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Effect of long dose exposure of *Podophyllum hexandrum* methanol extract on antioxidant defense system and body and organ weight changes of albino rats

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ABSTRACT

Objective: The present study was undertaken to investigate the effect of long dose administration of methanol rhizome extract of *Podophyllum hexandrum* and hydrogen peroxide on lipid peroxidation of erythrocytes, antioxidant enzyme status of rat liver, kidney, lung and brain tissue and body weight and organ weight changes of albino rats. **Methods:** The body and organ weight was monitored with digital scale balance and lipid peroxidation of RBC ghost membrane was monitored by measuring malonaldehyde (MDA). Antioxidant enzymes were assayed by standard procedures. **Results:** Our study showed that administration of H_2O_2 (0.1%) in drinking water of the rats for 25 weeks increased the malondialdehyde levels in erythrocytes of all the rats. However, rats receiving *Podophyllum hexandrum* extract and α – tocopherol had lower MDA levels in a dose dependent manner, which indicates decreased lipid peroxidation in these rats. Our results also showed decrease in the activity of glutathione reductase, glutathione–S–transferase and reduced glutathione levels in different organs of H_2O_2 treated rats. Rats receiving methanolic extract of *Podophyllum hexandrum* at the concentration of 5, 10 and 15mg% for 25 weeks increased the activity of glutathione reductase, glutathione–S–transferase and glutathione levels in different organs of the rats indicates the protective effect of the plant in combating oxidative stress undergone by the rats. No significant variation ($P< 0.05$) in the organ weights between the control and the treated groups was observed after 25 weeks of treatment. **Conclusions:** In conclusion, this study presents strong evidence of the nontoxic effect of the methanol extract of *Podophyllum hexandrum*. The findings also demonstrate that *Podophyllum hexandrum* methanol extract increased the levels of antioxidant enzymes and decreased lipid peroxidation in albino rats and explained the extensive utilization of the plant in traditional medicine.

1. Introduction

From prehistoric times, various communities and civilizations throughout the world have been using herbal medicines but its consumptions in the developing countries have shown an increasing momentum since several decades. They are traditionally considered as harmless as they belong to natural sources. Herbal formulations are fast attaining widespread acceptability as therapeutic agents like antidiabetics, antiarthritics, aphrodisiacs, hepatoprotective, cough remedies and memory enhancers. Although it is important to determine the efficacy of herbal products but their safety should also be assessed as all the herbal medicines are not harmless[1].

Podophyllum hexandrum Royle (Berberidaceae) grows in the Kashmir valley at high altitudes and is locally known as Banwangon. The dried rhizomes and roots of this plant form the source of medicinal activity. Podophyllin, a resin from the plant is commonly used as a cholagogue, purgative, emetic and a bitter tonic. The rhizome powder as such is used as a laxative or to get rid of intestinal worms and also used as poultice to treat warts and tumorous growths on the skin. Podophyllatoxin, a natural lignin from the plant is currently being used as a precursor to semi–synthetic anticancer drugs like etoposide, teniposide, and etopophos. These compounds have been used for the treatment of lung and testicular cancers as well as certain leukemias[2]. Cytotoxic lignins derived from Podophyllatoxin are currently used in cancer chemotherapy. Besides, extracts

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of the plant are used in topical medications for genital warts and some skin cancers. *Podophyllum hexandrum* has been extensively exploited in traditional Ayurvedic system of medicine for treatment of a number of ailments like Taenia capitis, monocytoid leukemia, Hodgkins disease, non-Hodgkin's lymphoma, cancer of brain, lung, bladder and venereal warts[3]. Traditional utility of *P. hexandrum* has also been reported against constipation, cold, biliary fever, septic wound, burning sensation, erysipelas insect bite, mental disorders, rheumatism, plague[4] and also to provide symptomatic relief in some of the allergic and inflammatory conditions of skin.

Cells use molecular oxygen as a terminal electron acceptor and produce reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and peroxyl radical. These ROS are very unstable and highly reactive, and are thus used to synthesize and degrade organic compounds. Moreover, ROS cause peroxidation of cell membrane lipids, denaturation of proteins and nucleic acids, and cardiovascular diseases[5-6].

Hydrogen peroxide (H_2O_2) has been shown to induce oxidative stress in both human and animal models, leading to the generation of potent reactive oxygen species (ROS) such as hydroxyl radical ($OH^{\bullet}$). Ward et al.,[7] have suggested that the cytotoxicity of H_2O_2 is associated with local multiplication of damaged sites on DNA. Such oxidative damage is known as one of the mechanisms leading to chronic diseases, such as atherosclerosis, aging, cancer and rheumatoid arthritis [8]. Almost all organisms are well protected against free radical damage by oxidative enzymes, such as superoxide dismutase and catalase. However, these enzymes are frequently insufficient when it comes to completely preventing ageing-associated diseases and health problems[9-10].

The present study is aimed to assess the effect of 25 week (6 month) exposure of experimental rats to methanol extract of the rhizome of *Podophyllum hexandrum* on body and organ weights. We have also examined the effect on the erythrocyte lipid peroxidation and some antioxidant enzymes in the H_2O_2 induced oxidative stress in different organs of albino rats.

2. Materials and methods

2.1. Plant material collection and extraction

The rhizome of *Podophyllum hexandrum* was collected from higher reaches of Aharbal, Shopian, J&K, India during May and June 2009, identified and authenticated by the Centre of Plant Taxonomy, Department of Botany, University of Kashmir. A reference specimen has been retained in the herbarium of the Department of Botany at the University of Kashmir under reference number KASH- bot/Ku/PH- 702-SAG.

The plant material (rhizome) was dried in the shade at 30 ±2℃. The dried rhizome material was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. The powder obtained was extracted with methanol using a Soxhlet extractor (60-80℃). The extract was then concentrated with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for further use.

2.2. Experimental Animals

Adult male albino rats of Wistar strain weighing 200-250 g used throughout this study were purchased from the Indian Institute of Integrative Medicine Jammu (IIIM). The animals had access to food and water ad libitum. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12 h light and dark cycle. The animals were maintained in accordance with the guidelines prescribed by the National Institute of Nutrition, Indian Council of Medical Research, and the study was approved by Institutional Animal Ethics Committee of the University of Kashmir.

Thirty-six adult albino rats, approximately 2 months old, weighing in the range of 200-250 g, were selected for the study. All the rats were healthy and not infected with virus or bacteria. The rats were housed for 25 weeks in separate cages, into six groups (six rats per group) and kept at 23± 2 °C, with a 12h light dark cycle. They were provided with a standard laboratory diet and water ad libitum. The rats were randomly divided in to six groups and treated for 25 weeks as follows:

Group 1 was given only normal diet.

Group 2 was given normal diet and 0.1% H_2O_2 in drinking water.

Group 3 was given normal diet, 0.1% H_2O_2 and 10 mg% of commercially available α-tocopherol in drinking water.

Group 4 was given normal diet, 0.1% H_2O_2 and 5mg% methanol extract of *Podophyllum hexandrum*.

Group 5 was given normal diet, 0.1% H_2O_2 and 10mg% methanol extract of *Podophyllum hexandrum*.

Group 6 was given normal diet, 0.1% H_2O_2 and 15mg% methanol extract of *Podophyllum hexandrum*.

Blood samples taken at the end of each month was collected from retro orbital plexus and drawn into the tubes containing EDTA as an anticoagulant for erythrocyte (ghost membrane) preparations. The weight of each animal in every group was also measured at the end of every month. At the end of the experiment, all the rats were sacrificed and liver, kidney, lung and brain were isolated, washed in ice cold 1.15% KCl and homogenized. The homogenate was centrifuged at 9000 *g* for 20 min to remove debris. The supernatant was further centrifuged at 15000 *g* for 20 minutes at 4 °C to get PMS (Post mitochondrial supernatant) hat has been used for the estimation Glutathione (GSH), glutathione reductase (GR) and glutathione-S-transferse (GST). Before homogenizing, liver, kidney, lung, heart, spleen and brain

tissues were excised and dabbed with filter paper to remove blood and other liquid. They were then weighed using a top loading balance. Protein concentration was estimated by the method of Lowry[11].

2.3. Lipid peroxidation assay

Lipid peroxidation of ghost membrane was measured by using the method of (Buege & Aust[12]. Ghost membranes (0.5 ml) were mixed with 5 μl of 10mM EDTA and 1 ml (TBA-TCA-HCl) solution and placed in a boiling water bath for 15 min. After cooling, the flocculent precipitate was removed by centrifugation at 6000 rpm for 10 min. The absorbance of malonaldehyde complex was then measured at 532 nm. Malonaldehyde formed from the degradation of poly unsaturated fatty acids, was then calculated by using an extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

2.4. Glutathione Reductase activity (GR)

GR activity was assayed by the method of Sharma[13]. The assay mixture consisted of 1.6 ml of sodium phosphate buffer (0.1 M pH 7.4), 0.1 ml EDTA (1mM), 0.1 ml of 1mM oxidized glutathione, 0.1ml of NADPH (0.02mM), 0.01 ml of 1mM H_2O_2 and 0.1 ml PMS in a total volume of 2 ml. The enzyme activity measured at 340 nm was calculated as nmoles of NADPH oxidized/min/mg of protein using € of $6.22 \times 103\ M^{-1}\ cm^{-1}$.

2.5. Glutathione-S-transferase (GST) activity

GST activity was assayed using the method of Haque[14]. The reaction mixture consisted of 1.67 ml sodium phosphate buffer (0.1 M pH 6.5), 0.2ml of 1mM GSH, 0.025ml of 1mM CDNB and 0.1ml of PMS in a total volume of 2ml. The change in absorbance was recorded at 340nm and the enzyme activity was calculated as nmoles of CDNB conjugates formed/min/mg protein using € of $9.6 \times 103\ M^{-1}\ cm^{-1}$.

2.6. Reduced glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman[15]. 0.5ml of homogenate precipitated with 100 μl of 25% TCA, was subjected to centrifugation at 300xg for 10 minutes to settle the precipitate. 100 μl of the supernatant obtained was added to the test tube containing the 2ml of 0.6mM DTNB (Ellman, s reagent) and 0.9ml of 0.2mM sodium phosphate buffer (pH 7.4). The yellow color obtained was measured at 412nm against the reagent blank which contained 100 μl of 25% TCA in place of the supernatant. Sulphydryl content was calculated using the DTNB molar extension coefficient of 13100.

2.7. Statistical analysis

The values are expressed as mean±standard deviation (SD). The results were evaluated by using the SPSS (version 12.0) and Origen 6 softwares and each experiment was performed at least thrice.

3. Results

3.1. Effect on lipid peroxidation in RBC ghost membrane

The localization of radical formation resulting in lipid peroxidation, measured as MDA in erythrocyte ghost membrane is shown in Table 1. Malondialdehyde (MDA) contents in erythrocyte ghost membrane were significantly increased in group II rats receiving H_2O_2 only. Effect of the H_2O_2 and *Podophyllum hexandrum* extract on RBC ghost membrane LPO was recorded month wise. At the end of the first month about 7.043±0.1887 nmoles of MDA were formed in the erythrocyte membrane, compared to normal group where only a value of 4.25±0.250nmoles of MDA were calculated. Lipid peroxidation was observed to be increased at the end of every month (Table 1). At the end of 6th month about 11.76±0.69nmoles of MDA was observed as compared to normal group where only 8.033±0.92nmoles of MDA was formed. Groups receiving methanol extract of *Podophyllum hexandrum* at the concentrations of 5, 10 and 15mg% decreased the MDA level in a dose dependent manner to 6.300±0.200, 5.30±0.180 and 4.95±0.180nmoles of MDA/mg protein in the first month and 10.68, 10.28 and 9.31nmoles

Table 1

MDA formation in RBC ghost membrane of different groups of rats in long term study (nmoles of MDA formed/mg protein)

Groups	Month1	Month2	Month3	Month4	Month5	Month6
Group 1Normal control	4.25±0.25	4.43±0.28	5.43±0.28	6.39±0.30	7.39 ±0.30	8.033±0.92
Group IIH2O2 treated	7.04±0.188	7.35±0.20	8.35±0.20	9.52±0.43	10.70±0.59	11.76±0.69
Group III5mg% extract	6.30±0.20abc	6.66±0.38abc	7.67±0.37abc	8.68±0.38abc	9.68±0.37abc	10.68±0.37abc
Group IV10%mg extract	5.30±0.18abc	6.15±0.16abc	7.32±0.35abc	8.30±0.34abc	9.32±0.31abc	10.28±0.23abc
Group V15mg% extract	4.95±0.18abc	5.48±0.25abc	6.48±0.25abd	7.41±0.30abd	8.31±0.38abd	9.31±0.38abd
Group VIVitamin E	4.30±0.18eb	4.98±0.12ab	6.11±0.25ab	7.09±0.28ab	7.66±0.72ab	8.66±0.72ab

Each value represents the mean±SD of 6 animals. a; $p < 0.001$, as compared with normal control group, b; $P < 0.001$ as compared with H_2O_2 group, c; $P < 0.001$ as compared with Vit.E, d; non significant as compared with Vit. E, e; non significant as compared with normal control. The data were presented as mean±S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t - test to detect inter group differences. Differences were considered to be statistically significant if $P < 0.05$.

Table 2

Effect t of Podophyllum hexandrum methanol extract on enzymatic and non enzymatic makers of Liver, Kidney, Lung and Brain tissues of H2O2 treated rats

Groups	Tissue Enzymatic markers								Tissue Non Enzymatic markers			
	GR(nmoles of NADPHutilized/minute/mg protein)				GST(nmoles of CDNB conjugated /minute/mg protein)				GSH (nm/g protein)			
	Liver	Kidney	Lung	Brain	Liver	Kidney	Lung	Brain	Liver	Kidney	Lung	Brain
1	89.25±9.26	64.26±5.28	48.81±4.16	25.22±0.89	17.6±3.2	13.5±1.7	8.817±0.2	11.99±0.20	80.6±3.5	63.1±2.7	33.3±2.5	26.5±2.1
2	12.49±0.8	12.94±2.3	6.58±1.5	5.34±1.1	4.21±1.0	6.17±1.0	3.87±0.2	3.68±0.1	64.97±2.1	27.6±2.6	3.2±0.7	6.0±1.4
3	75.1±4.5ab	56.0±12.5cb	36.3±1.0ab	22.7±0.6cb	15.1±3.0eb	11.1±0.5ab	6.9±0.8ab	10.8±0.382ab	79.7±1.4ib	56.5±2.0ab	24.8±2.3ab	19.1±1.775ab
4	22.1±0.9abc	50.6±3.0abd	10.0±0.8abc	6.8±0.7abc	8.3±0.2abc	4.8±0.7abc	4.8±07abc	5.5±0.4abc	74.6±7.90ebf	34.0±2.0abc	6.5±1.1abc	11.6±0.9abc
5	61.9±10.9abc	50.6±3.0abd	14.1±0.8abc	13.9±2.7abc	7.7±1.1agc	9.1±0.3abc	5.5±0.2abc	6.9±0.2abc	77.6±1.2ibf	42.4±2.4abc	11.9±2.1abc	12.6±0.5abc
6	72.1±5.3abd	51.3±3.8abf	22.0±2.2abc	16.9±0.8abc	12.7±2.1abd	10.7±2.1abd	10.7±0.62abd	6.7±0.3abd	8.8±0.3abc	78.3±3.3ibf	18.3±2.0abc	16.50±0.7abc

Each value represents the mean ±SD of 6 animals. a; p < 0 .001, as compared with normal control group, b; $P < 0.001$ as compared with H_2O_2 group, c;$P < 0.001$ as compared with Vit.E, e; non significant as compared with normal control, d; non significant as compared with Vit.E, f; do not test as compared with Vit.E, g; non significant as compared with H_2O_2 group, i; do not test as compared with control group. . The data were presented as mean ±S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t – test to detect inter group differences. Differences were considered to be statistically significant if $P< 0.05$.

of MDA/mg protein at the last month respectively. So we observed that LPO increased with the increase in the exposure time. However a concentration dependent protection was observed with the plant extract in all the months of the study including the terminal month.

3.2. Effect on antioxidant enzymes

3.2.1. Effect on Glutathione reductase

GR helps to maintain the redox/oxidative state of the cell/tissue, which is essential for cell survival. Long term treatment of rats with hydrogen peroxide resulted in the depletion of glutathione reductase in liver, kidney, lung and brain tissue homogenates as depicted in Table 2. The activity of the enzyme was reduced to 12.491, 12.947, 6.587 and 5.346 (nmoles of NADPH utilized/minute/mg protein) from their control values of 89.255, 64.262, 48.814 and 25.224 (nmoles of NADPH utilized/minute/mg protein) in all the tested organs respectively. In groups IV, V and VI concentration dependent increase in the activity of glutathione reductase was observed in all the tested organs by using methanol extract of *Podophyllum hexandrum* at the dose level of 5, 10 and 15mg% for 25 weeks. Vitamin E at the concentration of 10mg% also restored the enzyme activity to a large extent.

3.2.2. Effect on glutathione-S-transferase

The glutathione-S-transferase activity in liver, kidney, lung and brain tissues were significantly affected in H2O2 treated rats. As observed in Table 2, GST activity was restored in a dose dependent manner in all the tested organs. At the higher concentration of plant extract (15mg %), the GST activity was restored to 12.755 (liver), 10.726 (kidney), 6.778 (lung) and 8.860 (brain) as compared to H_2O_2 treated group where only 4.214 (liver), 6.172 (kidney), 3.874 (lung) and 3.680 (brain) activity was observed. Similar results were observed with vitamin E treated group.

3.2.3 Effect on total sulphydryl groups

In the normal control group, the GSH level was found to be 80.626 (Liver), 63.125 (Kidney), 33.385 (Lung) and 26.593nm/g protein (Brain). However, the group treated with H2O2, decreased the GSH level to 64.975 (liver), 27.636 (kidney), 3.218 (lung) and 6.056 (brain) nm/g protein. Treatment with methanol extract of *Podophyllum hexandrum* at a concentration of 5, 10 and 15mg% for 25 weeks increased the GSH content in a dose dependent manner (Table 2). At the higher concentration of plant extract (15mg %), the GSH levels restored to 78.320 (liver), 49.298 (kidney), 18.385 (lung) and 16.496nm/g protein (brain). Group III animals treated with α – tocopherol afforded good protection and increased the GSH level to 79.708 (liver), 56.553 (kidney), 24.885 (lung) and 19.186 (brain).

3.3. Body weight and organ weight changes

Male albino rats were recruited for long term studies (6 months) having the weight of 200-220g. Body weights were measured at the end of each month. Group I animals receiving drinking water and pellet observed large weight gain, followed by group II and group III animals which receives H_2O_2 and alpha tocopherol. There was no significant differences in the overall body weight gain for groups I, II and III (242.66, 238.33 and 241.66) at the end of first month and 310.83, 302.50 and 308.33 at the end of sixth month, respectively. A dose dependent decrease in the body weight was found in the rats of group IV, V and VI receiving extract of *Podophyllum hexandrum* at the dose level of 5, 10 and 15mg% (Table 3).

No significant difference was detected in liver weights between the control and the rats treated with H2O2. However, the rats treated with methanol extract of *Podophyllum hexandrum* for 25 weeks showed some decrease in the relative weight of the liver (Fig. 1).

The kidney, lung, heart and brain weight of both H_2O_2 and control groups did not show any significant difference, however the rats treated with methanol extract of *Podophyllum hexandrum* at the dose level of 5, 10 and 15mg% showed some decrease in organ weights in

Table 3

Effect of *Podophyllum hexandrum* methanol extract on body weight changes in different groups of albino rats.

Groups	Month1	Month2	Month3	Month4	Month5	Month6 Group 1
Normal control	242.66±14.71	249.16±14.63	268.50±3.93	284.16±7.35	295.83±12.41	310.833±11.58
Group IIH_2O_2 treated	238.33±13.29	240.00±23.45	261.66±13.99	281.66±20.65	293.51±10.60	302.50±9.35
Group IIIVitamin E	241.66±18.34 NSa	248.33±18.34NSa	262.15±10.61$a	279.01±12.35$a	294.61±13.17NSa	308.33±12.11dc
Group IV5mg% extract	232.50±20.91$a@	238.11±10.51$a@	258.33±13.66$ans	268.50±3.93$#ns	285.16±7.35$#@	295.31±9.36NScb
Group V10%mg extract	231.45±23.14$a@	235.00±16.18$a@	253.37±17.22$#@	261.66±13.29$#@	271.66±11.51$#@	290.66±10.68$cns
Group V15mg% extract	228.35±10.41$#@	232.56±20.65$#@	248.33±18.34$#@	258.03±10.17$#@	268.40±3.93$#@	284.33±9.35$a@

Each value represents the mean±SD of 6 animals. $; $P < 0.001$, as compared with normal control group, #; $P < 0.001$ as compared with H_2O_2 group, @; $P < 0.001$ as compared with V.E, NS; non significant as compared with normal control, ns; non significant as compared with V.E, a; non significant as compared with H2O2 group, c; do not test as compared with H_2O_2 group, d; do not test as compared with control group, b; dot not test as compared with V.E. The data were presented as means±S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t − test to detect inter group differences. Differences were considered to be statistically significant if $P< 0.05$.

comparison to the control group (Table 3).

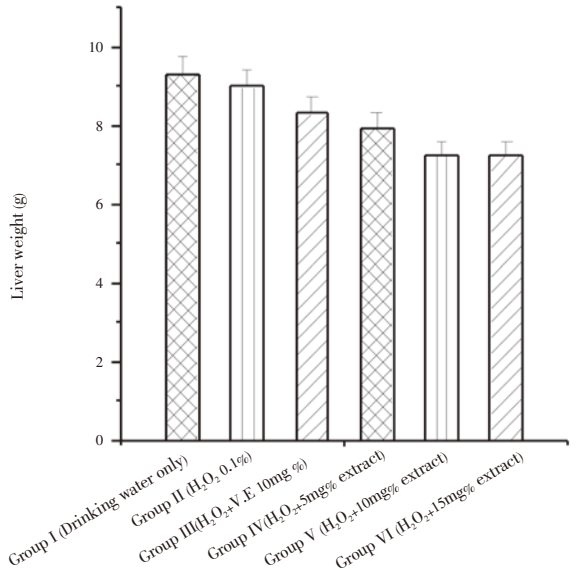

Figure 1. Histogram indicates marked variation in liver weights between control and treated groups. The data were presented as mean ±S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t − test to detect inter group differences. Differences were considered to be statistically significant if $P< 0.05$.

4. Discussion

Reactive oxygen species (ROS) are generated by many redox processes that normally occur during metabolism in aerobic cells. If not eliminated, ROS can attack important biological molecules, such as lipids, proteins, DNA, enzymes, and RNA. Thus, ROS are involved in a number of degenerative diseases such as cancer, cirrhosis, diabetes, and Alzheimer's disease. Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of chemo preventive drug discovery and development[16]. Lipid peroxidation is a free radical mediated process and acts as a potential marker of susceptibility of early and irreversible tissue damage. The levels of TBARS in RBC ghost membrane were elevated in H_2O_2 treated group when compared with control group. At the end of the study (6th month) a large MDA formation was observed in the group II animals receiving H_2O_2. We also observe some increase in MDA content in group I animals receiving drinking water only. This significant increase of MDA level in the H_2O_2 treated rats indicated the possibility of increased radical production, and higher rate of lipid peroxidation in these rats. However, the MDA increase experienced by normal rats may probably be associated with aging[17]. Nohl,[18] indicated that MDA levels were increased with age in healthy subjects and reported accumulation of lipid peroxidation products in during aging.

Our study reveals that administration of *Podophyllum hexandrum* methanol extract for six months significantly lowered the level of lipid peroxidation in H_2O_2 treated rats in a dose dependent manner and proved that *Podophyllum hexandrum* has excellent antioxidant activity.

Glutathione reductase is concerned with the maintenance of cellular level of GSH especially in the reduced state by effecting fast reduction of oxidized glutathione to reduced form. The enzyme is found both in cytosol and mitochondria. GR is inactivated after reduction by its own electron donor, NADPH. The activity of GR may reflect the physiological needs of the cell. For example, intracellular NADPH (40 to 50mM) inactivates GR in absence of GSSG and decreases glucose metabolism via HMP pathway. Lung GR activity depends on availability of NADPH through HMP shunt that depends on G6PD activity[19]. Results of the present study revealed that the GR activity of liver, kidney, lung and brain tissues were decreased drastically in the groups treated with H_2O_2 as compared to their normal control receiving drinking water only (Table 2). Administration of methanol extract of *Podophyllum hexandrum* restored the GR activity in a dose dependent manner in all the four tested organs.

Glutathione-S-transferase plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. Various forms of GST are distinguished by their ability to conjugate different xenobiotics with GSH. Present study showed the significant decrease in the GST activity in H_2O_2 treated rats as compared to the normal group in all the four tested organs. Methanol extract of *Podophyllum hexandrum* significantly increased the GST activity in a dose dependent manner in all the tested organs (Table 2). Similar results were reported by Ganie et al[20] by investigating the long dose exposure of H_2O_2 and *Podophyllum hexandrum* in albino rats.

Glutathione is considered to be the master antioxidant of the body and is found in almost all living cells. It removes free radical species such as hydrogen peroxide, superoxide

radicals and maintains membrane protein thiols. GSH depletion increases the sensitivity of organ to oxidative and chemical injury. Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion [21]. The depletion of GSH, also, seems to be the prime factor that permits lipid peroxidation in the H_2O_2 treated group. Treatment of *Podophyllum hexandrum* methanol extract reduced the depletion of GSH levels and provided protection to the different organs of H_2O_2 treated rats.

Generally, the reduction in body weight gain and internal organ weights is a simple and sensitive index of toxicity after exposure to toxic substances[22]. In our present study rats treated with 5, 10 and 15mg% doses of methanol extract of *Podophyllum hexandrum* had lowered weight in body and organ in comparison to that of control rats. This suggested that probably the animals taking the plant extract have resulted in reduced appetite and therefore significantly lowers the food intake of rats as compared to normal control group. Similar results reported by Spurlock[29] and Zainol[30], suggested that lowered body and organ weights achieved by Centella asiatica fed rats in comparison to that of control rats may be because of reduced appetite of plant extracts or by breaking down of fats.

Results from this study showed that level of MDA and thus lipid peroxidation was significantly increased in the H2O2 treated rats, but this result was not shared by rats fed either with *Podophyllum hexandrum* extract or α-tocopherol. In addition, glutathione reductase, glutathione-S-transferase activity of rats receiving both plant extract and α-tocopherol was found to be significantly higher than that of H2O2 fed rats. This increase in the enzyme activity indicates the antioxidant potential of the plant extract. The methanol extract of *Podophyllum hexandrum* also showed promising antioxidant activity in terms of increasing glutathione levels in H_2O_2 fed rats. In conclusion we have also observed that P. hexandrum rhizome extract have decreased the mean body and organ weight, probably by catabolism of lipids accumulated in adipose tissue or by reduced appetite and therefore significantly lowered the food intake of rats.

Conflict of Interest statements

The authors suggest that they have no conflict of interest.

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