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Isoprenaline Induced Hepatic Alterations and Modulation by Hydroalcoholic Extract of *Juglans regia* hull in Wistar Rats

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Abstract

The present study aimed at determining isoprenaline (ISO) induced hepatic alterations and effect of hydroalcoholic extract of *Juglans regia* hull supplementation in modulating those alterations in Wistar rats. Various biochemical, histopathological and antioxidant biomarkers in hepatic tissue viz. transferases (aspartate and alanine aminotransferases), Total Plasma Proteins (TPP), Albumins (ALB), Total Antioxidant Status (TAS), Total Thiols (TTH), Catalase (CAT), Superoxide Dismutase (SOD), Acetylcholinesterase (AChE), Arylesterase (AE), Glutathione Peroxidase (GP_x) and Glutathione Reductase (GR), and cellular damage indicators viz. Malondialdehyde (MDA) and Advanced Oxidation Protein Product (AOPP) were assessed. Intra-peritoneal administration of ISO produced significant increase in aspartate aminotransferase, AChE, MDA and AOPP levels and reduced TPP, ALB, TTH, TAS, AE and GP_x, activities in hepatic tissue of rats. Histopathologically, the liver sections of ISO treated rats revealed congestion, vacuolar degeneration and necrosis of liver parenchyma, bile duct hyperplasia and edema along with cellular infiltration in portal triad areas. Pretreatment with hull extract restored TAS, AOPP, AChE, CAT, AE activities and significantly reduced severity of histomorphological changes in hepatic tissue of ISO treated rats. The protective effect may be attributed to phytochemical constituents present in hull. Overall, our results show that hydroalcoholic extract of *J. regia* hull confers partial protection to hepatic tissue against ISO induced oxidative changes in Wistar rats.

Keywords: Hepatic Tissue, Isoprenaline, *Juglans regia,* Oxidative Damage

1. Introduction

Oxidative stress arises when excessive production of Reactive Oxygen and Nitrogen Species (ROS/RNS) cannot be taken care of by antioxidant capacity of the tissue. ROS/RNS damage lipids, proteins and genetic machinery causing cellular dysfunctions and even death. Liver is a versatile gland, with functions ranging from synthesis and metabolism of macromolecules such as fats, carbohydrates and proteins, to detoxification of exogenous and endogenous substances. It is the principal organ involved in biotransformation of xenobiotics by biochemical reactions involving oxidation, reduction or conjugation. Since these mechanisms generate large quantities of ROS/RNS, therefore liver is highly prone to oxidative injury. Excess ROS/RNS attack macromolecules

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(lipids, proteins and nucleic acid), leading to hepatic parenchymal degeneration, necrosis and inflammation[1,2] and ultimately derailing its physiological functions which cause direct or indirect damage to various other organ systems of body. Isoprenaline is non-selective β adrenergic receptor agonist used for the management of asthma, chronic bronchitis, mild and transient episodes of heart block in humans. ISO administration produces oxidative damage by increasing ROS/RNS genesis[3]. It can cause oxidative injury and pathological lesions in hepatic tissue[4-6].

Dietary supplementations of antioxidant counteract oxidative/nitrosative stress either by directly scavenging free radicals or by replenishing endogenous antioxidant apparatus. Antioxidants have been used for treatment or prevention of oxidative liver injury[7]. Natural phenolic compounds and flavonoids in plant based food sources can help replenish hepatic antioxidant stores and protect from oxidative hepatic damage[8,9]. A variety of naturally occurring antioxidants have shown promising results in treatment of oxidative stress induced hepatic abnormalities[10-12]. *Juglans*, the type genus of Family Juglandaceae, comprises of various species of deciduous trees which are widely distributed throughout the world. Its fruit is commonly called as walnut. The Persian or common walnut (*Juglans regia* L.) is the best-known member of genus *Juglans* which is found primarily in temperate areas and cultivated commercially throughout Southern Europe, Northern America, Eastern Asia, United States and Western South America[13,14]. *J. regia* traditional used since ancient times in traditional medicine for the treatment of various diseases like microbial infections, stomach ache, thyroid dysfunctions, cancer, heart diseases and sinusitis plant[15]. Its various forms like green walnuts and many of its components like shells, epicarps and leaves have been used in cosmetic and pharmaceutical industries[16,17].

Walnuts possess numerous medicinal properties and have been used traditionally for treatment of skin ailments, venous insufficiency, jaundice, parasitic diseases, bacterial infections, liver disorders and renal diseases[14,18-20]. *Juglans* consists of different phenolic compounds like gallic acid, tannins, quercetin, caffeic acid, coumarin, etc. which make it an inherently rich in antioxidant properties and capability to quench free radicals[21]. In the current study we evaluated hepatoprotective potential of hydroalcoholic extract of *J. regia* hull against isoprenaline induced hepatotoxicity in Wistar rats.

2. Material and Methods

2.1 Experimental Animals

The study was conducted on adult male Wistar rats (175-200 g, 12-14 weeks of age) procured from Indian Institute of Integrative Medicine (IIIM), Jammu, India. The animals were allowed to acclimatize for a week prior to the start of experiment. They were provided standard pelleted laboratory animal feed and water *ad libitum*. All the animals were maintained under standard managemental conditions (22 ± 3°C, 50-60% relative humidity and 12 h light-dark cycles). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) vide proposal no 7/IAEC-17/2017. Isoprenaline was used to induce toxicity. Hull of *J. regia* was evaluated for its potential in protecting liver from oxidative damage induced by ISO. All other chemicals used in the study were of analytical grade and purchased from different standard firms.

2.2 Collection and Preparation of Extract

The hull of the plant *J. regia* was collected from Kashmir region and was identified by Taxonomists, Department of Botany, University of Kashmir (EC voucher-specimen/CBT/20, dated 05/03/2020. After proper identification sufficient quantities of hull from fresh fruit were collected in polythene bags and transported to laboratory, where the hull was cleaned with moist cloth and air dried in shade with temperature not exceeding 40°C for 2-3 weeks prior to extraction process. Dried parts were pre-crushed and later pulverized into fine powder using an electric grinder. Powder was then subjected to hydro-alcoholic extraction. For this, powder was weighed and placed in thimble which was put in the flask of Soxhlet distillation apparatus and the extraction was done with hydroalcoholic (50:50) solvent. Extractions were done by maintaining hot plate temperature (70-80°C). The final drying was done in a rotatory evaporator. The dried extract was scrapped off and transferred to a glass container and stored in refrigerator under desiccation. The extract was freshly prepared in distilled water (60 mg/ml) every day prior to its administration by oral gavage in experimental

animals at the rate of 300 mg/kg. Dose of plant extract administration was based on the previous studies[15-17].

2.3 Experimental Design

The total duration of experiment lasted eight days. Thirty adult male Wistar rats were randomly allocated into five groups with six animals in each. Group I served as normal control, Group II animals were administered isoprenaline at the dose rate of 100 mg/kg b wt subcutaneously on 5th and 6th day, and Group III received hydroalcoholic extract continuously for 7 days. Group IV were given isoprenaline on 5th and 6th day (subcutaneously) along with plant extract, whereas Group V were given Quercetin (100 mg/kg) and isoprenaline (100 mg/kg). On eighth day, the animals were sacrificed by cervical dislocation and blood (2-3 ml) in heparin containing sterilized tubes and liver (1 gm) was collected in 10 ml ice-cold 0.5 M phosphate buffer (pH 7.4) for estimation of antioxidant parameters. Additionally, liver tissue was also collected in formal saline for histopathological study.

2.4 Plasma Biochemical Analysis

Activities of Alanine Amino Transaminase (ALT), Aspartate Amino Transaminase (AST), Total Plasma Proteins (TPP) and Albumin (ALB) of different groups were determined by standard kits (Transasia Bio-Medicals Ltd, HP, India) using Chemistry Analyzer (CHEM-7, ERBA, Mannheim).

2.5 Hepatic Oxidative Stress Analysis

Tissue homogenate (1%) was prepared by homogenizing hepatic tissue using Teflon coated homogenizer at 1000 rpm for 5-7 min at 4°C. The activity of Acetylcholinesterase (AChE) in hepatic tissue was expressed in nmol thiol group formed/min using reduced glutathione as standard curve[22]. Arylesterase (AE) activity was measured by using phenyl acetate (Sigma-Aldrich, USA) as a substrate[23]. Total Antioxidant Status (TAS) was determined spectrophotometrically by using 2, 2 etazinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS) according to the method previously described[24]. Total Thiols (TTH) were determined as per the method described[25] and expressed as millimolar (mM) using reduced glutathione as a standard. The enzymatic parameters viz. Catalase (CAT) and Glutathione Peroxidase (GP_x) were determined as per the standard methods[26,27]. The activities of Superoxide Dismutase (SOD) and Glutathione Reductase (GR) were determined as per the method described previously in hepatic tissue[28,29]. Similarly, Malondialdehyde (MDA) and Advance Oxidation Protein Product (AOPP) levels in hepatic tissue of different groups were determined using standard protocol and expressed in nmole of MDA formed/g/h and µM of Chloramine-T, respectively[30,31].

2.6 Histopathological Studies

Processing of liver for histopathological studies was carried out according to standard protocol. Formalin fixed livers from different groups were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H and E) and examined under microscope. Grading of lesions in hepatic sections was done as follows: no (-), mild (+), moderate (++) and severe (+++).

2.7 Statistical Analysis

The results were subjected to analysis of variance (ANOVA) in Completely Randomized Design (CRD) with statistical significance at $P<0.05$ being tested using the Duncan Multiple Range Test (SPSS 16.0).

3. Results

3.1 Activities of Plasma Aminotransferases

Levels of plasma transaminases in rats of various groups are presented in Figure 1. Significant ($P<0.05$) increase in activities of AST were observed after the sub-cutaneous administration of ISO in Wistar rats. Exclusive hull administration did not alter aminotransferase activities significantly in comparison to the control values. Co-administration of plant extract and ISO reduced activities of AST when compared to ISO only treated rats although the difference was non-significant. Quercetin on the other hand, could not reduce ISO-enhanced AST values.

3.2 Plasma Protein Profile

Significant decreases in total plasma proteins and albumin levels in ISO administered group were observed as compared to control group. But, after administration of plant extract along with ISO and quercetin along with ISO, a significant restoration of total plasma protein levels occurred. Also, even though albumin levels were raised

significantly by quercetin treatment to ISO administered groups, hull extract failed to revert the ISO-induced reduction in albumin level (Figure 1).

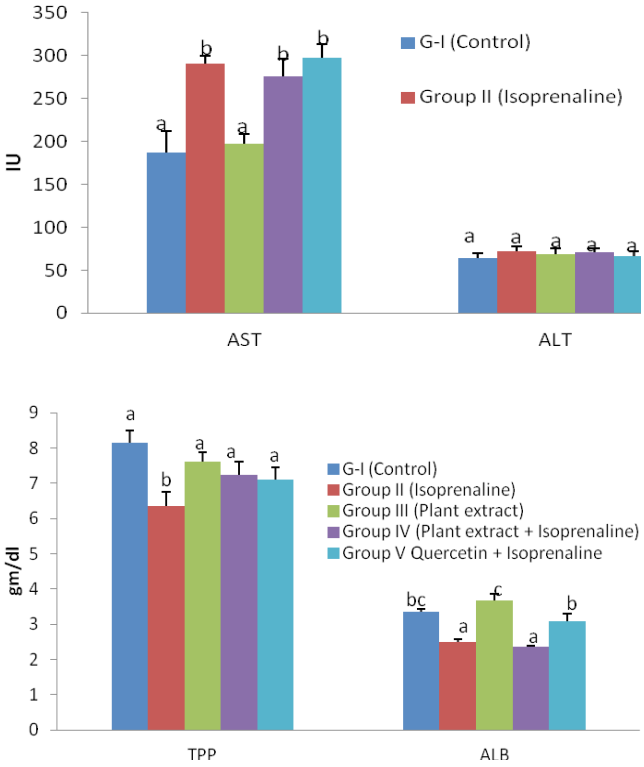


Figure 1. Alterations in the activities of plasma hepatic biomarkers *viz.* **(a)** Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), **(b)** Total Plasma Proteins (TPP) and Albumin (ALB) in different treated groups of rats. (Values having different superscripts (a, b, c) in a column are statistically different from one another at 5% level of significance)

3.3 Activities of SOD, GR and GPx in Hepatic Tissue

Variations in activities of SOD, GR and GP_x in different treatment groups are shown in Table 1. The activity of GP_X in hepatic tissue had significantly (P>0.05) decreased in ISO administered group as compared to control. These activities remained decreased (P>0.05) upon plant extract and ISO combined administration. But the GP_x activity was restored with quercetin in ISO treated group. Only, non-significant alterations in activities of SOD and GR in liver tissue were observed among rats in all groups.

3.4 Activities of AChE, AE and CAT in Hepatic Tissue

Levels of AChE, AE and CAT in livers of rats in different groups are presented in Table 2. The activities of AChE were elevated significantly (P<0.05) in ISO administered group when compared to control. However, pretreatment with hull extract significantly diminished the increments in activities of AChE in ISO administered rats. Similarly, after subcutaneous administration of ISO, a significant (P<0.05) reduction in mean AE level was observed in comparison to control animals and treatment with hull extract significantly increased activities of AE in hepatic tissue of ISO administered rats. Quercetin was also effective in restoring both AChE and AE levels in ISO administered rats. As far as values of CAT were concerned, ISO treatment didn't bring any significant change in their levels as compared to control rats.

Table 1. Levels of antioxidant biomarkers in hepatic tissue of rats in various groups

Group/Treatment	SOD	GR	GP_X
Control (1 ml/day/rat)	438.75^{a}±126.34	28.54^{a}±4.48	188.69^{d}±5.84
Isoprenaline (100 mg/kg)	337.35^{a}±98.94	26.33^{a}±2.21	153.16^{c}±9.63
Plant extract (300 mg/kg)	364.34^{a}±128.03	36.38^{a}±5.79	84.10^{b}±3.69
Plant extract (300 mg/kg) + Isoprenaline (100 mg/kg)	626.45^{a}±198.12	39.28^{a}±7.00	24.57^{a}±6.64
Quercetin (100 mg/kg + Isoprenaline (100 mg/kg)	339.60^{a}±209.06	311.56^{a}±2.99	219.14^{e}±10.76

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscripts (a, b, c) in a column are statistically different from one another at 5 % level of significance.
Values of SOD (Superoxide dismutase) expressed in Unit/ g of tissue.
Values of GP_x (glutathione peroxidase) are expressed in Unit/ g of tissue.
Values of GR (glutathione reductase) are expressed nmol of NADPH / min.

Table 2. Activities of Acetylcholinesterase (AChE), Arylesterase (AE) and Catalase (CAT) in hepatic tissue of rats in various groups

Group/Treatment	AChE	AE	CAT
Control (1 ml/day/rat)	$9544.1^{b}\pm609.81$	$17.32^{b}\pm3.74$	$5663.82^{ab}\pm868.30$
Isoprenaline (100 mg/kg)	$11615.9^{c}\pm442.88$	$4.87^{a}\pm0.77$	$8784.72^{b}\pm821.09$
Plant extract (300 mg/kg)	$7995.1^{ab}\pm743.39$	$16.31^{b}\pm2.21$	$4975.01^{a}\pm541.96$
Plant extract (300 mg/kg) + Isoprenaline (100 mg/kg)	$7739.0^{ab}\pm583.99$	$10.83^{b}\pm2.83$	$3391.79^{a}\pm250.30$
Quercetin (100 mg/kg + Isoprenaline (100 mg/kg)	$6665.0^{a}\pm696.54$	$19.54^{b}\pm5.11$	$6730.98^{ab}\pm430.91$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscripts (a, b, c) in a column are statistically different from one another at 5% level of significance.
Activity of AChE are expressed in nmole of thiol group produced/min/mg of tissue.
Activity of arylesterase (AE) is expressed in U/ml.
Activity of CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/g of tissue.

3.5 Levels of TAS and TTH in Hepatic Tissue

Following subcutaneous administration of ISO, significantly ($P<0.05$) decreased levels of TAS and TTH in hepatic tissue were observed as compared to control group (Table 3). The level of TAS was restored with hull extract treatment but levels of TTH remained significantly lower even after hull extract treatment as compared to control. However, significantly ($P<0.05$) increased levels of both TAS and TTH were observed in rats given quercetin along with isoprenaline as compared to ISO alone treated animals.

3.6 Cellular Damage Indicators in Hepatic Tissue

Values of MDA and AOPP in hepatic tissue of rats in various groups are presented in Table 3. MDA and AOPP levels in hepatic tissue were significantly ($P<0.05$) increased following subcutaneous administration with ISO as compared to control. A significant decrease was observed in AOPP levels following quercetin or plant extract administration in ISO injected rats. Increased MDA level was not restored in ISO administered rats after hull extract administration but quercetin reduced the ISO-altered levels of MDA significantly.

3.7 Histopathological Alterations in Hepatic Tissue

Lesion scores of hepatic tissue of rats from different treatment groups are presented in Table 4. Histopathological examination of liver in control rats revealed presence of normal hepatic parenchyma where polygonal hepatocytes containing a centrally located nucleus were arranged in cords. Adjacent hepatic cords were separated by sinusoids lined with endothelial cells (Figure 2a). Each portal triad contained a bile duct, a hepatic

Table 3. Levels of different antioxidant status and hepatic damage indicators in animals of various groups

Group/Treatment	TAS	TTH	AOPP	MDA
Control (1 ml/day/rat)	$21.09^{b}\pm1.41$	$3.68^{bc}\pm0.35$	$1.71^{a}\pm0.12$	$24.28^{a}\pm2.87$
Isoprenaline (100 mg/kg)	$14.05^{c}\pm0.67$	$2.05^{a}\pm0.75$	$3.83^{c}\pm0.17$	$153.40^{bc}\pm13.21$
Plant extract (300 mg/kg)	$24.53^{b}\pm0.99$	$4.19^{c}\pm0.69$	$2.38^{ab}\pm0.29$	$106.38^{bc}\pm21.20$
Plant extract (300 mg/kg) + Isoprenaline (100 mg/kg)	$23.01^{b}\pm0.77$	$1.92^{a}\pm0.10$	$1.90^{a}\pm0.14$	$187.46^{c}\pm38.81$
Quercetin (100 mg/kg + Isoprenaline (100 mg/kg)	$28.81^{a}\pm2.09$	$2.33^{b}\pm0.56$	$1.66^{a}\pm0.27$	$56.50^{a}\pm4.97$

Values are given as mean ± SE of 6 animals unless otherwise stated.
Values having different superscripts (a, b, c) in a column are statistically different from one another at 5 % level of significance.
Values of TAS (total antioxidant status) are expressed in mM.
Values of TTH (total thiols) are expressed in µM.
Values of AOPP (advanced oxidation protein product) are expressed in µM of Chloramine-T.
Values of MDA (malondialdehyde) are expressed in nmol MDA produced g of tissue/hr.

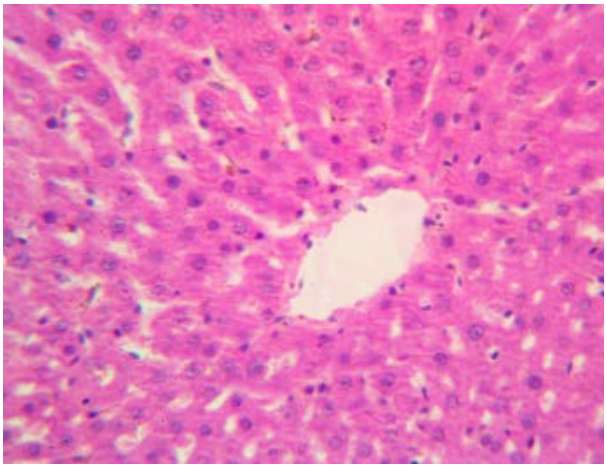
artery, hepatic vein and a lymphatic vessel. In Group 2 rats, severe pathological alterations were noticed. The hepatocytes revealed widespread vacuolar degeneration and necrosis. Necrotic hepatocytes had deeply eosinophilic cytoplasm and pyknotic or karryorrhectic nuclei. Vessel walls were often degenerated resulting in perivascular edema, haemorrhage and necrosis of hepatocytes (Figure 2b[1]). These areas frequently got infiltrated by inflammatory cells which were mostly lymphocytes and macrophages. The portal triads of ISO injected rats also revealed striking bile duct hyperplasia, perivascular edema, presence of brown hemosiderin pigment and infiltration by an admixture of lymphocytes, macrophages and fewer neutrophils. Inflammation from portal triad areas extended to engulf periportal hepatocytes at many sites (Figure 2b[2]). Group 3 rats had no noticeable histopathological alterations in their livers (Figure 2c). Group 4 livers revealed presence of only mild congestion, haemorrhage and edema. Hull extract attenuated the severity of hepatic injury due to ISO administration. Vacuolar degeneration along with mild necrosis and rarely foci of inflammatory cells however could also be appreciated (Figure 2d). Livers of group 5 animals only showed congestion and hepatocellular degeneration (Figure 2e).

4. Discussion

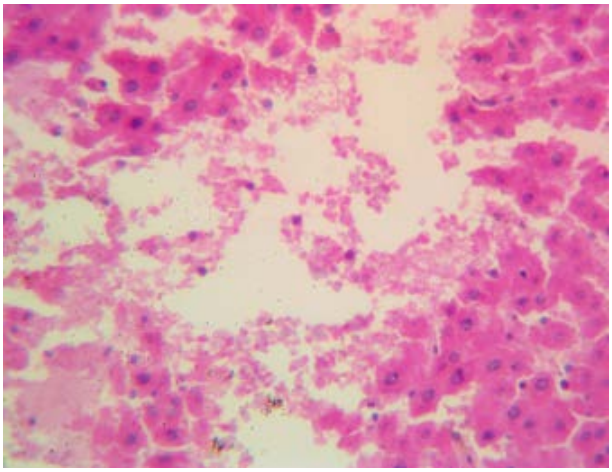
The crucial involvement of liver in metabolism, detoxification of xenobiotics and purification of blood received from gut along with phagocytic activity of resident Kupffer cells, continuously exposes hepatic tissue to a plethora of toxins and stressful oxidant

Table 4. Scores of histopathological lesion hepatic tissue of rats in different treatment groups

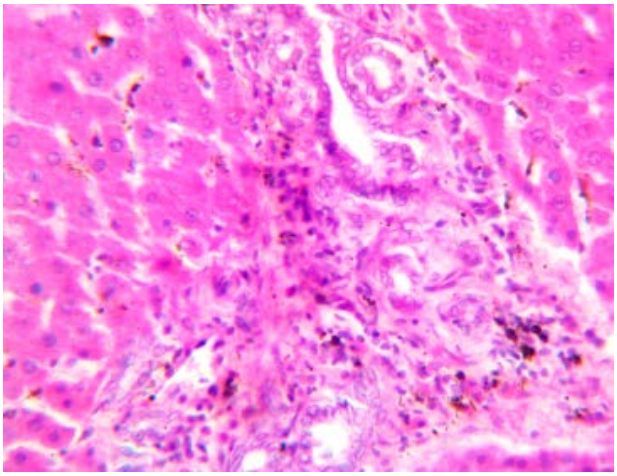
Lesion	Group I	Group II	Group III	Group IV	Group V
Congestion	-	++	-	+	+
Haemorrhage	-	++	-	+	+
Edema	-	++	-	+	+
Hepatocellular degeneration		+++		++	+
Hepatic necrosis	-	++	-	+	-
Inflammation	-	++	-	+	-



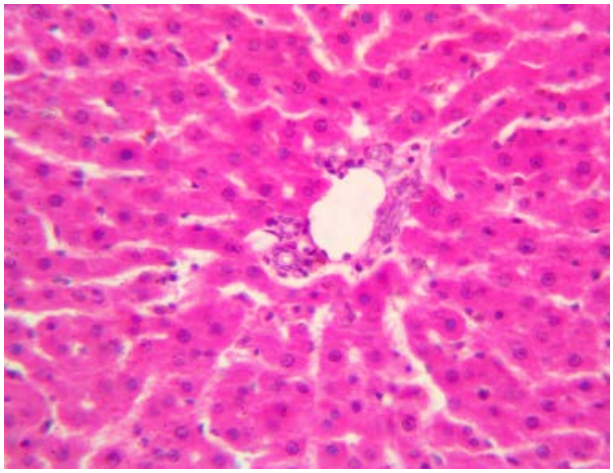
(a)



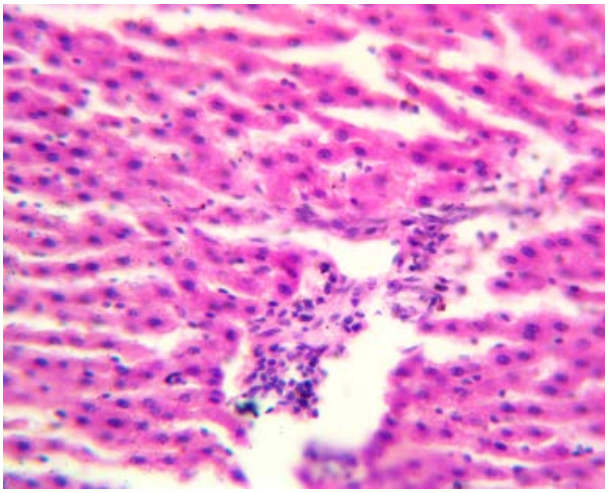
(b_1)



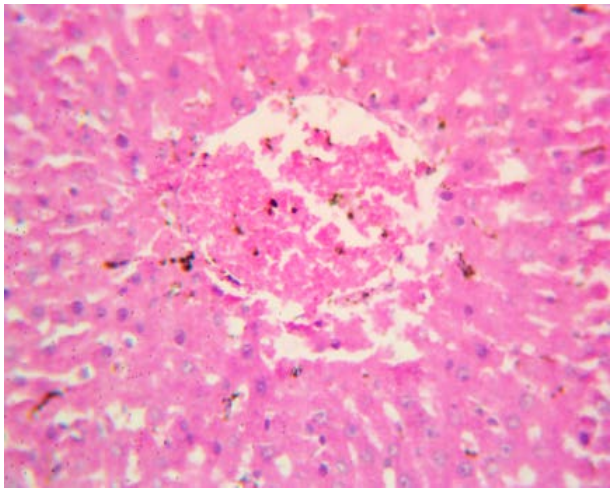
(b_2)



(c)



(d)



(e)

Figure 2. Histopathological alteration in hepatic tissue **(a)** normal histologic picture of liver with hepatocytes arranged in cords radiating from central vein **(b_1)** Edema, haemorrhage and necrosis of hepatocytes and **(b_2)** proliferation of bile ducts, edema, hemosiderin pigment, and infiltration in a portal triad in group 2, **(c)** group 3 Normal hepatocytes around a portal triad (arrow), **(d)** Mild vacuolar degeneration with occasional loss of hepatocytes and focal area infiltration by inflammatory cells (arrow) in group 4 and **(e)** Congested central vein and degeneration of surrounding hepatocytes in group 5 (H and E X400).

environment. Exposure of liver to extraneous toxins, endogenous metabolites, infectious agents including viruses, fungi or bacteria, environmental pollutants and chemotherapeutic agents disturbs homeostasis and increases the likelihood of development of liver diseases[32]. Use of medicinal plants for prevention and therapy of liver ailments has been rewarding in many studies[33-34]. Since plant derived dietary supplements are a rich source of phytochemicals, they provide protective cover to liver against oxidative damage induced by a wide variety of toxicants. For instance, carnosic acid bolstered cellular antioxidant defence system and provide protection against lipopolysaccharide-induce hepatic injury in rats[35]. Recent decades, the tendency towards using natural products rather than synthetic materials has increased. Walnut is rich in polyunsaturated fatty acids especially omega-3 and its regular consumption can increase good cholesterol in hyperlipidemic patients[36]. In addition, walnut oil is

a modulator of hepatic steatosis[37]. Moreover, different parts of *J. regia* have been used in traditional medicine to cure or prevent liver ailments since antiquity. Many recent studies have also demonstrated hepatoprotective potential of walnut and its leaves[14,38,39].

The present study assessed the ameliorative capacity of walnut hull extract against isoprenaline induced hepatotoxicity. Quercetin is one of the most common plant flavonoid endowed with rich antioxidant potential. It has been shown to be protective against ISO toxicity in rats. [40] In our study we used quercetin as a positive control[8,40] to compare the antioxidant capabilities of *J. regia* hull extract. Subcutaneous administration of ISO resulted in an increase in levels of ALT and AST in our study. These levels were lowered by administration of hull extract. Many other studies have shown that ISO administration results in elevation of hepatic transaminases. The elevated ALT and AST values indicate their leakage after hepatocellular damage. Quercetin could only reduce ALT levels but not AST levels. Similar results were also found that ALT and AST in plasma were increased after repeated CCl_4 administration and co-treatment with ethanolic extract attenuated these levels. *Tribulus terrestris* also protected liver from isoprenaline-induced damage as manifested by reversal of changes in ISO-induced hepatic aminotransferase contents. [41] Also, in another study, *Coptidis* rhizome extract caused decrease in ALT and AST contents and thus protected against carbon tetrachloride induced hepatotoxicity in rats[42]. Reduced TPP and ALB concentrations after ISO administration in our study, suggest impaired protein synthesis by liver. Similarly, changes in protein synthesis after liver toxicities have been also documented[14,43]. Hull extract could restore total protein levels but not the levels of albumin. But quercetin could restore both total protein as well as albumin levels. Our results are also in concurrence with the findings of other study[44] who reported that acetaminophen-induced hepatotoxicity in rats lead to a reduction in total protein level but co-treatment with *Vernonia amygdalina* negated this decrease of total protein levels.

Reduced levels of TTH and TAS reflect their increased utilization towards neutralization of excessive free radicals generated in response to increased cellular activities on chemical exposure. Total thiols (including proteins and non-protein thiols) play an important role in the amelioration of lipid peroxidation injuries inflicted by free radicals and this scavenging property is attributable to inherent sulfhydryl linkages (-SH groups) in their structures. Previous works have illustrated that ISO administration impedes hepatic antioxidant capacity in rats[6]. A significant reduction was observed in mean TTH levels after isoprenaline was administered but extract failed to normalize TTH levels in rats treated with ISO. Quercetin effectively counteracted the adverse effects of ISO on TTH levels. On the other hand, reduced TAS levels after isoprenaline treatment were resurrected after treatment with hull extract. Similar trend was observed for quercetin in attenuating ISO-induced changes in hepatic TSA content.

Hepatic cells possess a rich repertoire of enzymatic defenses against active free radicals. SOD and CAT play pivotal roles in protection against reactive oxygen species. SOD, the first line of defense against superoxide radicals generated in the mitochondria and endoplasmic reticulum during cellular respiration and metabolism, converts O_2^- and other superoxide radicals to H_2O_2 [8,14]. SOD levels in our study remained unaltered across all groups. Likewise CAT levels were not impaired after ISO treatment in rats. In contrast to our findings, carbon tetrachloride toxicity depleted SOD levels which restored after Coptidis rhizome extract administration in rats[42].

Acetylcholinesterase is an enzyme that mediates breakdown of neurotransmitter, acetylcholine. Alteration in AChE levels may cause disruptions in neurotransmission. Increase in AChE protein expression may occur in human livers affected with cirrhosis. [45] Subcutaneous administration of isoprenaline leads to a significant increase in mean AChE levels. But use of hull extract significantly reverted back these ISO-induced changes in AChE levels. Similar results were also seen after quercetin treatment. Arylesterase is a detoxification enzyme for hydrolysis of aryl esters. Activity of AE is a good indicator of liver protein synthesizing capability[46]. Any reduction in AR activity is reflected in a concomitant reduction in levels of total proteins. ISO inflicted a significant declined in AE activities in rat livers. This decrease in AE levels also correlated well with decreased total protein levels in the present study. Studies have shown that reduced AR levels could be associated with pathogenesis of chronic hepatitis. Studies have shown that reduced AR levels could be associated with pathogenesis of chronic hepatitis and can serve as useful markers for studying its progression[47,48]. Treatment with hull extract raised AE levels in ISO treated rats. Likewise, quercetin treatment also caused restoration of AE values in ISO treated rats. The activity of glutathione containing

enzymes-GP_X in hepatic tissue fell significantly after ISO administration. Hull extract could not replenish GP_x content while quercetin was able to significantly increase, the levels depleted after ISO treatment. Similar findings were also observed during other hepatotoxicity studies[8,14]. Values of glutathione containing enzyme-GR did no not manifest any significant changes among different treatment groups in this study.

Oxidation of Polyunsaturated Fatty Acids (PUFAs) associated with plasma membrane results in the formation of malondialdehyde, a Thiobarbituric Acid Reactive Substance (TBARS), which is the indicator of lipid peroxidation. Lipid peroxidation, which was found to be an important underlying mechanism for inducing cytotoxicity after ISO treatment in our study, as was illustrated by raised MDA levels, is also a common pathway and route to developing many different kinds of hepatic ailments. Significantly decreased levels were observed only after quercetin treatment but not after hull extract treatment in ISO administered groups. Similar findings with regards to MDA values were also observed during drug hepatotoxicity in Wistar rats[8,14]. Walnut extract was found to protect against carbon tetrachloride induced toxicity and reduced MDA levels in brain, kidney and liver tissue of Wistar rats[38]. In the same vein, thymoquinone also reduced MDA levels and was thus shown to possess protective effects against cholestatic oxidative stress and hepatic damage after biliary obstruction in rats[49]. AOPP are unique markers of oxidative stress used to assess oxidation of protein damage[31]. In our study, ISO treatment raised AOPP levels significantly. These elevations in AOPP levels were however counter balanced in rats pretreated with either hull extract or quercetin.

Isoprenaline induced severe pathological alterations in liver parenchyma of rats which included severe congestion, vacuolar degeneration, necrosis of hepatocytes and focal areas of infiltration. Additionally, portal triads had hyper-plastic bile ducts and were expanded by presence of inflammatory cells. ISO treatment induced of microscopic alterations such as congestion, degeneration and infiltration in liver parenchyma and portal triad areas in rat livers in studies conducted by other researchers also[6], which is in concurrence with the results of the present work. Others have also similarly reported that ISO administration caused fatty degeneration, dystrophic calcification and parenchymal necrosis in hepatocytes of chicken embryos[50]. High dose ISO treatment in rats also induced ultra-structural changes in hepatocytes[4]. Studies have also suggested that increased hepatic β adrenergic signaling is associated with hepatic steatosis[5].

The hull extract conferred significant degree of protection to the liver tissue and reduced severity of ISO-induced histopathological alterations in this study as revealed by presence of only mild necrosis and degeneration in liver parenchyma. The leaf extract of *J. regia* also reduced CCl_4-induced hepatocellular damage,[14] which is in agreement with our findings. Furthermore, leaf extract of *J. regia* when given along with *Nasturtum officinalis* could attenuate CCl_4 induced renal and hepatic toxicity[39]. ISO induced histopathological lesions in rat livers were ameliorated by betaine treatment[6], which is also in concordance with results. Likewise, *Tribulus terrestris* also protected hepatic architecture from oxidative damage in rats exposed to ISO toxicity[41]. Even though extract of *J. regia* hull significantly protected liver from ISO-induced hepatocellular damage, quercetin in comparison however, was found to be more effective in restoring histomorphology of liver which was severely damaged after ISO-induced oxidative stress in rats. Hull of *J. regia* is a byproduct after harvesting walnut fruit. The underlying antioxidant properties of this hitherto discarded waste product as exhibited by our findings should be further explored to tap its potential as a cheap ingredient in hepatoprotective antioxidant formulations.

In conclusion, ISO induced significant alterations in hepatic antioxidant parameters and hepatic histomorphology of rats. *J. regia* hull extract conferred a partial protection against ISO-induced toxic damages in liver parenchyma.

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6. Conflict of Interests

The author(s) declare(s) that they have no competing interests.

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