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Original Research Article

Protective Effect of *Bryophyllum pinnatum* on Mercuric Chloride Induced Oxidative Stress in Rats

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A B S T R A C T

Thirty six albino rats (Wistar strain) of 7-9 weeks age weighing around 130-140 g. All rats were divided in four groups of 9 rats each. The Group I without any treatment was kept as control, otherwise Group II (Mercuric chloride @ 2mg/kg BW), III (*Bryophyllum pinnatum* @ 200 mg/kg BW) and IV (Mercuric chloride @ 2mg/kg BW + *Bryophyllum pinnatum* @ 200mg/kg BW) were treated respectively. Lipid peroxidation (LPO) was observed. The LPO levels in liver, kidney and brain increased significantly in rats treated with mercury chloride (II) as compared to control. Lower level in Group IV was noted compared to Group II. The Superoxide dismutase (SOD) levels in liver, kidney and brain were decreased significantly ($P < 0.05$) in Group II as compared to control. These decreased levels were increased in Group IV. The present study suggested that *Bryophyllum pinnatum* given at a dose rate of 200 mg/kg, orally along with mercuric chloride showed mild protection against the toxic effects caused by mercury in rats.

Keywords

Mercuric chloride, *Bryophyllum pinnatum*, oxidative stress, albino rats

Introduction

Mercury (Hg) is ubiquitous in the environment and is inevitable in both human and animals to avoid its exposure in some form or forms of mercury on a regular basis. Mercury occurs widely in the biosphere (Sheikh *et al.,* 2011) and cause severe physiological and bio-enzymological alteration in the tissues of both animal and men (Lund *et al.,* 1993). Large populations worldwide are exposed to relatively low levels of Hg, especially via the use of pesticides in agriculture and of fluorescent light bulbs as well. In this context, Hg exists in a wide variety of physical and chemical states, each of which has specific characteristics for target organs. Exposure to Hg vapour as well as to organic Hg compounds specifically affects the central nervous system, while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuric chloride (Merzoug *et al.,* 2009).

Mercury promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxide. Mercuric chloride $HgCl_2$ causes

oxidative damage in normal cells. Normally $HgCl_2$ has been widely used to study the hemodynamics changes, functional alteration and tissue damages in animals. $HgCl_2$ induced oxidative damage is generally attributed to the formation of highly reactive hydroxyl radical (OH), the stimulator of lipid peroxidation and the sources of destruction of damage to the cell membrane. $HgCl_2$ is one of the most toxic forms of mercury that easily forms organo mercury complexes with proteins, leading to functional and structural alterations in many organs (Vanithasri and Jagadeesan, 2013).

In recent years, an extensive research work has been carried out on chemical protection. Some chemical substances, i.e. sodium 2, 3-dimercapto propane-1- sulphonate, 2, 3-dimercapto succinic acid and dimercaprol (also known as BAL or British antilewisite) have been tested for their protective activity against heavy metal toxicity and found to be promising in the field of chemical protection, but all these chemicals could not have practical utility in human beings due to their inherent toxicity at effective dose. Recently interest has been generated to develop the potential drugs of plant origin for the modification of heavy metal toxic effects. Several naturally occurring dietary or non-dietary constituents, as well as parts of several species of edible plants having pharmacological activity, influence the antioxidant enzymes and provide protection against free radical induced damage (Lakshmi *et al.*, 2014).

The leaves of *Bryophyllum pinnatum* plant have been reported to possess antimicrobial, antifungal, anti-ulcer, anti-inflammatory, analgesic, antihypertensive, antidiabetic and antimutagenic activities. A number of active compounds, including flavonoids, glycosides, steroids, bufadienolides and organic acids have been identified in *Bryophyllum pinnatum* that have been shown individually to possess variety of activities such as antibacterial, antitumor, cancer preventive and insecticidal actions (Afzal *et al.*, 2013). Keeping in view the pharmacological properties of *Bryophyllum pinnatum* the present investigation has been undertaken to assess the protective effect of alcoholic extract of *Bryophyllum pinnatum* on mercuric chloride induced toxicity in wistar rat by using the markers of oxidative stress and antioxidant in liver, kidney and brain tissue.

Materials and Methods

Mercuric chloride ($HgCl_2$), was procured from Merck India Ltd. (Mumbai, India), and dissolved in distilled water and administered *per OS* route. The leaves of *Bryophyllum pinnatum* were obtained from Purba Medinipur district of West Bengal. The leaves were cleaned and chopped into small pieces and dried in shade. The dried plant material was powdered and passed through a coarse sieve. This powder (500 g) was macerated in 95% ethanol for 2 days with occasional shaking 4-5 times daily. It was then filtered using a filtered paper (Whatman size No.1) and the filtrate was evaporated to dryness in water bath at 37ºC. A brownish colour residue weighing 28.5 g was obtained. This was kept in air tight bottle in a refrigerator until use.

Albino rats (Wistar strain) of 7-9 weeks age weighing around 130-140 g were obtained from licensed dealer(Chakraborty Enterprise, 3/1D Vidyaratna Lane, Kolkata), and maintained in an air-conditioned room (25 ± 3 ºC) with a 12 h light/12 h dark cycle. The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal feed Pranav agro-Industries Ltd. Pune, India). All experimental studies were conducted in the

Department of Vety. Pathology, Faculty of Animal and Veterinary Science, W.B.U.A.F.S., Kolkata. The experimental study was approved by the Ethical Committee.

A total of thirty six albino rats of Wistar strain were used in this study and were divided into four groups equally. At the commencement of the study the weight variation of animals was minimized and did not exceed ± 10 per cent of mean weight of each group. The experiment was conducted for a period of 90 days.

Group I: Control, maintained on commercial rat pellet diet and water

Group II: Mercuric chloride (2mg/kg bw) orally (Gavage)

Group III: *Bryophyllum pinnatum* (200 mg/kg bw) orally (Gavage)

Group IV: Mercuric chloride + *Bryophyllum pinnatum* (2mg/kg bw + 200mg/kg bw respectively) orally (Gavage)

Three rats from each group were sacrificed by cervical dislocation at monthly intervals. Detailed necropsy was conducted on rats sacrificed at regular intervals. The whole liver, kidney and brain tissue was isolated immediately from the animals in the cold room and then used for estimation of lipid peroxidation (LPO) by adopting the method of Rehman, (1984) and superoxide dismutase (SOD) by the method of Madesh and Balasubramanian, (1998).

Statistical analysis was performed by two way analysis of variance (ANOVA) and the groups were compared by Duncan's Multiple Range Test (DMRT) using SPSS Software Package, version 20.0. Results were expressed as means ± standard error for three rats in each group at each time interval. A value of $P \leq 0.05$ was considered to be statistically significant.

Results and Discussion

The mean values of LPO in Liver, kidney and tissues of different groups of rats at different time interval are given in Table 1. On 30^{th} day, significantly higher level of LPO in liver was recorded in Group II and Group IV as compared to control (Group I) but significantly higher level of LPO in kidney was recorded in only Group II as compared to control. On 60^{th} and 90^{th} day significantly higher level of LPO in liver and kidney was recorded in Group II and Group IV as compared to control, whereas non-significant variation was observed in Group III. Within the groups there was non-significant time dependant variation in level of LPO was observed. It was also observed that there were no significant variations in LPO level of brain tissue between different groups and within different groups at 30^{th}, 60^{th} and 90^{th} day of experiment. However, the highest value of LPO was recorded in Group II and followed by Group IV and Group III as compared to control.

The mean values of SOD in liver and kidney tissues of different groups of rats at different time interval are given in table 2. On 30^{th} day there was no significant variation in level of SOD in liver and kidney tissues between different groups. On 60^{th} day significantly lower level of SOD was recorded in Group II as compared to control, whereas lower but non-significant variation was observed in Group IV. On 90^{th} day significantly lower level of SOD was recorded in Groups II and IV as compared to control. Within the groups there was significant lower level of SOD in Group II on 60^{th} and 90^{th} day as compared to 30^{th} day of experiment. On the other hand no

significant time dependant variation in level of LPO was observed in Groups I, III and IV. On 30th and 60th day there was no significant variation in level of brain tissue SOD between different groups. On 90th day significantly lower level of SOD was recorded in Group II as compared to Group I, whereas lower but non-significant variation was observed in Group IV.

Within the groups there was non-significant time dependant variation in level of SOD was observed.

In the present experimental study, significantly increased level of LPO was observed in the liver, and kidney tissues of Wistar rats, when treated with mercuric chloride whereas in brain tissue minute increase of LPO was recorded. Our finding is in accordance with the report of Jagadeesan and Pillai (2007) who observed significant increase level of LPO in mercury intoxicated liver tissue of rats. The present finding was also supported by Kavitha and Jagadeesan (2006) who observed significant increase level of LPO in mercury intoxicated kidney tissue of mice. The significant increase in lipid peroxidation level in mercuric chloride intoxicated rat could lead to the damage of plasma membrane of the respective tissue which is causing oxidative stress induced by mercuric chloride (Bharathi *et al.*, 2012 and Rao *et al.*, 2000)·The increase in the levels of LPO indicates enhanced lipid peroxidation leading to liver, kidney and brain tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals. During the recovery span, the level of LPO content in the respective tissue was found to be near normal level in *Bryophyllum pinnatum* treated on mercuric chloride intoxicated rats. Administration of *Bryophyllum pinnatum* treatment normally decreases the production of LPO in respective tissue of mercury intoxicated rat, which in turn might protect the system against the toxic manifestation of OH radical and H_2O_2.

Table.1 Lipid Peroxidation (nM MDA/g) in liver, kidney and brain tissues of rats of different groups at different time intervals (n=3 in each group)

Organs		**30 Days**	**60 Days**	**90 Days**
Liver	Group I	19.25 ± 0.75^{A}	20.12 ± 1.01^{A}	20.52 ± 1.24^{A}
	Group II	24.34 ± 0.85^{Ca}	28.75 ± 1.32^{Bb}	30.25 ± 0.34^{Bb}
	Group III	19.80 ± 1.16^{AB}	20.56 ± 1.25^{A}	20.76 ± 1.14^{A}
	Group IV	23.12 ± 1.47^{BC}	26.15 ± 1.61^{B}	28.16 ± 1.25^{B}
Kidney	Group I	21.20 ± 0.83^{A}	21.36 ± 1.14^{A}	21.52 ± 0.91^{A}
	Group II	26.84 ± 1.14^{B}	28.65 ± 1.09^{C}	29.54 ± 0.91^{B}
	Group III	21.25 ± 1.21^{A}	21.41 ± 0.69^{A}	21.49 ± 1.05^{A}
	Group IV	24.36 ± 1.02^{AB}	25.24 ± 1.07^{B}	25.96 ± 1.45^{B}
Brain	Group I	4.35±0.40	4.49±0.29	4.52±0.31
	Group II	4.81±0.17	4.96±0.19	5.19±0.18
	Group III	4.47±0.45	4.50±0.16	4.86±0.16
	Group IV	4.61±0.29	4.76±0.21	5.01±0.18

Mean±S.E bearing at least one common superscript (A, B, C and a, b, c) do not differ significantly between groups and days respectively ($P<0.05$).

Table.2 Superoxide Dismutase (U) in liver, kidney and brain tissues of rats of different groups at different time intervals (n=3 in each group)

Organs		30 Days	60 Days	90 Days
Liver	Group I	19.04±1.02	$18.65±0.17^{B}$	$18.68±0.27^{C}$
	Group II	$17.58±0.10^{b}$	$17.15±0.05^{Ab}$	$16.36±0.18^{Aa}$
	Group III	19.05±0.79	$18.60±0.13^{B}$	$18.74±0.34^{C}$
	Group IV	18.19±0.24	$17.96±0.50^{AB}$	$17.72±0.11^{B}$
Kidney	Group I	$8.54±0.21^{B}$	$8.49±0.21^{B}$	$8.55±0.95^{B}$
	Group II	$7.65±0.05^{A}$	$7.01±0.10^{A}$	$5.98±0.05^{A}$
	Group III	$8.62±0.32^{B}$	$8.59±0.16^{B}$	$8.70±0.13^{B}$
	Group IV	$7.96±0.10^{AB}$	$7.87±0.46^{AB}$	$7.95±0.21^{B}$
Brain	Group I	3.38±0.60	3.49±0.35	$3.41±0.29^{B}$
	Group II	3.01±0.21	2.94±0.03	$2.16±0.55^{A}$
	Group III	3.46±0.27	3.43±0.15	$3.49±0.29^{B}$
	Group IV	3.16±0.08	3.04±0.23	$2.87±0.01^{AB}$

Mean±S.E bearing at least one common superscript (A, B, C and a, b, c) do not differ significantly between groups and days respectively ($P<0.05$).

A significantly decreased level of SOD in the present experimental study was observed in the liver, kidney and brain tissues of Wistar rats, when treated with mercuric chloride. Our finding is in agreement with the report of Jagadeesan and Pillai, (2007) who observed significant decreased level of SOD in mercury intoxicated liver tissue of rats.

The present finding was also supported by Kavitha and Jagadeesan (2006) reported decreased level of SOD in mercury intoxicated kidney tissue of mice. Vanithasri and Jagadeesan (2013) have reported decrease level of SOD in mercury intoxicated brain tissue of rats.

The decreased levels of SOD activities promote the oxygen intolerance in the respective tissues and triggers several deleterious reactions. It is clearly indicated that ROS acting as a cytotoxic material gets accumulated and leads to cellular damage (Rajakrishnan *et al.,* 1996). During the recovery span, the level of SOD content in the respective tissue was found to be near normal level in *Bryophyllum pinnatum* treated on mercuric chloride intoxicated rats.

This result suggest that the *Bryophyllum pinnatum* can reduce the reactive oxygen free radicals and also improve the antioxidant enzymes activities in the mercuric chloride intoxicated rat tissues (Harlalka and Patil, 2007 and Jain *et al.,* 2010).

In conclusion, mercuric chloride proved to be hepatotoxic, nephrotoxic and neurotoxic effect and administration of *Bryophyllum pinnatum* can reduces the resulting damage probably due to its ability to neutralize or scavenge the free radicals that are generated by mercuric chloride.

This study elucidated the protective role of *Bryophyllum pinnatum* against mercuric chloride toxicity, but the present result suggests that administration of *Bryophyllum*

pinnatum cause mild protection against mercuric chloride toxicity.

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