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Research Paper

Prophylactic Efficacy of *Podophyllum hexandrum* in alleviation of immobilization stress-induced oxidative damage in rat

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ABSTRACT

Free radicals are generated in the body by means of various conditions such as exercise, pollution, stress, poor diet, and smoking. They are known to attack the cell components and increase the risk of heart disease, ulcer, stroke, or cancer. In this study, we evaluated whether podophyllum treatment has a prophylactic effect on the prooxidant–antioxidant state following immobilization stress treatment in rats. Immobilization stress caused an increase in free radical production as evidenced by an increase in the levels of malondialdehyde (MDA) and a decrease in the levels of glutathione (GSH) and superoxide dismutase (SOD) in erythrocytes, liver and kidney. Both AL and AQ restored the increased LPO and decreased GSH and SOD significantly in immobilized rats and non-significantly in normal rats. Alcoholic extract was more efficacious in preventing excess free radical production as compared to aqueous extract. The potentiated antioxidant potential of erythrocytes due to Podophyllum administration can play an important role in the protective mechanism against immobilization induced oxidative damage.

Keywords: Podophyllum; Immobilization; Oxidative stress.

INTRODUCTION

Stress is one of the basic factors in the development of any disease and has been shown to be associated with altered homeostasis that may lead to oxidant-antioxidant imbalance. Immobilization is a typical psycho-physiological stress. Psychological stress triggers changes in various biochemical parameters in man and animals and induces the formation of ROS and lipid peroxidation in plasma.

Increased production of O_2^- by neutrophils leading to oxidative stress has been reported in rats in response to psychological stress (Kang and McCarthy, 1994). Among different markers of oxidative stress, malondialdehyde (MDA) and the natural antioxidants, metaloenzymes Cu, Zn-superoxide dismutase (Cu, Zn-SOD) and selenium-dependent glutathione peroxidase (GSHPx), are currently considered to be the most important. Malondialdehyde (MDA) is a three-carbon compound formed from

peroxidized polyunsaturated fatty acids, mainly arachidonic acid. Since MDA levels are increased in various diseases with excess of oxygen free radicals, many relationships with free radical damage were observed (Ohkawa, et al., 1979, Guichardant, et al. 1994). Cu,Zn-SOD is widespread in nature. It is present in all oxygen-metabolizing cells. Cu,Zn-SOD is an intracellular enzyme, which dismutates the extremely toxic superoxide radical into potentially less toxic hydrogen peroxide. GSHPx, an intracellular enzyme, belongs to several proteins in mammalian cells that can metabolize hydrogen peroxide and lipid hydroperoxides.

Plants are known to produce plethora of secondary metabolites showing wide array of pharmacological properties. The natural combination of compounds present in the whole plant extract often contained several molecules, which nullify the side effects produced by the active biomolecules or their combinations. Plant products, due to their synergistic action and comparatively non-toxic nature are being exploited worldwide for their antioxidative potential.

Podophyllum, the Himalayan Mayapple is a rhizomatous herbaceous perennial found in the high altitude wild. *Podophyllum* is a medicine of most extensive service; its greatest power lies in its action upon the liver and bowels. It is a gastro-intestinal irritant, a powerful hepatic and intestinal stimulant. *Podophyllum* is a powerful medicine exercising an influence on every part of the system, stimulating the glands to healthy action. Its most beneficial actions are obtained by the use of small doses frequently given. It is highly valuable in dropsy, biliousness, dyspepsia, liver and other medical conditions. Mayapple acts admirably upon all the secretions, removing obstructions, and producing a healthy condition of all the organs in the system.

During the last twenty or thirty years, attention has be drawn by pharmacologists and medical researches to the fact that *Podophyllum* contains chemical agents responsible for anti-cancer activity. Many tribes consume or drink brew from the powder as a laxative or to treat intestinal worms. In Maine, Indians used mayapple in poultice form for external use of wart tumors on the skin. In America, the plant was used as an anti-rheumatic, cathartic, dermatological aid, ear medicine, insecticide, and laxative. Others used the root as a purgative, vermifuge, for the treatment of warts and as an anthelmintic. In New England, the root was used to stimulated glands and for gastrointestinal disorders. The root was also used as a tonic for liver, lung, and stomach ailments. A decoction was made by boiling the roots in water and was used to treat rheumatism. Many of these medicinal properties have been assigned to the antioxidant properties of Podophyllum.

The present study was designed to investigate the efficacy of *Podophyllum hexandrum* rhizome extracts in alleviating the immobilization stress in rats. To investigate the extent to which the antioxidants in erythrocytes and other tissues play a role against the ROS produced by stress, the peripheral distribution of blood cells and antioxidants present in plasma and erythrocytes along with tissues are estimated.

MATERIALS AND METHODS

***Plant Material*:** The mature roots of *Podophyllum hexandrum* were collected from the Valley of Flowers and authenticated by Dr. D.S. Rawat, Botany Deptt., of same institution. The rhizomes were dried and powdered. The powder was soaked in absolute alcohol for 48 h with intermittent stirring at room temperature with the help of magnetic stirrer. The infusion was filtered through several layers of muslin cloth and centrifuged at 3000 rpm for 15 minutes to get the supernatant. The filtrate was dried in fan incubator at 45ºC and lyophilized to get the final extract (AL).

The matt left behind was now soaked in water for 48 h with intermittent stirring at room temperature with the help of magnetic stirrer. The infusion was filtered through several layers of muslin cloth and centrifuged at 3000 rpm for 15 minutes to get the supernatant. The filtrate was dried in fan incubator at 45ºC and lyophilized to get the final extract (AQ). Both the extracts were stored at -20ºC till further use.

***Animal model*:** Forty two albino rats (Sprague Dawley) of 2 to 2.5 months of age, weighing between 150 to 200 g, were procured from Laboratory Animal Resource Centre, IVRI, Izzatnagar, two weeks before the commencement of the experiment. The animals were divided into seven groups- control (C)(I), immobilisation stress (IS)(II), IS + AL@25 mg/kg (ALI)(III), IS + AQ@25 mg/kg (AQI)(IV), AL@25 mg/kg (V), AQ@25 mg/kg (VI) and vitamin C @25 mg/kg (V)(VII). The animals were kept in plastic cages and were acclimatized for two weeks in the experimental laboratory animal shed of the Department of Pharmacology, College of Veterinary and Animal Sciences, Pantnagar, under standard managemental conditions.

The extracts were given to 3rd, 4th, 5th and 6th group animals daily in the morning orally by gavage for 28 consecutive days. Starting from the 21st day, the rats belonging to group II, III and IV were subjected to immobilization stress for 8 h daily for 7 days. On 29th day, all the rats were sacrificed. Blood and vital body tissues such as kidney and liver were collected and the antioxidative parameters were evaluated. Absorbance of all the estimations was read using Double beam UV-VIS spectrophotometer.

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; Yagi, 1989). The washed erythrocyte pellets were suspended in PBS of pH 7.4 and kept at 4ºC till further analysis. This 33% packed RBC was used for the estimation of lipid peroxidation and reduced glutathione. The 1:10 dilution of packed erythrocytes in PBS (pH 7.4) was used for the estimation of catalase and superoxide dismutase. Catalase was assayed in erythrocytes by spectrophotometric method as described by Bergmeyer (1983). 1:10 dilution of haemolysate was used for estimation of Catalase. Superoxide dismutase (SOD) was estimated as per the method described by Madesh and Balasubramanian (1998).

Membrane peroxidative damage in erythrocytes was determined in terms of malondialdehyde (MDA) production by the method of Rehman (1984). Reduced glutathione (GSH) was estimated by the 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) method of Prins and Loos (1969).

***Preparation of tissue homogenate*:** Frozen liver and kidney 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). Reduced Glutathione (GSH) was estimated by estimating free -SH groups, using DTNB method of Sedlak and Lindsay (1968). For GSH, 10% homogenates were made in 0.02 M EDTA.

Statistical analysis: Statistical analysis of the data was done using ANOVA technique according to the method described by Snedecor and Cochran (1967). Comparisons

among the treatment groups were made. Statistically significant difference was considered at 5% level.

RESULTS

Both AL and AQ restored the increased LPO (table 1) and decreased GSH and SOD (table 2) significantly in immobilized rats and non-significant in normal rats. Similar patterns were observed for erythrocytes, liver and kidney.

Table-1: Effect of *Podophyllum hexandrum* on antioxidative parameters in erythrocytes, liver and kidney of immobilization stressed rats.

	ERYTHROCYTES		LIVER		KIDNEY	
Parameters	LPO (nM MDA/ml)	GSH (mM/ ml)	LPO (nM MDA/ml)	GSH (mM/ ml)	LPO (nM MDA/ml)	GSH (mM/ ml)
Control	15.92±1.05	0.83±0.10	122.63±5.05	0.67±0.05	55.68±5.34	0.67±0.03
AQ	14.58±1.02	0.88±0.01	127.29±4.27	0.7±0.05	53.54±1.70	0.61±0.01[a]
Vit C	12.85±0.91[a]	0.94±0.02[a]	112.08±2.97[a]	0.86±0.03[a]	42.69±1.68[a]	0.83±0.03[a]
AL	13.57±0.50	0.85±0.07	130.55±1.03	0.67±0.02	43.04±2.16	0.62±0.05
IS	20.59±2.81[a]	0.61±0.04	150.87±2.15[a]	0.57±0.01	80.07±2.93[a]	0.49±0.04
AQI	16.15±2.46	0.80±0.02[b]	133.39±1.79[b]	0.61±0.01[b]	76.49±1.91[a]	0.51±0.03
ALI	15.15±0.67[c]	0.76±0.12[c]	137.67±3.50	0.60±0.05	71.92±4.24[c]	0.54±0.02[c]

- Values in table are Mean±S.E.(n=6)
- [a]P<0.05 vs control within same column
- [b]P<0.05 vs group AQ within same column
- [c]P<0.05 vs group AL within same column

Table- 2: Effect of *Podophyllum hexandrum* on SOD level of immobilization stressed rats.

Parameters	SOD(RBC)	SOD(LIVER)	SOD(KID)
Control	61.72±2.43	44.49±3.73	51.83±5.41
AQ	64.36±3.45	50.82±4.83[a]	56.72±6.34[a]
Vit C	68.16±4.29[a]	57.82±5.86[a]	60.55±4.81[a]
AL	59.83±4.43	53.18±6.29	54.29±5.51
IS	50.41±5.19[a]	29.86±3.06[a]	39.83±2.92[a]
AQI	54.36±4.97	35.49±4.02[b]	48.15±2.99[b]
ALI	56.44±5.18[c]	39.81±5.77[c]	46.41±5.44[c]

- Same as given in table-1.

DISCUSSION

All organisms have their own cellular antioxidative defence system, composed of both enzymatic as well as non-enzymatic components. Enzymatic pathway consists of SOD, CAT and GPx. Under normal physiological condition, animals maintain a balance between generation and Neutralization of reactive oxygen species (ROS). The O_2^- are dismutated by SOD to H_2O_2 which is reduced to water and molecular oxygen by CAT or is neutralized by GPx. However when organisms are subjected to any kind of oxidative stress, rate of production of ROS, such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and peroxyl radical (ROO^-) exceeds their scavenging capacity.

Immobilization stress resulted in an overall reduction in the antioxidant defense system in leading to disruption of pro-antioxidant balance in the immobilized body. Increase in LPO in RBC might be due to peroxidation of unsaturated fatty acids in plasma membrane phospholipids of RBC. Elevation of LPO in RBC suggests formation of free radicals and participation of free radical-induced oxidative cell injury in immobilized rats. Increased LPO in RBC is suggestive 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). LPO was also measured in vital organs such as liver and kidney in present study. There was significant increase in LPO in these tissues in immobilized groups. In tissues, increased LPO suggests increased production of MDA, which signifies generation of ROS in these tissues through oxidative damage to cell membrane.

While LPO increased, there was marked reduction in reduced glutathione in erythrocytes, liver and kidney in immobilized rats. This was further associated with decrease in activity of antioxidant enzymes GSH levels in RBC. GSH plays an important role in the protection of cells against oxidative damage caused by ROS. GSH also reacts nonenzymatically with ROS. Oxidation-Reduction coupling of GSH is central to the cellular response to oxidative stress. The balance between oxidation of GSH to glutathione disulfide (GSSG) and the rapid reduction of GSSH by glutathione reductase contributes to the maintenance of the cellular homeostatic GSH:GSSG ratio of about 300:1 (Alpert and Gilbert, 1985). It is authenticated that oxidative stress (Kumar, et al., 2005) reduces GSH level by depleting -SH groups. Since GSH is utilized for detoxification of free radicals, increased sensitivity to oxidative stress also occurs when cells are depleted of GSH (Chen, et. al*.,* 1997).

The erythrocytic antioxidant metabolites and enzymes act as imp antioxidant in whole blood. Superoxide dismutase is a natural antioxidant of the body. Significant decrease in RBC, liver and kidney SOD is seen due to immobilization stress. However it was restored back to a significant level by AL and AQ. However, total activity of the enzymes in whole blood did not change appreciably because there was a dramatic decrease in RBC number. These findings may suggest that both AQ and AL may enhance the natural antioxidant efficiency of the body. Our findings regarding SOD in the present study are well supported with the findings of Khopde*,* (2001), Bhattacharya, et al., (2000), Benov et al., (1990) and Panda and Kar, (2003).

In the present study, *P. hexandrum* extracts, both AL and AQ showed marked decrease in lipid peroxidation and increase in GSH content in erythrocytes and hepatic and kidney tissues of normal and stressed rats. *Podophyllum* consists of active flavonoids, which cause marked increase in antioxidant potential by an increase in the activities of SOD, LPO and glutathione peroxidase (Bhattacharya, et al., 1997).

CONCLUSION

Current studies have reveled that oral administration of AQ and AL can render protection against the oxidative stress induced by immobilization and may prove useful in providing antioxidant therapy necessary for the prevention of free radical-mediated oxidative cell injury. The extracts also provided significant protection to the vital organs. However, further detailed studies in this direction are warranted to use this fraction in humans against free radical induced injuries.

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