NO may play a role in the progression of hepatic injury. After pretreatment with an ethanolic extract of *I. staphylina* (100 and 200mg/kg), the levels of TNF-α, IL-6, iNOS and NO in the liver tissue were significantly reduced (table 3).

DISCUSSION

The AHF caused due to D-GalN/LPS is a well-known and widely used model for anti-hepatotoxic drug evaluation. Hepatic injuries caused by D-GalN/LPS are similar to viral hepatitis (Nakagiri *et al.,* 2003). As a result, Wistar rats with D-GalN/LPS-induced hepatic damage were chosen as an AHF experimental model. The main facts of D-GalN/LPS-induced hepatocellular artifacts are increased ROS production and oxidative stress, both of which add to AHF (Sheik and Thiruvengadam, 2013; Czekaj *et al.,* 2022). D-GalN increased the permeation of hepatocyte membranes by impairing ionic pumps. In addition, damage to the calcium pump raises intracellular calcium, causing cell sense (Nakagiri *et al.,* 2003; Zhao *et al.,* 2021).

Hepatic antioxidant enzymes may be inhibited by D-GalN/LPS (Zhou *et al.,* 2008). Pretreatment of D-GalN/LPS intoxicated animals with the extract increases the activity of hepatocellular antioxidant enzymes such as GPx, CAT and SOD (Zhou *et al.,* 2008). Besides, LPS cause increase in oxidative stress via Nrf2 pathway (Sun *et al.,* 2020). Free radical formation can be induced by excessive production of hydroxyl free radicals from hydrogen peroxide, which is suppressed by endogenous antioxidant enzymes such as reduced GSH, which produces a first-line defence against oxidative stress and a drop in GSH level. Aside from that, CAT also involves the detoxification of hydrogen peroxide. Membrane lipid peroxidation is caused by excessive hydrogen peroxide production, which leads to an increase in MAD formation (Zelber-Sagi *et al.,* 2020). GSH depletion, on the other hand, can lead to an increase in MAD formation due to lipid peroxidation in the membrane (Salminen and Vihko, 1983).

Table 1: Effect of ethanolic extract of *I. staphylina* on serum SGOT, SGPT, ALP, GGT, total bilirubin and total protein in D-GalN/LPS intoxicated rats

Serum	Normal Control	D-GalN/LPS	D-GalN/LPS + Silymarin (100 mg/kg)	D-GalN/LPS +*I. staphylina* (100 mg/kg)	D-GalN/LPS+*I. staphylina* (200 mg/kg)
SGOT U/L	72.19±3.85	257.82±8.86$^{###}$	101.04±3.16***	127.38±2.96***	113.86±3.83***
SGPT U/L	41.13±2.63	122.67±1.61$^{###}$	54.53±1.77***	74.47±2.67***	57.96±1.58***
ALP U/L	61.47±2.23	194.96±6.90$^{###}$	71.06±3.35***	97.06±3.94***	80.63±3.38***
GGT U/L	1.02±0.07	3.72±0.33$^{###}$	1.57±0.10***	1.92±0.08***	1.61±0.05***
Total Bilirubin mg/dL	0.55±0.03	1.25±0.09$^{###}$	0.76±0.02***	0.95±0.05**	0.85±0.03**
Total Protein mg/dL	8.40±0.41	4.52±0.29$^{###}$	7.24±0.27***	6.26±0.33**	7.14±0.17***

Data are displayed as mean±standard error mean (n=6).
$^{###}$p<0.001 – Normal Control vs D-GalN/LPS
**p<0.01 and ***p<0.001 – D-GalN/LPS + Silymarin (100mg/kg), D-GalN/LPS + *I. staphylina* (100 mg/kg) and D-GalN/LPS + *I. staphylina* (200 mg/kg) vs D-GalN/LPS

Table 2: Effect of ethanolic extract of *I. staphylina* on hepatic endogenous antioxidants in D-GalN/LPS intoxicated rats

Parameters	Normal Control	D-GalN/LPS	D-GalN/LPS + Silymarin (100 mg/kg)	D-GalN/LPS + *I. staphylina* (100 mg/kg)	D-GalN/LPS + *I. staphylina* (200 mg/kg)
LPO (ηm of MDA per mg of protein)	4.48±0.29	16.59±0.49$^{###}$	6.92±0.39***	9.02±0.48***	7.13±0.48***
GPx (µMole of the oxidized GSH/min/mg of protein)	14.02±0.87	5.19±0.51$^{###}$	13.03±1.16***	9.77±0.53***	12.83±0.83***
CAT (µm H_2O_2/mg of protein)	14.99±1.12	4.50±0.54$^{###}$	12.92±1.58***	10.56±0.90***	11.26±0.90***
SOD (U/mg of protein)	13.80±1.14	4.52±0.54$^{###}$	10.36±0.69***	8.50±0.55***	10.27±0.88***
MPO (U/g of tissue)	10.81±0.77	37.39±2.71$^{###}$	17.69±1.15***	20.64±1.32***	19.00±1.31***

Data are displayed as mean±standard error mean (n=6).
$^{###}$p<0.001 – Normal Control vs D-GalN/LPS
***p<0.001 – D-GalN/LPS + Silymarin (100 mg/kg), D-GalN/LPS + *I. staphylina* (100 mg/kg) and D-GalN/LPS + *I. staphylina* (200 mg/kg) vs D-GalN/LPS

Table 3: Effect of ethanolic extract of *I. staphylina* on hepatic TNF-*α*, IL-6, iNOS and NO levels in D-GalN/LPS intoxicated rats

Parameters	Normal Control	D-GalN/LPS	D-GalN/LPS + Silymarin (100 mg/kg)	D-GalN/LPS + *I. staphylina* (100 mg/kg)	D-GalN/LPS + *I. staphylina* (200 mg/kg)
TNF*α* (pg/ml)	17.97±1.13	165.61±5.06$^{###}$	51.30±3.81***	78.76±5.23***	61.92±3.32***
IL6 (pg/ml)	19.78±1.91	140.09±6.92$^{###}$	37.45±3.92***	71.87±2.99***	44.82±2.45***
iNOS (U/mg)	7.78±0.78	51.40±1.86$^{###}$	17.49±1.61***	30.38±1.32***	24.39±1.43***
NO (µmol/mg	5.57±0.73	13.61±1.03$^{###}$	7.43±0.39***	10.88±0.97*	9.43±0.49**

Data are displayed as mean±standard error mean (n=6).
$^{###}$p<0.001 – Normal Control vs D-GalN/LPS
*p<0.01, **p<0.01 and ***p<0.001 – D-GalN/LPS + Silymarin (100 mg/kg), D-GalN/LPS + *I. staphylina* (100 mg/kg) and D-GalN/LPS + *I. staphylina* (200 mg/kg) vs D-GalN/LPS

Furthermore, lowering SOD activity may increase the production of superoxide anion (O2•-) in the mitochondria (Dennis and Witting 2017). Giving D-GalN/LPS to rats augmented LPO by causing renal MDA generation and reduction of GSH, according to the findings. CAT and SOD, were also established to be depleted in hepatocytes of D-GalN/LPS challenged rats (Ighodaro and Akinloye, 2019). Treatment with the extract considerably reduced LPO while increasing the action of GPx, CAT and SOD.

Because many antioxidants reveled to have inflammation slowing action (Geronikak and Gavalas, 2006) we investigated the effect of the extract on hepatic TNF-α and IL-6 levels in the current study. TNF-α is an essential proinflammatory cytokine that promotes liver damage in

rats exposed to D-GalN/LPS. (Liu *et al.,* 2001; El-Beshbishy, 2008). Besides, it has been reported that TNF-α triggers apoptosis by activating caspase-8 and caspase-3 through various apoptotic pathways (Mignon *et al.,* 1999). In this study, TNF and IL-6 levels in the livers of D-GalN/LPS intoxicated rats were found to be considerably higher. After pretreatment with the extract (100 and 200 mg/kg), TNF-α and IL-6 levels were appreciably reduced. Thus, the extract's antioxidant properties and ability to lower TNF-α and IL-6 levels may be contributing to its hepatoprotective activity.

The role of iNOS in hepatocellular artifacts is a topic of debate (Lekic *et al*., 2013). NO is formed by iNOS, which then acts in response with ROS to produce peroxynitrite, which damages the liver (Stadler *et al.,* 2008). During this process, NO, on the other hand, act as ROS scavengers (Loguercio and Federico, 2003). D-GalN/LPS therapy dramatically boosted iNOS activity and NO generation in the liver. Pretreatment with silymarin or extract reduced iNOS activity and NO production to the point where the extract protected oxidative stress from the production of NO. MPO is also thought to be a useful tissue marker for determining neutrophil infiltration (Zhang *et al.,* 2018). Our findings revealed that intoxicated rats with D-GalN/LPS had a significant increase in this marker, whereas pretreatment with silymarin or extract significantly reduced MPO activity in a dose-dependent manner.

These findings show that an ethanolic extract of *I. staphylina* reduces hepatic damage in rats with D-GalN/LPS-induced liver damage by reducing oxidative stress and inflammation. We also found that the effect of ethanolic extract of *I. staphylina* is in a dose dependent manner. The higher dose of the extract was found to show more effectively improve the antioxidants enzymes activity and reduced the proinflammatory markers.

CONCLUSION

According to our findings, the ethanolic extract of *I. staphylina* protected rats against D-GalN/LPS-induced AHF. The beneficial findings are due to the antioxidant action and the reduction of inflammatory hepatocellular artifacts.

REFERENCES

Auzinger G and Wendon J (2008). Intensive care management of acute liver failure. *Semin. Live. Dis*. **28**: 188-200.

Bag AK and Mumtaz SMF (2013). Hepatoprotective and nephroprotective activity of hydroalcoholic extract of *Ipomoea staphylina* leaves. *Bangladesh. J. Pharmacol*., **8**: 263-268.

Banerjee A and Firdous SM (2015). Antiulcer activity of hydroalcoholic extract of *Ipomoea staphylina* plant in rats. *Bangladesh. J. Pharmacol*., **10**: 652-653.

Bernal W and Wendon J (2013). Acute liver failure. *N. Engl. J. Med*. **369**: 2525e34.

Bruck R, Aeed H, Shirin H, Matas Z, Zaidel L, Avni Y and Halpern Z (1999). The hydroxyl radical scavengers dimethylsulfoxide and dimethylthiourea protect rats against thioacetamide-induced fulminant hepatic failure. *J. Hepatol*. **31**(1): 27-38.

Czekaj P, Król M, Limanówka Ł, Skubis-Sikora A, Kolanko E, Bogunia E, Hermyt M, Michalik M, Sikora B, Prusek A, Grajoszek A and Pająk J (2022). Dynamics of acute liver injury in experimental models of hepatotoxicity in the context of their implementation in preclinical studies on stem cell therapy. *Front. Biosci (Landmark Ed)*. **27**(8): 237.

Coen M, Hong YS, Clayton TA, Rohde CM, Pearce JT, Reily MD and Nicholson JK (2007). The mechanism of galactosamine toxicity revisited: A metabonomic study. *J. Proteome. Res*., **6**: 2711-2719.

Decker K and Keppler D (1972). Galactosamine induced liver injury. *Prog. Liver. Dis*., **4**: 183-199.

Dennis JM and Witting PK (2017). Protective role for antioxidants in acute kidney disease. *Nutrients*. **9**(7):718.

El-Agamy DS, Makled MN and Gamil NM (2014). Protective effects of agmatine against D-galactosamine and lipopolysaccharide-induced fulminant hepatic failure in mice. *Inflammopharmacol*., **22**: 187e94.

El-Beshbishy HA (2008). Aqueous garlic extract attenuates hepatitis and oxidative stress induced by galactosamine/lipopolysaccharide in rats. *Phytother. Res*., **22**: 1372e9.

Emerit I, Huang CY, Serejo F, Filipe P, Fernandes A, Costa A, Freitas J, Baptista A and Carneiro de Moura M (2005). Oxidative stress in chronic hepatitis C: A preliminary study on the protective effects of antioxidant flavonoids. *Hepatogastroenterol*., **52**(62): 530-536.

Farber JL and El-Mofty SK (1975). The biochemical pathology of liver cell necrosis. *Am. J. Pathol*., **81**: 237-250.

Firdous SM and Koneri R (2012). *In vivo* and *in vitro* antiinflammatory activity of leaves of *Ipomoea staphylina*. *Int. J. Pharm. Pharm. Sci*., **4**(5): 339-343.

Firdous SM and Koner R (2014). Antidiabetic and antioxidant activities of *Ipomoea staphylina* leaves in Streptozotocin (STZ) induced diabetic mice. *J. Pharma. Sci. Tech*., **3**(2): 77-84.

Firdous SM and Singh A (2016). Effect of *Ipomoea staphylina* leaves on streptozotocin-nicotinamide induced type-II diabetes in wistar rats. *Asian Pac. J. Health. Sci*., **3**: 30-44.

Geronikaki AA and Gavalas AM (2006). Antioxidants and inflammatory disease: Synthetic and natural antioxidants with anti-inflammatory activity. *Comb. Chem. High. Throughput. Screen*, **9**(6): 425-442.

Ghosh S and Firdous SM (2014). Evaluation of analgesic activity of hydroalcoholic extract of *Ipomoea staphylina*. *Thai. J. Pharm. Sci.,* **38**(2): 57-60.

Ighodaro OM and Akinloye OA (2019). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria. J. Med*., **54**: 287-293.

Inoue T, Horiai H, Aoki C, Kawamura I, Ota M, Mizuhara H, Tomoi M and Mutoh S (2003). Insulin-like growth factor-I prevents lethal acute liver failure induced by dgalactosamine and lipopolysaccharide in rats. *In vivo*. **17**: 293e9.

Lee WM (2013). Drug-induced acute liver failure. *Clin. Liver. Dis*., **17**: 575-586.

Lekic N, Canova NK, Horinek A and Farghali H (2013). The involvement of heme oxygenase 1 but not nitric oxide synthase 2 in a hepatoprotective action of quercetin in lipopolysaccharide-induced hepatotoxicity of D-galactosamine sensitized rats. *Fitoterapia*. **87**: 20e6.

Liu TZ, Lee KT, Chern CL, Cheng JT, Stern A and Tsai LY (2001). Free radical-triggered hepatic injury of experimental obstructive jaundice of rats involves overproduction of proinflammatory cytokines and enhanced activation of nuclear factor kappab. *Ann. Clin. Lab. Sci.* **31**: 383e90.

Loguercio C and Federico A (2003). Oxidative stress in viral and alcoholic hepatitis. *Free. Radic. Biol. Med*., **34**: 1e10.

Marklund A and Marklunf G (1974). Involvement of superoxide anion radical in the autoxidation of pyrogallol and convenient assay for superoxide dismutase. *Eur. J. Biochem*., **47**: 469-474.

Mignon A, Rouquet N, Fabre M, Martin S, Pages JC, Dhainaut JF, Kahn A, Briand P and Joulin V (1999). LPS challenge in D-galactosamine-sensitized mice accounts for caspase-dependent fulminant hepatitis, not for septic shock. *Am. J. Respir. Crit. Care. Med*., **159**(4 Pt 1): 1308-1315.

Muriel P and Rivera-Espinoza Y (2008). Beneficial drugs for liver diseases. *J. Appl. Toxicol*., **28**(2):93-103.

Nakagiri R, Hashizume E, Kayahashi S, Sakai Y and Kamiya T (2003). Suppression by hydrangeae dulcis folium of D-galactosamine-induced liver injury *in vitro* and *in vivo. Biosci. Biotechnol. Biochem*., **67**(12): 2641-2643.

OECD (2002). Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 3.03.1996. *In*: Eleventh Addendum to the OECD guidelines for the testing of chemicals organisation for economic co-operation development, Paris, June, 2000.

Ohkawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal. Biochem*., **95**: 51-8.

Ramachandran J, Devanesan AA and Thilagar S (2019). Hepatoprotective and antioxidant activity of *I. staphylina* Linn. *Clin. Phytosci*., **5**(18): 1-11.

Reddy DP, Kota R, Renuka S, Anarthe SJ and Raghavendra NM (2013). Isolation, characterization of phytoconstituents and pharmacological screening of *Ipomoea staphylina*. *Asian. J. Pharm. Clin. Res*., **6**(1): 30-33.

Roturck JT, Pope Al, Ganther HL and Swanson AB (1973). Selenium: Biochemical role as a component of glutathione peroxidise. *Sci*., **179**: 588-590.

Salminen A and Vihko V (1983) Lipid peroxidation in exercise myopathy, *Exp. Mol. Pathol*., **38**: 380-388.

Sheik AS and Thiruvengadam D (2013). Effect of lycopene on oxidative stress induced during D-galactosamine/lipopolysaccharide-sensitized liver injury in rats. *Pharm. Biol*. **51**: 1592-1599.

Sinha AK (1972). Colorimetric assay of catalase. *Anal. Biochem*. **47**: 389-394.

Stadler K, Bonini MG, Dallas S, Jiang J, Radi R, Mason RP and Kadiiska MB (2008). Involvement of inducible nitric oxide synthase in hydroxyl radical-mediated lipid peroxidation in streptozotocin-induced diabetes. *Free. Radic. Biol. Med*. **45**: 866e74.

Su GL (2002). Lipopolysaccharides in liver injury: Molecular mechanisms of Kupffer cell activation. *Am. J. Physiol. Gastrointest. Liver. Physiol*. **283**: G256e65.

Sun Y, Wu Y, Wang Z, Chen J, Yang Y and Dong G (2020). Dandelion extract alleviated lipopolysaccharide-induced oxidative stress through the Nrf2 pathway in bovine mammary epithelial cells. *Toxins (Basel)*. **12**(8): 496.

Wang JB, Wang HT, Li LP, Yan YC, Wang W, Liu JY, Zhao YT, Gao WS and Zhang MX (2015). Development of a rat model of D-galactosamine/ lipopolysaccharide induced hepatorenal syndrome. *World. J. Gastroenterol*. **21**(34): 9927-9935.

Zhang S, Yang N, Ni S, Li W, Xu L, Dong P and Lu M (2014). Pretreatment of lipopolysaccharide (LPS) ameliorates D-GalN/LPS induced acute liver failure through TLR4 signaling pathway. *Int. J. Clin. Exp. Pathol*. **7**: 6626e34.

Zhang H, Jia R, Wang F, Qiu G, Qiao P, Xu X and Wu D (2018). Catalpol protects mice against Lipopolysaccharide/D-galactosamine-induced acute liver injury through inhibiting inflammatory and oxidative response. *Oncotarget*. **9**(3): 3887-3894.

Zhou Y, Park CM, Cho CW and Song YS (2008). Protective effect of pinitol against D-galactosamine-induced hepatotoxicity in rats fed on a high-fat diet. *Biosci. Biotechnol. Biochem*., **72**(7): 1657-1666.

Zelber-Sagi S, Ivancovsky-Wajcman D, Fliss-Isakov N, Hahn M, Webb M, Shibolet O, Kariv R and Tirosh O (2020). Serum malondialdehyde is associated with non-alcoholic fatty liver and related liver damage differentially in men and women. *Antioxidants (Basel)*. **9**(7): 578.

Zhao X, Wang H, Yang Y, Gou Y, Wang Z, Yang D and Li C (2021). Protective effects of silymarin against D-Gal/LPS-induced organ damage and inflammation in mice. *Drug Des Devel. Ther*., **15**: 1903-1914.