International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; SP-9(3): 238-241

www.veterinarypaper.com
Received: 10-03-2024
Accepted: 14-04-2024

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Antioxidant effect of *Panax ginseng* in cold restraint stressed rats

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Abstract
The study was conducted to assess the ameliorating potential of *Panax ginseng* against cold-restraint stress in rats. Cold-restraint stress was induced by keeping the rats at 4 °C for 2 hours from 21st day to 28th day of the study. Oxidative parameters in RBC and tissues of liver and kidney were recorded in rats. Rats exposed to cold-restraint stress showed a significant elevation in lipid peroxidation (LPO) in RBC, liver and kidney. The GSH level and SOD activity in RBC, liver and kidney were reduced significantly. The rats treated with *Panax ginseng* root extract along with stress showed a significant decrease in LPO in RBC, liver and kidney compared to rats treated with stress alone. The GSH level and SOD activity of liver, kidney and RBC was found to be elevated. These observations suggest the protective role of *Panax ginseng* against cold-restraint stress in rats.

Keywords: *Panax ginseng*, cold restraint stress, oxidative parameters, liver, kidney, RBC

Introduction
Asian ginseng or *Panax ginseng* provides several health benefits including immunomodulation, neuroprotection, anti-septicemia, anti-diabetic and anti-cancer properties (Lobina *et al*., 2014) [11]. Research on the characteristics and molecular study of extracts revealed the presence of ginsenosides, glycosides, and tetracyclic triterpenoid saponins which are responsible for much of their pharmacological activity (Leung and Wong, 2010) [10].
Stress is an inevitable component of life and a state in which equilibrium may be compromised due to environmental, physiological, or psychological stresses (Ravindran *et al*., 2005) [18]. As described in the studies, the body may develop a variety of illness states if it is incapable to manage prolonged stress (Verma and Khosa, 2009) [24]. Cold restraint stress is the model used to see the damaging effect of chronic stress on the whole body system. Considering the wide use of ginseng in adaptogenic condition, the present study investigates the action of aqueous and alcoholic extract of *Panax ginseng* root extract in rats to ascertain the scientific basis for the use of this root extract in cold –restraint stress.

Materials and Methods
Root of the plant material *Panax ginseng* were purchased from JKH Herbs and Spices, Navimumbai. The dried root of *Panax ginseng* was powdered. The powder was soaked in absolute alcohol for 24 hours with continuous stirring at 40 °C. The mixture was filtered through muslin cloth and whatmann filter paper no.42, the solvent was concentrated in a rotatory vacuum evaporator at 40-50 °C. The final extract was produced after drying the filtrate in incubator with fan (40 °C) and lyophilized. The same procedure was followed for the preparation of aqueous extract (except the root which was soaked in distilled water). The percentage of yield of extract was calculated.
Experiments were performed on adult wistar rats at a weights ranging from 150-250 g and were procured from Laboratory Animal Resource Centre, IVRI, Izatnagar, Uttar Pradesh, India. The animals were kept in plastic cages and acclimatized for two weeks in the experimental lab animal shed of the College of Veterinary and Animal Sciences, Pantnagar, under standard managemental conditions. Standard rat feed and water was provided *ad libitum* throughout the experimental period.

All the experimental animals were kept under constant observation during entire period of study.

Cold-restraint stress was induced by keeping the rats at 4 °C for 2 hours for a period of 7 days (Bhattacharya and Ghosal, 1994) [2]. All the rats were randomly divided into seven groups of 6 rats each. The aqueous and alcoholic root extract of *P. ginseng* was given to groups II and III, respectively at the dose rate of 50 mg/kg b.wt, whereas Vitamin C was given to group V for 28 days. The dose of *Panax ginseng* was chosen according to previous study on *Panax ginseng* (Singh *et al.*, 1991, Ritu Shukla and Madhu Kumar, 2009) [20, 23]. In Groups IV, V, VI and VII, cold-restraint stress was induced by keeping the rats at 4 °C for 2 hours from 21st day to 28th day of the study. In group VI stress along with aqueous extract was provided while Group VII received alcoholic extract. On 28th day all the rats were sacrificed. Blood, liver and kidney samples were collected to evaluate antioxidative and histopathological parameters. After the completion of experiment, all the rats were buried scientifically.

Oxidative parameters in erythrocytes

Separation of erythrocytes: The heparinised blood samples were centrifuged at 2000 rpm for 15 min. Plasma and buffy coat were removed. The resulting erythrocyte pellet was washed thrice with 0.15 M NaCl. The 33% dilution of the packed RBC was made in PBS (pH 7.4). The washed erythrocyte pellets were suspended in PBS of pH 7.4 and kept at 4 °C till further analysis. This 33% packed RBC was used for the estimation of lipid peroxidation and reduced glutathione. Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde (MDA) production by the method of Rehman (1984) [19]. Reduced glutathione (GSH) was estimated by the 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) method of Prins and Loos (1969) [17].

Preparation of tissue homogenate: Frozen liver, kidney and brain samples were partially thawed and 200 mg of sample was weighed and taken in 2 ml of ice-cold saline. 200 mg of sample was weighed separately and taken in 2 ml of 0.02 M EDTA for GSH estimation. Organ homogenates were prepared using IKA homogenizer, Germany under cold condition. The homogenate was centrifuged for 10 min at 3000 rpm. The supernatant was used for different biochemical estimations. The extent of lipid peroxidation was evaluated in terms of MDA (malondialdehyde) production, determined by the thiobarbituric acid (TBA) method (Rehman, 1984) [19]. Reduced Glutathione (GSH) was estimated by estimating free –SH groups, using DTNB method of Sedlak and Lindsay (1968) [22]. For GSH, 10% homogenates were made in 0.02 M EDTA. SOD was estimated as per the method described by Madesh and Balasubramanian (1998) [12].

Statistical Analysis

The data were statistically analyzed using analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison test. Differences were considered to be significant at $p<0.05$. The data were presented as Mean ± SE. All statistical tests were performed using Graph Pad In Stat Software Inc., v.4.00, San Digeo, USA.

Results and Discussion

The present study showed that exposure to cold-restrain stress resulted in increase in LPO levels in erythrocytes as evidenced by the increased production of malondialdehyde (MDA). This was further associated with decrease in activity of antioxidant enzymes GSH level in RBC (Table 1). Increased level of LPO in RBC indicates free radicals formation and participation of free radical-induced oxidative cell injury in mediating the effect of stress. Corry *et al*., (1970) [6] have reported that increase in LPO in RBC might possibly be due to peroxidation of unsaturated fatty acids in plasma membrane phospholipids of RBC. Thus, increased LPO in RBC is indicative of progressive increase in cellular deformity, increase in membrane permeability and rigidity and disruption of structural and functional integrity of cell organelles (Corry *et al*., 1970) [6]. Antioxidant defense system in cold-restraint stress is reduced which lead to interference of pro-antioxidant balance in the body. Similar results were recorded by Alptekin and Cevikbas, (2003) [1] during cold stress to rats.

LPO was also measured in vital organs such as liver and kidney in present study (Table 2 & 3). There was significant increase in LPO in these tissues in stressed groups. Our finding is in agreement with Burhan Ates *et al*., (2006) [3] and Cigdem *et al*., (2009) [5] where both recorded an increase in the level of lipid peroxidation in liver of cold-exposed rats. Ifor D *et al*., (1983) [8] have reported similar findings that an increased level of LPO in liver of rats of various ages. Increased LPO in the present study in RBCs, liver and kidney by cold-restraint stress could be attributed to the generation of ROS through oxidative damage to cell membrane.

Glutathione antioxidant system plays a pivotal role in cellular defence against reactive free radicals and other oxidant species (Meister and Anderson, 1983) [13]. GSH with its –SH group functions as a catalyst for disulfide exchange reactions, and it plays a major role in H2O2 detoxification. In addition to serving as a substrate for glutathione related enzymes, GSH acts as a free radical scavenger, a generator of α-tocopherol and plays an important role in the maintenance of protein sulphydryl group (Ookhtens and Kaplowitz, 1998) [15]. Hepatic GSH plays a crucial role in both scavenging reactive oxygen species and the detoxification of xenobiotics (Haque *et al*., 2003) [7]. While LPO increased, there was marked reduction in reduced glutathione in erythrocytes, liver and kidney of cold-restrain stressed rats. Same finding has also been recorded by Alptekin and Cevikbas, (2003) [1] in cold stressed rats. Yegen *et al*., (1990) [25] and Mira *et al*., (2009) [14] recorded a decrease in GSH levels in liver of cold-restraint stressed rats.

The present study has shown a decrease in SOD activities in liver and kidney of rats during cold-restraint stress (Table 2 & 3). This decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation (Pigeolot *et al*., 1990) [16]. The increase in level of antioxidants (SOD and GSH) after administration of *Panax ginseng* may be due to direct reaction of Ginseng with ROS. The antioxidant activity may be due to the flavonoids present in the plant extract (Chevallier, 2000) [4].

Panax ginseng root extract pretreatment has been reported to decrease the LPO and increase the SOD and GSH level in kidney of rats exposed to gentamicin sulphate induced toxicity (Karadeniz *et al*., 2008) [9]. Co-administration of *Panax ginseng* decreased the harmful effects of gentamicin sulphate by inhibiting free radical formation and by restoration of the antioxidant systems. The decrease in the level of antioxidants (SOD and Catalase) and increase in LPO indicates an enhancement in peroxidation, leading to a loss of membrane integrity and oxidative modifications of amino acid side chains, etc (Rivero *et al*., 2005) [21]. In present study, *Panax ginseng* has been found to have the potential to reduce oxidative damage induced by cold-restraint stress in rats.

Table 1: Effect of *Panax ginseng* on antioxidative parameters in erythrocytes of cold-restraint stressed rats

Groups	Dose (mg/kg)	LPO (Nm MDA/ml)	GSH (mM/ml)	SOD (Units)
I	--	16.19±0.52	0.85±0.04	6.27±0.33
II	--	20.11±0.30[a]	0.58±0.06[a]	4.96±0.37[a]
III	50	17.65±0.30[a,b]	0.76±0.06[b]	5.74±0.37[b]
IV	50	16.34±0.47	0.87±0.05	6.31±0.36
V	50	16.69±0.35	0.88±0.05	5.89±0.34
VI	50	16.39±0.39[b]	0.83±0.03[b]	5.98±0.41[b]
VII	50	16.57±0.50[b]	0.81±0.07[b]	6.32±0.38[b]

Values in table are Mean ± SE (n=6)
[a] $p<0.05$ vs group I within same column
[b] $p<0.05$ vs group II within same column
Groups (I-Control, II-Cold-restrain stress, III-Vitamin C + Cold-restraint stress, IV-Aqueous extract, V-Alcoholic extract, VI-Aqueous + Cold-restraint stress, VII-Alcoholic +Cold-restraint stress)

Table 2: Effect of *Panax ginseng* on antioxidative parameters in liver of cold-restraint stressed rats

Groups	Dose (mg/kg)	LPO (nM MDA/g)	GSH (mM/g)	SOD (Units)
I	--	30.27±2.11	2.91±0.10	5.37±0.20
II	--	50.62±1.54[a]	1.86±0.09[a]	4.62±0.26[a]
III	50	37.18±2.82[a,b]	2.46±0.16[a,b]	5.30±0.17[b]
IV	50	31.69±2.15	2.97±0.10	5.39±0.27
V	50	31.65±2.35	2.99±0.09	5.35±0.23
VI	50	32.88±1.14[b]	2.91±0.17[b]	5.38±0.24[b]
VII	50	33.99±0.88[b]	3.09±0.15[b]	5.24±0.35[b]

Values in table are Mean ± SE(n=6)
[a] $p<0.05$vs group I within same column
[b] $p<0.05$vs group II within same column

Table 3: Effect of *Panax ginseng* on antioxidative parameters in kidney of cold-restraint stressed rats

Groups	Dose (mg/kg)	LPO(nM MDA/g)	GSH (mM/g)	SOD (Units)
I	--	40.07±1.35	2.46±0.16	5.80±0.5
II	--	59.64±2.75[a]	1.41±0.06[a]	4.02±0.23[a]
III	50	48.21±2.31[a,b]	1.93±0.09[b]	5.22±0.60[b]
IV	50	41.26±1.44	2.65±0.13	5.62±0.45
V	50	42.53±1.39	2.76±0.14	5.83±0.32
VI	50	42.41±1.91[b]	2.49±0.18[b]	5.75±0.31[b]
VII	50	43.21±1.52[b]	2.70±0.10[b]	5.66±0.31[b]

Values in table are Mean ± SE(n=6)
[a] $p<0.05$ vs group I within same column
[b] $p<0.05$ vs group II within same column

Conclusion

The findings of this study demonstrate that exposure to cold-restraint stress leads to increased lipid peroxidation (LPO) levels in erythrocytes, liver, and kidney tissues, as indicated by elevated malondialdehyde (MDA) production. This oxidative stress was accompanied by a significant reduction in the activity of antioxidant enzymes like superoxide dismutase (SOD) and glutathione (GSH). The imbalance between reactive oxygen species (ROS) production and antioxidant defense systems suggests a heightened susceptibility to oxidative damage in stressed rats. Interestingly, administration of Panax ginseng extract showed potential in mitigating these effects by lowering LPO levels and enhancing antioxidant defenses, thereby protecting against oxidative stress-induced cellular injury. Further research into the mechanisms underlying these protective effects could offer valuable insights for therapeutic interventions targeting oxidative stress-related disorders.

Acknowledgements

The authors are thankful to Life Science Research Board, New Delhi for providing financial support for this study. The authors gratefully acknowledge the technical assistance and facility provided by Director, Experiment Station, G.B. Pant University of Agriculture and Tech and Dean, College of Veterinary and Animal Sciences, Pantnagar, Uttarakhand.

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How to Cite This Article
Kandasamy K, Karthikeyan S, Ahmad AH, Pandey HK, Kumar A, Elamaran A. Antioxidant effect of *Panax ginseng* in cold restraint stressed rats. International Journal of Veterinary Sciences and Animal Husbandry. 2024;SP-9(3):238-241.