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Neuroprotective potential of hydroethanolic hull extract of *Juglans regia* L. on isoprenaline induced oxidative damage in brain of Wistar rats

Priyanka Sharma [a], Pawan K. Verma [a,*], Shilpa Sood [b], Nrip K. Pankaj [a], Sanjay Agarwal [c], Rajinder Raina [a]

[a] Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Science and Animal Husbandry, R S Pura, 181102, India
[b] Division of Veterinary Pathology, Faculty of Veterinary Science and Animal Husbandry, R S Pura, 181102, India
[c] Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Science and Animal Husbandry, R S Pura, 181102, India

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ABSTRACT

The study was aimed at assessing isoprenaline (ISO) induced oxidative damage in brain of Wistar rats and its protection by hydroethanolic hull extract of *Juglans regia*. Administration of ISO significantly increases catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), malondialdehyde (MDA) and advanced oxidation protein product (AOPP) levels and significantly reduced activities of antioxidant status (TAS), total thiols (TTH), acetylcholinesterase (AChE), arylesterase (AE), and glutathione peroxidase (GPx) in rat brain. Histopathologically, neuronal degeneration, spongiosis and gliosis were seen in cerebral cortex after ISO administration. Pretreatment with hull extract restored TAS, TTH, AChE, CAT and SOD values. Additionally, significant reductions were noted in levels of MDA, AOPP, and severity of histomorphological changes in cerebral cortex following hull extract treatment. Altered antioxidant biomarkers along with histopathological changes indicate oxidative injury in rat brain following ISO administration. Repeated administration of *J. regia* hull extract demonstrating presence of neuroprotective properties against ISO induced oxidative damage in rat brain.

1. Introduction

Oxidative damage as a result of exposure to chemicals or drugs is involved in pathogenesis of a large number of neurological disorders including neurodegenerative, ailments such as Alzheimer's, insomnia, dementia, aging, *etc* [1]. Although, chemical agents can directly impact brain, but indirect neuronal injuries can also occur due to interference in vascular supply and metabolism. Isoprenaline (ISO) is a non-selective β adrenergic receptor agonist used for the management of asthma, chronic bronchitis as well as mild and transient episodes of heart block in humans [2]. It also acts as a reversible inhibitor of AChE [3], therefore it can adversely affect the cholinergic transmission. At higher doses, ISO incites oxidative stress and inflammatory reactions in rat brain which are mediated by activation of NADPH oxidase (NOX) [4]. Activated NOX causes excessive accumulation of reactive oxygen species (ROS) mainly in astrocytes triggering endoplasmic reticulum stress and activation of nuclear factor κB (NF-κB). ROS ultimately diffuses into the neural network thus initiating as well as aggravating cerebral injury.

The diverse array of bioactive nutrients present in natural products especially fruits and nuts play crucial roles in countering effects of chemically induced oxidative stress in multiple organ systems including brain. Furthermore, multiple evidences exist in literature to suggest that naturally occurring phytochemicals like polyphenolic antioxidants hinder degenerative processes in brain and improve cognitive functioning [5,6]. Walnut (*Juglans regia* L.) belongs to family Juglandaceae, and is extensively used in Indian Ayurvedic and other traditional systems of medicine for the treatment of various disorders [7,8]. It is a rich and cholesterol-free source of nutrients including quality proteins, high concentrations of alpha linolenic acid, phenolic acids, flavonoids, tetralones and naphthoquinone, are the main components [8,9]. Presence of pharmacological properties such as antifungal, astringent action, hepatoprotective, cardioprotective, neuroprotective, laxative action, antioxidant potential make it an ideal food for consumption [10,11]. Walnut enriched diets are neuroprotective as they can boost antioxidant enzymatic machinery and guard against oxidative stress, free radical mediated lipid peroxidation and inflammation [12]. Addition of walnut in diets has been also found to be beneficial against development and progression of Alzheimer's disease [13]. The basic mechanisms behind

* Corresponding author.
E-mail address: drpawankv@yahoo.co.in (P.K. Verma).

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the curative effects rely on the action of the phytonutrients in safeguarding pathways associated with protein folding and neuroinflammation *in vitro* [9,14]. Hull is the outermost green covering of the fruit and loss during the extract of walnut seed from fruits. Qualitative and quantitative studies indicate that hull is rich in polyphenolic compounds and alkaloids having various pharmacological properties. To maximize the utilization of hull as a feed and fodder supplement in animals and humans [7–10]. Therefore, present study was under taken to determine the possible antioxidant and neuroprotective potential of *J. regia* hull extract against ISO induced oxidative damage in brain of Wistar rats.

2. Materials 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, Jammu. The animals were allowed to acclimatize for a week prior to the start of experiment. They were provided standard pelleted rat 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. All experimental rats received humane care in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

2.2. Collection and preparation of hull extract

The hull of the plant *J. regia* L. 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 green 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 a fine powder using an electric grinder. Powder was then subjected to hydroethanolic extraction. For this, powder was weighed and placed in a thimble which was put in the flask of soxhlet distillation apparatus and the extraction was done with hydroethanolic (50:50) solvent by maintaining hot plate temperature between 70 °C–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 dissolved in distilled water 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 [8,15,16]. All other chemicals used in the study were of analytical grade and were purchased from different standard firms.

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 ISO at the dose rate of 100 mg/kg body weight subcutaneously on 5^{th} and 6^{th} day, group III received hydroethanolic hull extract continuously for 7 days (300 mg/kg, oral gavage). Group IV were given ISO on 5^{th} and 6^{th} day (subcutaneously) along with plant extract, group V were given Quercetin (100 mg/kg, oral gavage, daily for 7 days) and ISO (100 mg/kg, subcutaneously on 5^{th} and 6^{th} day). On 8^{th} day, the animals were scarified by cervical dislocation and brain (1gm) was collected in 10 mL ice-cold 0.5 M phosphate buffer (pH 7.4) for estimation of antioxidant parameters. Part of brain was also collected in 10 % formal saline for histopathological study.

2.4. Antioxidant biomarkers analysis in brain

Tissue homogenate (1%) was prepared by homogenizing brain tissue using Teflon coated homogenizer at 1000 rpm for 5−7 min at 4 °C. The activity of acetylcholinesterase (AChE) in brain tissue was expressed in nmol thiol group formed/min using reduced glutathione as standard curve [17]. Arylesterase (AE) activity was measured by using phenyl acetate (Sigma-Aldrich, USA) as a substrate [18]. Total antioxidant status (TAS) was determined spectrophotometrically by using 2,2etazinobis (3-ethylbenzothiazoline 6-sulphonate) (ABTS) according to the method previously described [19]. Total thiols (TTH) were determined as per the method described [20] and expressed as millimolar (mM) using reduced glutathione as a standard. The enzymatic parameters *viz.*, catalase (CAT) and glutathione peroxidase (GPx) were determined as per the standard methods [21,22]. The activities of superoxide dismutase (SOD) and glutathione reductase (GR) were determined as per the method described previously [23,24]. Similarly, malondialdehyde (MDA) and advanced oxidation protein product (AOPP) levels in brain of different groups were determined using standard protocol and expressed in nmole of MDA formed/g/h and μM of Chloramine-T, respectively [25,26].

2.5. Histopathological studies

For histopathological studies, formalin fixed specimens from cerebrum of different groups were embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E), as per the standard technique. Microscopic lesions in cerebral cortex were noted and graded as per the severity witnessed: no (-), mild (+), moderate (++) and severe (+++).

2.6. Statistical analysis

All data were expressed as mean ± standard error of mean of six animals. 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. Effect on activities of AChE and AE

The alteration in the activities of AChE and AE of rat brains in control and treatment groups is presented in Fig. 1. After subcutaneous administration of ISO, a significant ($P < 0.05$) decline in mean AChE levels with a parallel increase in mean AE values was observed in rat brains. Exclusive administration of hull extract alone showed no significant alteration in AChE and AE activities. Administration of hull extract or quercetin restored the activity of AChE while values of AE remained significantly ($P < 0.05$) higher despite the treatment with quercetin or hull extract in brain of rats injected with ISO as compared to control animals.

3.2. Effect on activities of antioxidant enzymes

Activities of different antioxidant enzymes namely CAT, SOD, GR and GPx of rat brains in different groups are presented in Table 1. Subcutaneous administration of ISO significantly ($P < 0.05$) increased the activities of CAT, SOD and GR, while activity of GPx significantly ($P < 0.05$) declined in brain tissue. Treatment with hull extract alone did not show any significant alteration in activities of CAT, SOD, GR and GPx when compared to normal control. The significantly ($P < 0.05$) enhanced CAT activity level in ISO treated group however, significantly reduced after treatment with hull extract and the levels became insignificantly different from the control group. Quercetin also significantly reduced CAT values in rats treated with ISO, but the values could not be

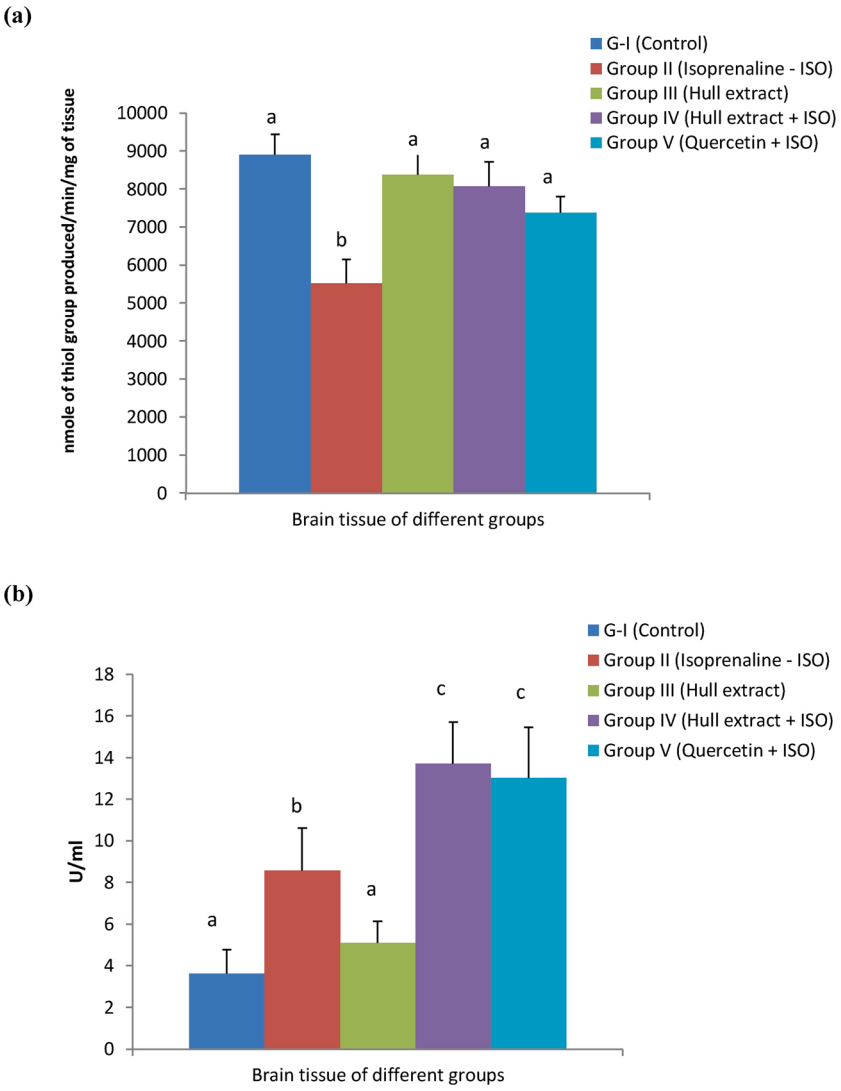

Fig. 1. Effect of hydroalcoholic extract of *J. regia* hull on activities of (a) AChE and (b) AE in brain tissue of rats treated with ISO.

Table 1
Effect of hydroalcoholic extract of *J. regia* hull administration on enzymatic antioxidant biomarkers in brain tissue after ISO induced neurotoxicity in rats.

Group	CAT	SOD	GR	GP_X
Group I	$2901.50^{a}\pm342.68$	$420.68^{a}\pm79.21$	$27.34^{a}\pm2.91$	$276.69^{b}\pm6.28$
Group II	$4801.88^{c}\pm474.50$	$803.61^{b}\pm57.1$	$34.99^{b}\pm2.19$	$139.29^{a}\pm5.28$
Group III	$3134.71^{ab}\pm554.32$	$530.44^{a}\pm73.76$	$25.13^{a}\pm3.84$	$157.57^{a}\pm8.47$
Group IV	$3437.32^{ab}\pm372.34$	$697.97^{c}\pm42.67$	$41.98^{b}\pm7.72$	$125.84^{a}\pm9.56$
Group V	$4061.57^{b}\pm334.10$	$587.74^{a}\pm81.35$	$24.86^{a}\pm2.96$	$108.84^{a}\pm9.95$

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 CAT (Catalase) are expressed in µmol H_2O_2 decomposed/ min/g of tissue.
Values of SOD (Superoxide dismutase) expressed in Unit/ g of tissue.
Values of GPx (glutathione peroxidase) are expressed in Unit/ g of tissue.
Values of GR (glutathione reductase) are expressed nmol of NADPH / min.

normalised and remained higher than the control group. Similarly, elevations in activity of SOD in ISO administered rat brains were also significantly reduced after hull extract treatment although the levels were still significantly higher than control group. In contrast, quercetin administration completely normalized the SOD values in ISO treated rats. The ISO induced significant increases in the activity of GR were reduced after quercetin supplementation but not by hull extract administration as compared to the control animals. The reduction in activity of GPx in brain tissue seen after ISO administration however, could not be restored upon administration of either hull extract or quercetin and remained significantly lower in comparison to the values in control animals.

3.3. Effect on TAS and TTH

Subcutaneous administration of ISO significantly ($P < 0.05$) decreased the mean levels of TAS and TTH in brain tissue of rats as compared to control. Exclusive treatment with plant extract did not alter the values of TAS or TTH when compared with control levels. However, hull extract restored the levels of both TAS as well as TTH after ISO treatment. Treatment with quercetin yielded similar outcomes (Table 2).

Table 2
Effect of hydroalcoholic extract of *J. regia* hull administration on antioxidant parameters in brain tissue after ISO induced neurotoxicity in rats.

Group	TAS	TTH	AOPP	MDA
Group I	$24.94^{b}\pm0.78$	$3.94^{b}\pm0.64$	$0.64^{a}\pm0.21$	$45.27^{a}\pm11.70$
Group II	$19.39^{a}\pm0.98$	$1.19^{a}\pm1.01$	$2.15^{b}\pm0.27$	$148.82^{b}\pm35.83$
Group III	$24.36^{ab}\pm0.90$	$3.27^{b}\pm0.62$	$1.03^{a}\pm0.19$	$86.54^{ab}\pm13.28$
Group IV	$25.29^{b}\pm0.11$	$1.92^{b}\pm1.06$	$1.67^{c}\pm0.29$	$89.57^{c}\pm11.89$
Group V	$22.53^{ab}\pm0.35$	$2.26^{b}\pm0.26$	$1.12^{c}\pm0.33$	$88.58^{a}\pm10.35$

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.

3.4. Effect on cellular damage biomarkers

MDA and AOPP levels in brain tissue of different groups of animals are shown in Table 2. A significant ($P < 0.05$) increase in mean AOPP and MDA levels occurred after ISO administration. Hull extract by itself had no effect on levels of AOPP or MDA content when compared with control rats. But the treatment with hull extract significantly reduced the values of AOPP in rats, which were given ISO. Administrations of quercetin was also effective in restoring ISO raised AOPP values in ISO administered rats. Similarly, hull extract as well as quercetin could mitigate the increases in MDA in ISO treated groups.

3.5. Effect on histopathological alterations in brain

Lesion scores of cerebral cortex from different experimental groups are presented in Table 3. Microscopic examination of sections from cerebral cortex in group I revealed presence of normal architecture of brain including neurons, neuronal processes and glia cells. The neurons were polygonal and had a centrally located vesicular nucleus (Fig. 2). Cerebral cortex in group II rats revealed presence of multifocal areas of liquifactive necrosis which appeared as areas containing fibrillar necrotic material dotted with glial cells (Fig. 3). Severe spongiosis was also noticed. Wide spread neuronal degeneration and nerve cell necrosis was observed wherein neurons were deeply eosinophilic, shrunken and angular (Fig. 4). Additionally, at multiple places neuronophagia was noticed where injured or dead neurons were being phagocytosed by microglia cells. In group III, no pathological lesions were observed (Fig. 5). In group IV, although neuronal damage in the form of degeneration and necrosis persisted but was less severe as compared to group II animals. Lesions were seen mostly around arterioles with degenerated vessel walls. Also, spongiosis was limited to perivascular areas and neurons in the surrounding areas also exhibited degeneration or necrosis (Fig. 6). Overall, neuronal changes and spongiosis were markedly attenuated in group IV rats. Likewise, cerebral sections of group V rats also revealed vacuolation and neuronal damage but the severity of lesions was significantly less when compared to group II animals (Fig. 7).

Table 3
Lesion scores in cerebral cortex of rats in control and treatment groups.

Brain alterations	Treatment groups				
	1	2	3	4	5
Neuronal degeneration	–	+++	–	++	++
Neuronal necrosis	–	+++	–	++	++
Gliosis	–	++	–	+	+
Foci of Liquefactive necrosis	–	++	–	–	–
Blood vessel wall degeneration	–	+	–	+	–
Spongiosis	–	+++	–	+	+

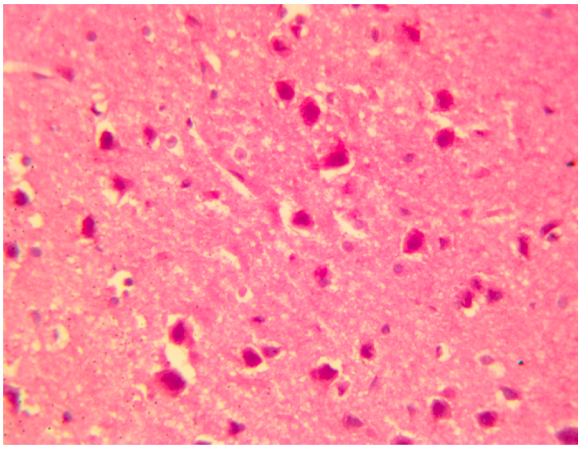

Fig. 2. Cerebral cortex from control rats with normal neurons having a centrally placed nucleus and glial cells. (H&E 400X).

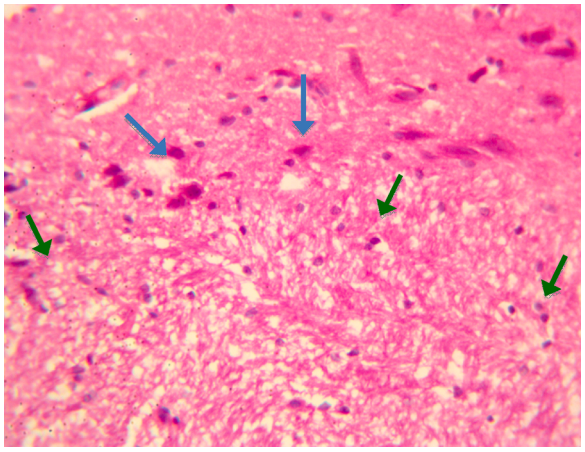

Fig. 3. A well demarcated area of liquefactive necrosis (boundary is marked by green arrows) containing fibrillary necrotic material and glial cells in cerebral cortex surrounded by degenerated and dead neurons (blue arrow) in Group II. (H&E 400X).

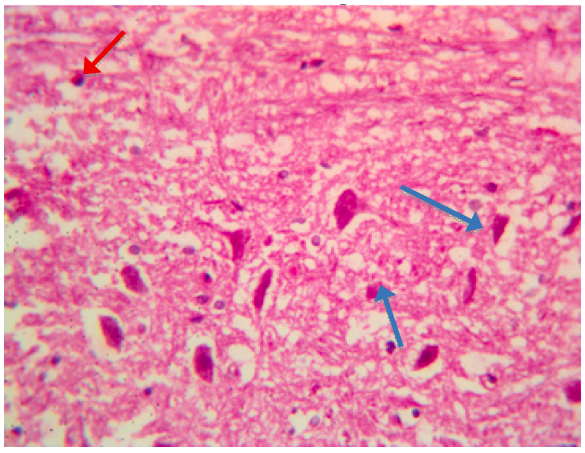

Fig. 4. Spongiosis (red arrow) and neuronal necrosis (blue arrow) in cerebral cortex of group II rats (H&E 400X).

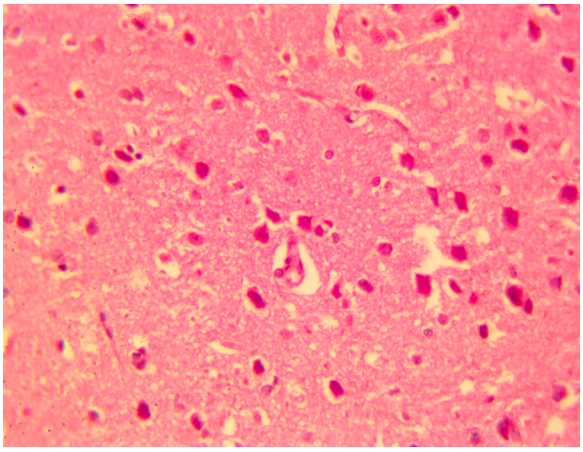

Fig. 5. Normal architecture of cerebral cortex in rats of group III.

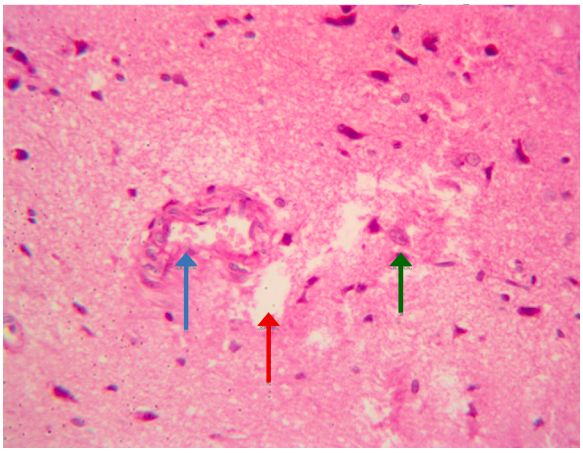

Fig. 6. Brain showing degeneration of arteriolar wall (blue arrow), spongiosis (red arrow) and degeneration of neurons in the vicinity (green arrow) of group IV rats.

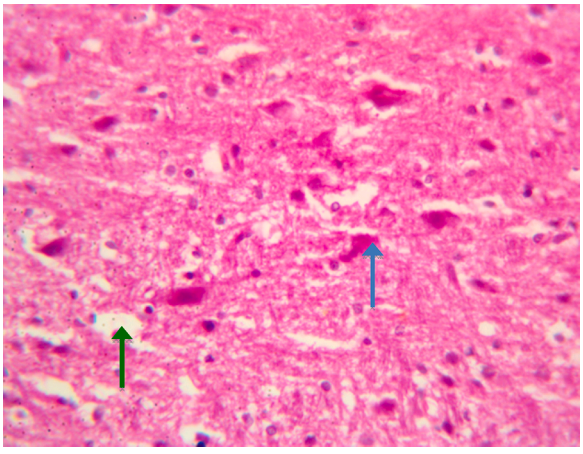

Fig. 7. Neuronal degeneration and necrosis (blue arrow) and vacuolation (green arrow) in cerebral cortex of group V.

4. Discussion

Oxidative stress is an important player in pathogenesis and progression of neurological disorders. Extremely high metabolic rate of nervous tissue predisposes it to oxidative stress which has been implicated as a major contributing factor in ageing, cerebrovascular disorders, cognitive disabilities and neurodegenerative diseases like Alzheimer's [27]. ISO administration induces activation of NOX and endoplasmic reticulum stress in rat brain resulting in excessive production of ROS/RNS which is the key reason for the decline of neural capacity and cognitive functions in rats [4]. In the present investigation also, significant derangements in brain antioxidant system and lipid peroxidation were seen after ISO administration reinforcing the fact that ISO causes oxidative damage to tissues. Walnuts being a rich source of polyphenolic compounds and antioxidants (ellagic acid, melatonin, vitamins, selenium), diets supplemented with walnut can check inflammation and reduce the risk of development or forestall progression of ageing and neurodegenerative diseases by virtue of its scavenging action on free radicals as well as protection of cellular antioxidant machinery [13,28,29].

During oxidative stress, thiols (-SH group) not only serve as a cornerstone of the antioxidant defence network but also regulate molecular response to oxidants. Thiols detoxify extra and intra-cellular ROS/RNS radicals either by a direct action on ROS or by catalyzing GPx mediated ROS decomposition to produce glutathione disulphide (GSSH). TTH levels are also important for proper maintenance of redox state of protein thiols involved in DNA synthesis and repair. Significant reduction of TTH levels in brain tissue in the current study indicated increased utilization of thiols in scavenging free radicals generated after ISO administration. Similar observation was also made by Essa et al. [14]. Supplementation of hull extract restored the level of TTH which may be due to a reduced generation of ROS/RNS or to an increased production or recycling of GSSH to reduced glutathione (GSH). Since this process is carried out by the by action of GR and activity of GR was not reduced by the administration of ISO or hull extract in the present investigation. Therefore, our observations suggest that hull extract reduced production of free radicals or scavenged them to restore the levels of TTH in brain tissue of ISO administered rats. Similarly, *Sonchus asper* extract administration also protected against cognitive decline by countering the oxidative damage in rat brain [30].

SOD and CAT are widely distributed in all animal tissues and protect them from highly reactive hydroxyl radicals. SOD is the first line of defense against the action of superoxide radicals produced in the mitochondria and endoplasmic reticulum. Increased levels of these antioxidant enzymes are indications of activation of compensatory mechanism of cell to scavenge free radicals to mitigate toxicant induced oxidation and peroxidation of protein and lipids [31]. In our study also, CAT and SOD content significantly increased after ISO treatment. A number of other experimental and clinical studies have also reported alterations in these antioxidant enzymes in the brain tissue during different neurological disorders [14,32]. The *J. regia* hull extract supplementation effectively suppressed these elevations possibly by reducing the free radical concentration in brain and thus the requirement for these antioxidant enzymes also went down. A recent report showed that *J. regia* L. possesses a thermostable SOD, and therefore was recommended as a potential nutraceutical [33]. It can be postulated from our results that the inherent SOD activity of *J. regia* hull might have played a role in attenuating the enhancements in SOD concentrations seen after ISO administration. Moreover, it has been demonstrated previously that peptides from walnut protein lysates have neuroprotective activity *in vivo* as well as *in vitro* because a normalization in the altered brain antioxidant SOD and CAT, and elevated MDA occurred after their administration [34]. The normal firing of central cholinergic system is vital for higher brain traits like behaviour and cognition [35,36]. In the present study, significant inhibition of neural AChE resulted after ISO administration. The toxicity of organophosphorus compounds for example profenofos, imidacloprid and deltamethrin is also associated with reduced cholinesterase in brain of albino rats [37–39].

Since abnormal regulation of neurotransmitter release in brain can result in behavioural abnormalities, cognitive deficits, neuropsychosis and neurodegeneration, hence, neurotransmission imbalance as a result

of preponderance of acetylcholine due to inhibition of AChE in nervous tissue as observed in our study may also disrupt neurophysiology leading to neuropathies [36,40]. Hull extract pretreatment completely ameliorated the decline in levels of AChE that resulted after ISO administration. Similarly, treatment of Vitamins C and E exerted an antioxidative effect and protected against profenofos induced decrease in AChE in brain of albino rats [37].

The activity of glutathione containing enzyme GP_X, in brain tissue had significantly decreased in ISO administered group. But hull extract and quercetin failed to restore the GPx activity. On the contrary, contents of glutathione containing enzyme GR got elevated after ISO administration but administration of hull extract failed to decrease the levels and so they remained higher than the animals in control group. Quercetin in contrast was effective in reinstating GR values to levels comparable with control animals.

Oxidation of PUFAs associated with plasma membrane results in the formation of MDA, a thiobarbituric acid reactive substance, which is the indicator of lipid peroxidation. Increased lipid peroxidation is an important feature of cytotoxicity caused by ISO. Studies have suggested that lipolysis after administration of ISO raises the concentration of unesterified fatty acids in plasma, which in turn causes decreased binding of tryptophan to albumin resulting in a hike in plasma concentration of free tryptophan and other aromatic amino acids [41]. Enhancement in tryptophan directly increases the serotonin levels in brain that can in result neurological manifestations in affected subjects. Subchronic exposure to chlorpyriphos and atrazine also caused tissue destruction and significant increase in MDA, in brain of the common carp [42]. Concentrations of lipid peroxidation products and AOPP in brain were significantly elevated in response to oxidative damage after acute respiratory hypoxia [43]. This in agreement with our findings of high levels of MDA and AOPP in brain of ISO administered rats. Treatment with hull extract and quercetin although significantly attenuated these changes, but could not entirely normalize the levels when compared with the corresponding values of control animals. Similar changes were also observed by Wang et al. [32]. Aryl esterase, a hydrolase is an important free radical scavenger whose levels may fluctuate under oxidative stress conditions [44]. In the present investigation, aryl esterase levels shot up in response to ISO treatment. Hull extract or quercetin however could not prevent this surge.

Pro-inflammatory cytokines generated as a result of lipid peroxidation and oxidative stress are well known aggravators of a pre exiting cerebral injury [27]. In our study, ISO caused severe neuronal degeneration and necrosis, gliosis, neuronophagia, spongiosis, and foci of liquefactive necrosis in rat cerebral cortical sections likely as a result of significant perturbations in oxidant /anti-oxidant homeostasis in brain of ISO administered rats. Likewise, necrosis, hyperemia, gliosis, and spongiosis were salient histopathological findings in brain after exposure to nickel oxide and nickel oxide nanoparticle in rat brain [45]. Similar lesions in brain have also been observed by Wang et al. [32] upon ISO treatment and Essa et al. [14] in a mouse model of neurodegeneration. Subacute neural toxicity by administration of lead resulted in neuronal degeneration, cellular swelling and disruption of different cell layers in cerebrum [46]. Both hull extract and quercetin attenuated the severe pathological changes incurred by ISO. Similar neuroprotection was also observed by Wang et al. [32] and Essa et al. [14] after supplementation with melatonin and walnut respectively. Additionally, peptides from walnut protein lysates exhibited anti-apoptotic action against glutamate induced apoptosis of PC12 cells, a classical neuronal cell model [34]. Moreover, walnut peptides retarded cognitive impairment and inhibited neuro-inflammation and oxidative stress induced by LPS in rats and also conferred protection to BV2 cells, the immortalized microglial cell line *in vitro* [47]. Also, corvitin and 2-oxoglutarate afforded cardio and neuroprotection against isoproterenol induced myocardial damage in rats [48]. In addition, severe oxidative stress in brain, lipid peroxidation, necrosis and inflammation in cerebral cortex induced by sodium valproate were ameliorated by 1-cysteine [49]. Hesperidin conferred significant neuroprotection after radiation induced brain as was appreciated by improvements in MDA and total antioxidant levels and reductions in degeneration and inflammation in histological sections of brain [50]. Nebivolol reduced aluminum induced neuronal degeneration in cerebral cortex and oxidative stress in brain [51].

In the present study quercetin was used as protective agent against ISO induced neurological alterations and its activity was compared with our *J. regia* hull extract. Quercetin a flavonoid is commonly used for the treatment of cardiovascular and neurological disorders owing to its abilities to check ROS production, inhibit the activity of oxidative enzymes (lipoxygenase and xanthine oxidase) and suppress inflammation [52]. However, poor intestinal absorption and solubility as well as rapid neutralization after oral administration limit the bioavailability of quercetin. Evidence exists that antioxidant activity of quercetin ameliorates ISO-induced myocardial oxidative stress [53]. Here we found that quercetin was also effective against ISO-induced cerebral damage. The perturbations induced by exposure of number of phytochemicals present in hull extract against ISO induced damage in rat brain need to be investigated for their applications [52–54].

Overall, when contrasted with the neuroprotection conferred by quercetin, hull extract also possessed remarkable oxidative stress and neuronal damage attenuating potential as indicated by restoration in values of AChE, CAT, SOD, TAS, TTH, AOPP and MDA and reduction in severity of pathological lesions in cerebrum. The ethanolic hull extract of *J. regia* produced protective effects which may be due to high levels of polyphenolic compounds having high scavenging potential of free radicals or ROS [55]. In present study, treatment with walnut hull extract could significantly reduce the cortical architectural damage in cerebrum and preserve antioxidant system in rats challenged with ISO and these properties may be attributed to presence of polyphenolic components in hull extract of *J. regia*. Similar neuroprotective properties have been found in many other herbal ingredients against a wide variety of neurotoxic agents [56,57]. Even though, walnut hull is a byproduct but owing to its rich antioxidant makeup plus presence of excellent neuroprotective properties as shown in the current study, future research should be carried out to explore its neuroprotective potential against various nervous disorders.

5. Conclusion

The *J. regia* hull extract harbors neuroprotective properties against ISO induced neurotoxicity as it ameliorated changes in AChE, CAT, SOD, TAS, TTH, AOPP and MDA levels seen after ISO administration and also provided significant protection to brain tissue against ISO-induced pathological damage in rats.

Authorship statement

P. Sharma, P.K. Verma: Conception and design of study.

P. Sharma, Shilpa Sood, N.K. Pankaj: Acquisition of data.

R. Raina, S. Agarwal, P.K. Verma: Analysis and/or interpretation of data.

P.K. Verma, P. Sharma, R. Raina, S. Agarwal: Drafting the manuscript.

S. Sood, N K Pankaj: Revising the manuscript critically for important intellectual content.

P. Sharma, P.K. Verma, S. Sood, N.K. Pankaj, S. Agarwal, R. Raina: Approval of the version of the manuscript to be published.

Declaration of Competing Interest

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

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