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Effect of *Juglans regia* hull extract on isoprenaline induced testicular toxicity in Wistar rats

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Abstract
The current study evaluated the potency of hydro-alcoholic *Juglans regia* hull extract against oxidative damage to testes in Wistar rats generated after isoprenaline (ISO) treatment. Thirty apparently healthy rats were divided into five groups with each group containing six animals. Various enzymatic and non-enzymatic parameters in testicular tissue like total antioxidant status (TAS), catalase (CAT), total thiols (TTH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), acetylcholinesterase (AChE), arylesterase (AE), malondialdehyde (MDA), advanced oxidation protein product (AOPP) were estimated. Impact of various treatments on sperm health related parameters and testicular histology was also evaluated. Subcutaneous administrations of ISO in rats caused significant rise in CAT, MDA, GPx, AOPP levels and fall of TTH, TAS, GR, AChE, AE levels in rat testicular tissue. Sperm abnormalities, declined sperm concentration along with histopathological changes in testicular tissue were also recorded in ISO treated rats. Pre-exposure with *J. regia* extract caused a significant reduction in ISO induced oxidative injury in testicular tissue as manifested by improvements in altered oxidative stress parameters and histopathological lesion scores. Observations of the present study revealed that extract prepared from hull of *J. regia* bestows a partial defense to testicles of rats against oxidative changes generated after treatment with ISO.

Keywords: *Juglans regia*, Wistar rats, testicular toxicity

Introduction
Isoprenaline (ISO), a non-selective β-adrenergic receptor agonist, is used for treating ailments like chronic bronchitis, asthma and heart block in man ISO administration produces stress induced hypogonadism [1-3] by increasing reactive oxygen and nitrogen species (ROS/RNS) genesis [4]. These reactive species have been found to be one of the most prominent causes of infertility in male animals and humans. In spite of presence of the antioxidant activity in seminal plasma, spermatozoa as well as epididymis, oxidative insult mediated impairment of sperm functions and DNA integrity frequently occurs. Since, testicular tissue has limited capability to repair DNA and is plentiful in poly unsaturated fatty acids (PUFA), so they are extremely prone to ROS spurred oxidative injury [5]. Exposure to external or internal oxidants leads to dose dependent increment in lipid peroxidation and ROS levels in testes as well as in epididymal spermatozoa [6, 7].

Plants are a rich source of phytochemicals which have diverse pharmacological properties. Walnut (*Juglans regia* L; family Juglandaceae) has been cultivated since ancient times not only due to its precious fruits but also wood. Its seed, which is basically a nut, is highly valued for its nutritional, health and sensory attributes [8]. Its kernel is enriched with unsaturated fatty acids, tocopherols and phytosterols [9]. The active ingredients present in the walnut may vary depending upon environmental conditions and cultivars [10-11]. Presence of anti-carcinogenic, ant-mutagenic, antioxidant and cardioprotective [12] activities in walnut has been validated through numerous previous experimental studies. Researchers have also shown occurrence of a high concentration of natural antioxidants in walnut which may be responsible for conferring protection against cancer, cardiovascular diseases and other disorders [13-16]. Hull, present as the outermost covering of walnut fruit, is discarded at the time of initial fruit collection. Quercetin, a most common naturally occurring polyphenolic compound is present in high concentrations in fruits, nuts having high antioxidant potential. Therefore, we designed our study to evaluate the efficacy of extract made out of *J. regia* hull against oxidative injury to testicular tissue of Wistar rats incurred after giving ISO.

Materials and methods

Extract Preparation: Hulls from *J. regia* were collected from Kashmir (India) and were identified by taxonomists working for University of Kashmir (EC voucher-specimen/CBT/20 dated 03/05/2020). The fresh green hull was packed in polythene bags in sufficient quantities and then taken to laboratory. The hull was properly cleaned, dried and crumbled into fine powder using electric grinder. Powdered part was subjected to the process of hydro-alcoholic extraction in Soxhlet's distillation apparatus at 70-80 °C. The extract was dried in rotatory evaporator (5-6 rpm, 55 °C). The dried product was collected and stored in fridge until use. The extract used for oral administration in the study was preparedfresh.

Chemicals and Reagents: Isoprenaline, 2,2′-azinobis (3-ethylbenzothiazoline 6- sulphonate) (ABTS), phenyl acetate, Chloramine-T supplied by Sigma-Aldrich. Other chemicals used for the current research work were procured from reputable firms.

Experimental Animals: For the purpose of this study, 30, 12-14 week old healthy male Wistar rats (160-180gm) were used. The use of rats in the current study received approval from Institutional Animal Ethics Committee of the authors' institution (10/IAEC-17/2019). The animals were given one week acclimatization to the laboratory conditions along with standard feed and tap water *ad-libitum*.

Experimental Design: The experiment lasted for eight days. Animals were equally allocated to five groups. Control rats (Group I) were given distilled water (1 ml $day^{-1}rat^{-1}$, oral gavage), animals in group II were treated with 100 $mgkg^{-1}$ b wt ISO by subcutaneous route on day 5 and day 6, rats of group III were administered hydro-alcoholic hull extract (300 mg kg^{-1}) via oral gavage continuously for seven days. Group IV animals were given plant extract at same dose rate for seven consecutive days along with ISO subcutaneously on 5^{th} and 6^{th} day whereas, animals in group V received quercetin (100 mg kg^{-1}, PO), for 7 days with ISO administering on day 5^{th} and $6^{th}$of quercetin treatment. After 24 h of administration of last dose of ISO, were euthanized by dislocation of at occipital joint. 1g testes added to 10 ml cold phosphate buffer saline (pH 7.4) for estimation of antioxidant enzymes. The Teflon coated homogenizer was used to prepare 1% testicular tissue homogenate at 1000 rpm for 5-7 min at 4 °C.

Estimation of concentration and motility of sperms: Sperm motility was determined by diluting a small drop of semen with 0.5ml phosphate buffer saline (pH as 7.4) on a pre-warmed glass slide. Percent motile spermatozoa were determined by counting motile and immotile sperms under five random fields. The sperm count was estimated by Neubauer's counting chamber and phase-contrast microscopy. Eosin diluting fluid and RBC diluting pipette was used for dilution and results were expressed as million ml^{-1}.

Viability and abnormality test: The viability of sperm was assessed by Eosin-Nigrosin staining [18].

Hypo osmotic swelling test (HOST): The test was done as per the previously described technique [19]. An aliquot of 0.1ml sperm suspension from cauda epididymis was incubated with 1.0ml hypo-osmotic solution after thorough mixing (37 °C for one hour). Two hundred sperms per sample were counted in different fields under phase microscope at x400 and the number of HOST positive sperms having coiled tails was estimated.

Determination of parameters in testes: The AChE activity was expressed using reduced glutathione as standard curve in nmol thiols group formed min^{-1} [20]. Activity of AE (U ml^{-1}) [21], Total antioxidant status (TAS) [22], Total thiols (TTH) level (µM) [23], catalase (CAT) [24] and glutathione peroxidase (GPx) [25] were assessed. Similarly, superoxide dismutase (SOD), glutathione reductase (GR), malondialdehyde (MDA) and advanced oxidation protein product (AOPP) in testicular tissue of all groups were assessed as per methods described previously [26, 27, 28, 29].

Histopathological studies: Testes were collected in 10% formalin, sectioned, stained with hematoxylin and eosin (H&E) as per the standard method. The observed histopathological lesions were scored [no lesions (-), mild lesions (+), moderate lesions (++) and severe lesions (+++)].

Statistical analysis: Data were analyzed for variance at 5% level of significance ($P< 0.05$) by Duncan Multiple Range test (SPSS 21.0).

Results

Sperm parameters: The sperm viability, motility, abnormality and HOST numbers were significantly reduced in all groups of rats in comparison to rats of control group. Although supplementation of quercetin in ISO administered rats led to higher viability as well as motility of sperms and HOST values as compared to ISO alone treated group, the corresponding values in group administered plant extract along with ISO were lower than those in ISO alone administered group but no significant variation was recorded in these levels among above three groups. Although total sperm abnormalities were significantly higher in ISO alone treated rats as compared to the respective levels in the control rats, ISO with either quercetin or hull extractcaused a significant reduction in the percentage of abnormal sperms as compared to ISO only treated rats (Table 1). Further, quercetin caused a significant higher improvement in sperm abnormalities as compared to plant extract in ISO poisoned rats.

Acetylcholinesterase (AChE) and Arylesterase (AE): ISO alone caused a significant decline in mean AChE and AE activities in testicular tissue of group II rats (Table 2). However, a significant enhancement was observed in activities of AChE and AE after giving ISO along with extract supplement as compared to ISO alone administration and no significant difference was observed between values of former group as compared to the levels in control group. Supplementation with quercetin although restored AE values but failed to bring about any improvement in AChE levels.

Catalase (CAT) and Superoxide dismutase (SOD): CAT activity was significantly higher in rats treated only with ISO as compared to control animals. Pre-treatment with plant extract but not quercetin restored the activity of CAT in ISO treated animals (Table 2). Although SOD activity declined in animals after subcutaneous administration of ISO, but no significant alteration occurred in SOD activity among any of the treatment groups (Table 3).

Glutathione peroxidase (GP_X) and glutathione reductase (GR): The activities of GP_X and GR in testicular tissue were significantly elevated and declined respectively in ISO treated group when compared to corresponding control group levels. The repeated oral administration of plant extract restored the ISO induced elevations in GPx and decline in GR activities in testicular tissue of rats (Table 3) in contrast quercetin could only restore altered GR but not GPX.

Total antioxidant status (TAS) and total thiols (TTH): ISO caused a significant decrease in the levels of TAS and TTH in testicular tissue. On the contrary, in groups given either quercetin or plant extract in conjunction with ISO, a significant rise was recorded in TAS as well as TTH levels when compared with respective levels after ISO only administration (Table 4). Moreover, plant extract caused higher improvement in TTH and TAS levels as compared to the quercetin

Cellular Damage Indicators: Tissue levels of MDA and AOPP were significantly higher in ISO treated rats as compared to control, however, treatment with plant extract or quercetin significantly reduced the ISO raised MDA and AOPP levels Although the values of MDA in co-treated groups remained significantly higher than that of the control animals but AOPP levels did not significantly vary between the corresponding levels of control and co-treated groups.

Histopathological alterations in testes: Histopathological lesion scores of testes from rats in different experimental groups are presented in Table 5. Sections from control (Fig. 1a) rats showed normal testicular parenchyma consisting of seminiferous tubules having a central lumen rimmed by basement membrane. Seminiferous tubules contained normal populations of different germ cell maturation stages and leydig cells were present in the interstitial spaces. Similar picture was also seen in group III rats. ISO caused severe testicular pathology as evidenced by presence of interstitial edema, haemorrhage, congestion along with severe degeneration of sperm cells (Fig. 1b & c). In some sections sperm cells were replaced by pink fibrillar material due to necrosis of entire cell population of seminiferous tubules (Fig. 1d). In some animals severe necrosis of sperm cells and disruption of seminiferous tubular basement membrane was seen. In group IV animals, plant extract ameliorated damage caused by ISO administration to a significant extent and severe necrotic lesions as appreciated in group II animals were not seen. However, moderate degree of degeneration and mild degree of interstitial edema and congestion were still noticed (Fig.1e). Haemorrhage along with mild necrosis was also seen in some sections. Similarly, animals pre-treated with quercetin followed later by administration of ISO showed much less severity of degeneration, necrosis, haemorrhage or congestion (Fig.1f) as compared to the animals given ISO alone. Also, group treated with ISO and plant extract showed comparable degree of pathological alterations to that seen in group treated with ISO and quercetin.

Discussion

ISO is many times used for treating bradycardia, heart block or septic shock and rarely also used in cases of asthma in humans. Its treatment often is associated with various side effects such as tachycardia, palpitations and dizziness. ISO administration also has been shown to inflict extensive oxidative injury in different organ systems in body [30-31]. It's a well known fact that acute model of ISO toxicity simulates stress induced cardiomyopathy [32]. However, the effects of ISO administration on male fertility have not been elucidated till date. Collective experimental and clinical evidence corroborates that oxidant injury is an important underlying contributor to male infertility [33]. Toxic compounds disrupt electron transport in the mitochondria of mammalian sperm resulting in the excessive accumulation of free radicals. These liberated radicals in large quantities could have potentially undermined the inherent protective antioxidants in rat testicles leading to sperm damage, deformity in the present study [34-35]. Indeed the reduced percentage of motile sperms and sperm count in ISO treated animals observed in the present study are comparable with results of preceding studies wherein exposure to different chemical toxicants induced aberrations in seminal parameters [36-38].

Even under physiological conditions the spermatozoa are continuously exposed to hostile oxidizing environment as they undergo repeated cell divisions as a part of normal process of spermatogenesis leading to accelerated generation of free radicals, which under ordinary circumstances are scavenged by cellular antioxidant system [34-35]. But the exposure to any extraneous insult may derail the delicate balance between oxidant and antioxidant machinery and put the testicular tissue at an increased risk of undergoing oxidative injury as was seen to occur after ISO administration in our study. We observed reduced AE, AChE values in testes after ISO administration.

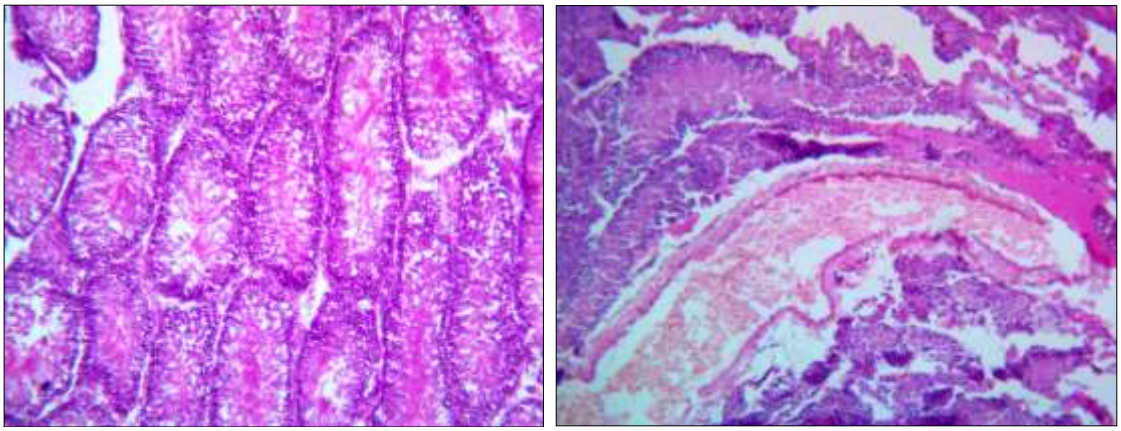
Testes being rich in polyunsaturated fatty acids (PUFA), are inherently prone to peroxidative injury due to generation of hydroxy and peroxy esters which are ordinarily metabolized by intra and extracellular esterases such as AE and AChE [39]. It can be inferred from the reduction seen in activities of AE and AChE after ISO administration in our study that a free radical driven damage of cellular macromolecules (membrane lipid and proteins), which was also reflected in enhanced values of AOPP and MDA, was presumably brought about by extensive oxidative damage caused by ISO. This lipid peroxidation and protein oxidation was seen to happen in conjunction with reduction in TTH and TAS in testicular tissue which points towards overwhelming of endogenous testicular antioxidant system in rats exposed with high dose of ISO. Since, TTH not only protects the cellular components from oxidative stress but is also needed for replenishment of SOD and GPx, therefore reduction of TTH levels probably triggered a cascade of reduction in activities of other antioxidant enzymes dependent on its availability for their activities [40]. Significant reduction in GR activity, which is responsible for the regeneration of reduced glutathione for maintaining the cellular redox status, as seen in our study also happened on exposure to many other toxicants in previous studies [41].

Usually reduction in levels of reduced glutathione tends to interfere with the activities of CAT, SOD and GPx because of its inherent role as a reducing agent [40]. The decreased levels of GPx, CAT and SOD were also recorded in the present study after ISO treatment. Overall, a significant oxidative stress unfolded upon ISO administration in testes likely induced oxidative damage of cellular membranes of spermatozoa thus further affecting their numbers and quality [41-42]. Similar testicular oxidative damage has been induced by agrochemicals and environmental contaminants in other experimental studies [42]. In the present study, very high doses of ISO were used to induce acute toxicity in testicles of rats which caused significant derailment of testicular oxidative

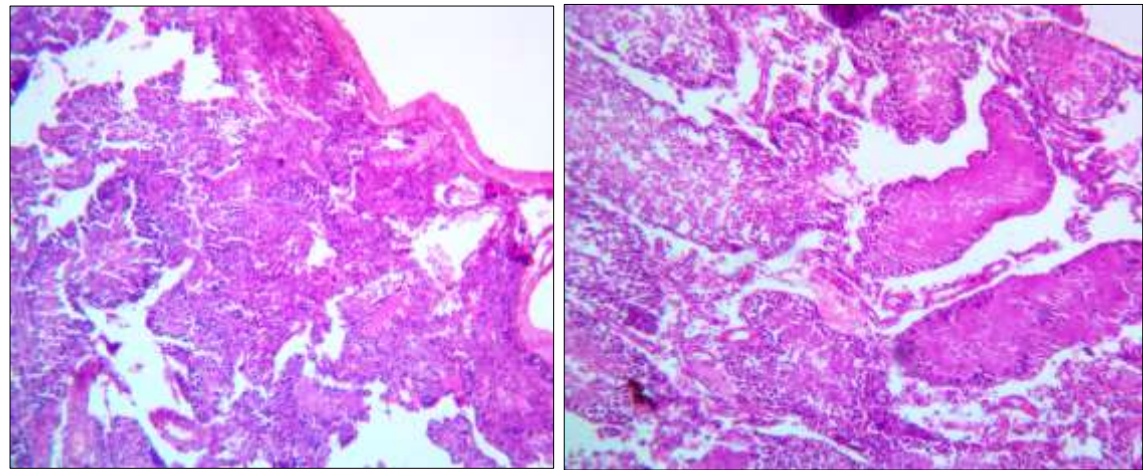
stress parameters, sperm health as well as count. Severe acute testicular toxicity was also induced after administration of melamine alone or along with cyanuric acid in mice [43].

The rise in mean percentage of morphologically abnormal sperms upon ISO treatment was found to be attenuated by quercetin [34]. On the contrary; administration of *J. regia* hull extract was not beneficial in restoring sperm count and sperm quality in ISO injected rats. *J. regia* hull extract administration however significantly ameliorated levels of CAT, GPx, GR, TAS, TTH, MDA and AOPP in rats that received ISO treatment. In-fact, plant extract conferred higher protection than quercetin in remediation of ISO induced oxidative stress in testes. Despite the persistence of sperm abnormalities during the course of our study, *J. regia* hull extract exhibited striking antioxidant potential as it countered ISO induced oxidative stress in testes to an appreciable extent. But keeping in view of the fact that in the present study the duration of *J. regia* administration was relatively short and very high doses of ISO was used to induce toxicity in our study, further work needs to be conducted in calibrating dose and duration of extract administration for having a corrective effect on sperm quality affected by oxidative damage to testes. Nonetheless, the findings of our study demonstrate valuable antioxidant properties in hull extract of walnut which is hitherto discarded as a by-product. Further studies need to be conducted to explore its potential as an antioxidative agent against different etiological agents including other toxicants inducing testicular damage.

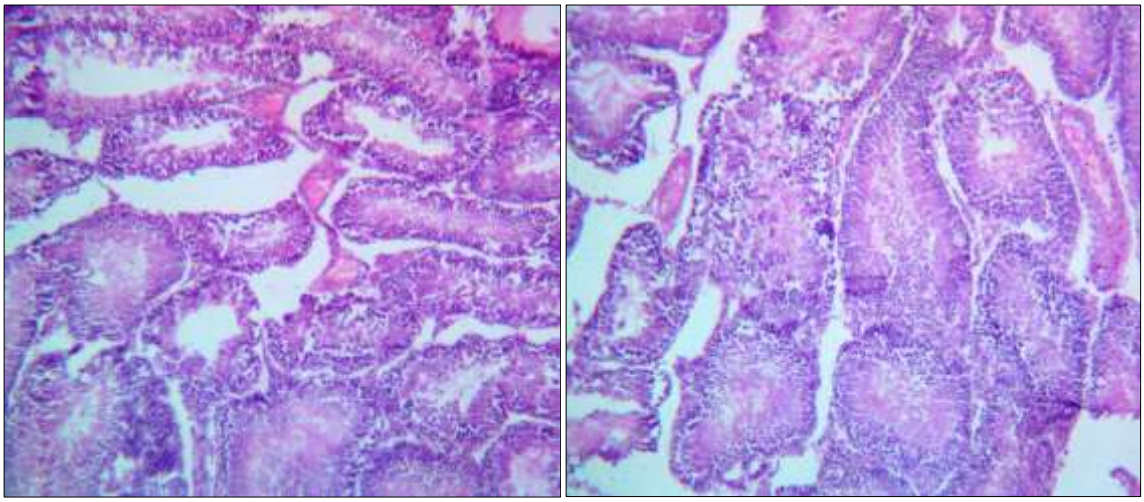
The ability of walnut extract to reduce oxidative damage in testicular tissue is probably owing to high phenolic content [44]. Polyphenolic compounds have high ROS scavenging capacity which may have imparted partial protection against ISO induced oxidative injury in testicular tissue as seen in the current work [13-14,45]. Apart from sperm abnormalities, patho-morphological alterations can also result from oxidative stress imposed on testes after exposure to a variety of causes such as environmental toxicants, chemotherapy, and ionizing radiation [46]. Depletion of antioxidants can reduce the ability of cells to withstand oxidative damage leading to cell death [47]. Oxidative stress can be gonado-toxic and chemical toxins that lower antioxidant capacity of the testis or increase lipid and protein peroxidation can inflict considerable damage to testicular tissue [48]. Our results also show that ISO induced considerable damage to testicular parenchyma as revealed by degeneration and necrosis of seminiferous tubules in addition to interstitial congestion and haemorrhage. Since testicular tissue is more susceptible to free radicals injuries, supplementation of antioxidants has been recommended to fight against oxidative stress [49]. Administration of compounds with antioxidant properties has been shown to circumvent the oxidative damage inflicted to testes by a wide variety of agents. For example, ghrelin, lemon fruit extract and curcumin were shown to protect against testicular histological alterations induced by cyclophosphamide [50-52]. Treatment with both quercetin and hull extract restored much of the histomorphology of testicles in ISO treated rats. Similarly, curcumin could confer protection to testis of mice from cadmium induced acute histopathological damage [13, 53]. Also, quercetin countered cadmium induced oxidative damage to testicular architecture in rats in a previous study [54]. Interestingly, hull extract was found to be equally effective as quercetin in offsetting ISO induced derangements in testicular histology in our study. Overall, the findings of this study bring forth the fact that acute ISO toxicity can unleash significant testicular oxidative damage, depreciation in sperm numbers and quality along with pathological changes in testicles. This can have implications for people undergoing ISO treatment for various reasons and therefore, its potential to cause infertility related problems must be taken into consideration. ISO induced alterations could be partially restored by pre-treatment with hull extract of *J. regia*. We recommend more studies be conducted in future to determine its potential as a dietary agent to offset deleterious effects of divergent chemicals capable of causing testicular damage.



(a) **(b)**



(c) **(d)**



(e) **(f)**

Fig 1: H&E stained section of testes indicating seminiferous tubules with germ cells in various stages of spermatogenesis in testes of group I rats (a); ISO treatment showed interstitial edema, haemorrhage, along with degeneration and necrosis of seminiferous tubules in testes (b), severe necrosis (c) and replacement by eosinophilic fibrillar mass in the seminiferous tubular lumen (d); mild interstitial congestion along with degeneration of seminiferous tubules in testes of group IV (e) and (f) rats (100X).

Table 1: Epididymal sperm parameters in Wistar rats of control and different treatment groups.

Treatment groups (n=6)	Live sperm (%)	Motile sperm (%)	Abnormal sperm (%)	HOST (%)
Control (1ml DW day^{-1}rat^{-1})	92.31^{b}±5.27	76.81^{b}±0.96	5.13^{a}±0.44	48.29^{b}±3.95
Isoprenaline (100 mg kg^{-1})	62.70^{a}±5.15	51.87^{a}±1.00	7.52^{c}±0.79	33.99^{a}±4.07
Plant extract (300 mg kg^{-1})	65.41^{a}±7.58	51.26^{a}±2.71	5.87^{ab}±0.45	31.64^{a}±5.40
Plant extract (300 mg kg^{-1}) + Isoprenaline (100 mg kg^{-1})	60.48^{a}±8.82	51.55^{a}±1.81	6.91^{b}±0.28	32.54^{a}±6.08
Quercetin (100 mg kg^{-1} + Isoprenaline (100 mg kg^{-1})	71.83^{a}±9.46	61.06^{a}±5.52	5.35^{a}±0.54	38.95^{a}±4.50

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

Table 2: Alterations in the activities of acetylcholinesterase (AChE), arylesterase (AE) and catalase (CAT) in testicular tissue of control and treated rats.

Group/Treatment	AChE	AE	CAT
Control (1ml DW day^{-1}rat^{-1})	13138.38^{b}±1603.90	5.66^{a}±1.21	1544.41^{a}±717.79
Isoprenaline (100 mg kg^{-1})	6902.88^{a}±336.62	3.93^{b}±0.26	2969.78^{b}±319.32
Plant extract (300 mg kg^{-1})	11201.38^{b}±1603.90	6.56^{a}±1.21	1642.11^{a}±512.23
Plant extract (300 mg kg^{-1}) + Isoprenaline (100 mg kg^{-1})	10138.38^{b}±1603.90	5.12^{a}±0.91	1574.41^{a}±723.21
Quercetin (100 mg kg^{-1} + Isoprenaline (100 mg kg^{-1})	3754.25^{c}±610.58	7.54^{a} ±2.36	2174.63^{b}±729.40

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 thiols group produced^{-1}min^{-1}mg of tissue^{-1}
Activity of arylesterase (AE) is expressed in U ml^{-1}
Activity of CAT (Catalase) are expressed in µmol H_2O_2 decomposed min^{-1}g of tissue^{-1}

Table 3: Superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) activities in testicular tissue of control and different treated rats.

Group/Treatment	SOD	GR	GPx
Control (1ml DW day^{-1}rat^{-1})	410.64^{a}±91.14	34.22^{a}±6.28	154.78^{a}±8.71
Isoprenaline (100 mg kg^{-1})	315.21^{a}±83.16	21.37^{b}±3.70	195.47^{b}±9.50
Plant extract (300 mg kg^{-1})	467.14^{a}±67.11	39.21^{a}±6.58	141.53^{a}±27.71
Plant extract (300 mg kg^{-1}) + Isoprenaline (100 mg kg^{-1})	510.64^{a}±91.14	31.21^{a}±5.21	143.20^{a}±27.71
Quercetin (100 mg kg^{-1} + Isoprenaline (100 mg kg^{-1})	521.44^{a}±81.97	22.03^{a}±2.38	235.11^{c}±3.86

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^{-1}
Values of GPx (glutathione peroxidase) are expressed in Unit g of tissue^{-1}
Values of GR (glutathione reductase) are expressed nmol of NADPH min^{-1}

Table 4: Antioxidant biomarkers and cellular damage indicators in testes of control and treatment groups.

Group/Treatment	TAS	TTH	AOPP	MDA
Control (1ml DW day^{-1}rat^{-1})	26.45^{b}±0.04	3.36^{b}±0.69	1.31^{a}±0.57	20.79^{a}±2.11
Isoprenaline (100 mg kg^{-1})	18.82^{a}±2.03	0.53^{a}±0.23	2.63^{b}±0.12	124.59^{b}±8.51
Plant extract (300 mg kg^{-1})	36.25^{b}±0.04	3.55^{b}±0.78	1.28^{a}±0.87	33.72^{a}±2.18
Plant extract (300 mg kg^{-1}) + Isoprenaline (100 mg kg^{-1})	31.40^{b}±0.04	3.45^{b}±0.79	1.34^{a}±0.56	39.29^{c}±2.23
Quercetin (100 mg kg^{-1} + Isoprenaline (100 mg kg^{-1})	25.53^{b}±0.24	2.78^{b}±0.46	1.39^{a}±0.44	49.09^{c}±14.91

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
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^{-1} hr^{-1}

Table 5: Scoring of histopathological lesions in testes of rats in control and treated groups.

Lesion	Group I	Group II	Group III	Group IV	Group V
Interstitial congestion	-	+++	-	+	+
Interstitial hemorrhage	-	+++	-	+	+
Seminiferous tubular degeneration	-	+++	-	++	++
Seminiferous tubular necrosis	-	+++	-	+	+

Conclusions

ISO administration inflicted significant damage to testicular tissue as was demonstrated by significant aberrations in spermatic parameters, alterations in oxidative stress indicators and histological changes. Even while only a partial restoration of epididymal sperm health parameters was recorded, a commendable restoration in levels of antioxidant biomarkers as well as cellular damage indicators and histopathological alterations was appreciated in rats given isoprenaline after *J. regia* hull extract pre-treatment. Therefore, hydro-alcoholic extract of *J. regia* hull can at least in part prevent oxidative stress related damage induced by isoprenaline administration in testicular tissue in Wistar rats.

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Conflict of Interests: The authors have no conflict of interest.

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