Modulating role of *Panax ginseng* in phase - II reaction of hepato - biotransformation in Albino rats following mercuric chloride intoxication

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ABSTRACT

Background: The fate of xenobiotics that is present, increasing day by day. The increasing fates altered or inhibit the metabolic activities like detoxification and biotransformation.

Methods: The present study highlights this slow biotransformation and detoxification on the basis of specific enzymes which have a say in assessment of mercuric chloride toxicity and modulation by *Panax ginseng* root extract.

Results: The results revealed that mercuric chloride caused significant increase in liver weight, glutathione - s - transferase (GST), glutathione peroxidase (GP_X), glutathione reductase (GR) and significant decrease in glutathione (GSH) level, while *Panax ginseng* alone and combination with mercuric chloride caused significant decrease in liver weight, glutathione - s - transferase (GST), glutathione peroxidase (GP_X), glutathione reductase (GR) and glutathione (GSH) level.

Conclusion: The results suggest a modulating role of this root powder extract against raised enzyme levels and liver weight induced by mercuric chloride in albino rat.

Key word: Liver weight, Glutathione - s - transferase, Glutathione peroxidase, Glutathione reductase, Glutathione

INTRODUCTION

Ginseng (*Panax ginseng*) has been used for traditional medicine in China, Korea, Japan and other Asians countries for the treatment of various diseases. Ginseng saponins (ginsenosides) have been regarded as the principal component responsible for the pharmacological activities. Ginsenosides are glycosides containing an aglycone with a dammarane skeleton and have been shown to possess various biological activities, including the enhancement of cholesterol biosynthesis(1), stimulation of serum protein synthesis (2), immunomodulatory effect[3)] and anti-inflammatory activity (4). *Panax ginseng* has been reported to have neuroprotective effects on stroke animal model(5,6). Among its diverse effects on the central nervous system, ginseng is known to have improved learning and memory (7) and also reveal oxygen free radical induced lipid peroxidation. It has been strongly suggested to play an important role in the pathogenesis of delayed neuronal damage after global cerebral ischemia (8). Different preparations of *Panax ginseng* are used as a medicine to delay the process of ageing. It prevents weight loss and is considered to be a strong anabolic drug. Recently some workers have reported that *Panax ginseng* has anti-mutagenic and anti- carcinogenic property (9).

The major oxidative free radical scavenging enzymes of phase - II reaction the glutathione - s - transferase (GST) ,

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glutathione reductase (GR), glutathione peroxidase (GP_X) and glutathione (GSH) are instrumental in assessing the modulation by *Panax ginseng* following mercuric chloride intoxication. Deficient functioning of these enzymes leads to accumulation of toxic oxidative free radicals. Such a modulating role of *Panax ginseng* has been highlighted.

MATERIALS AND METHODS

Collection of plant material: The plant root powder was gifted by Prof. A. Kumar, Cancer and Radiation Biology Laboratory, Department of Zoology, Rajasthan University, Jaipur (Rajasthan).

Experimental animals: *Rattus norvegicus* (Berkenhout) weighting approximately (120-130) gm of both the sexes were procured from inbreed colony and acclimatized to the laboratory condition for 2 weeks. The animals were fed with a standard balanced diet (Hindustan Lever Ltd, Mumbai) and water was provided *ad libitum.*

Experimental compound: Experimental compound (mercuric chloride) was obtained from Bayer India Ltd. Mumbai, while other chemicals were procured from Sigma chemical, Germany and SRL, Mumbai. The acute oral LD_{50} was determined in albino rats. The mercuric chloride was dissolved in distilled water and introduced orally by gavage tube. The data were statistically analyzed by probit analysis(10) for LD_{50} determination (Table 1). Rats from the control set were given distilled water only.

Experimental protocol: Animals were divided into 5 groups of 5 rats each. Group I (control) received 1 ml of distilled water and 10 µl tween - 20, group II received *Panax ginseng* (10 mg/kg body weight), group III received mercuric chloride after LD_{50} (9.26 mg/kg body weight) determination for acute (0.926 mg/kg body weight) and sub-acute (0.033 mg/kg body weight) sets, group IV received *Panax ginseng* followed by mercuric chloride, while group V received mercuric chloride followed by *Panax ginseng*. The intake gap between *Panax ginseng* following by mercuric chloride and mercuric chloride following by *Panax ginseng* was 2 hours in IV and V groups respectively. The details of groups and treatment are given below (Table 2, 3 and 4).

Estimation of liver enzymes: The rats were sacrificed and liver was excised out immediately. Liver was homogenized in potter elvenhjem type homogenizer to a concentration of 10% with ice cold KCl (0.15 M) and ultra centrifuged for separating mitochondrial, microsomal and cytosolic fractions (11). The enzyme activities of glutathione-s-transferase (GST)(12), glutathione peroxidase (GP_X)(13), glutathione reductase (GR) (14) and glutathione (GSH) (15) were assayed in cytosolic fraction. Body and liver weight were also recorded.

Statistically significant values between experimental and control were calculated according to Fisher student‘t’ test (16).

RESULTS

Liver weight were significantly ($p<0.001$) increased in acute and sub-acute mercuric chloride treated groups, while it was significantly ($p<0.01$) decreased in *Panax ginseng* treated groups. On the other hand, it was non-significantly ($p>0.05$) increased in mercuric chloride followed by *Panax ginseng* treated and sets *Panax ginseng* followed by mercuric chloride treated set (Table 2).

Increased liver weight in mercuric chloride treated groups may be due to hepatic fibrogenesis (17), while it was decreased in *Panax ginseng* treated group probably due to their antioxidant potential.in Mercuric chloride followed by *Panax ginseng* and *Panax ginseng* followed by mercuric chloride sets, liver weight was decreased in comparison to the mercuric chloride treated set may probably due to released of free radicals of mercuric chloride and sub-sequent scavenging by *Panax ginseng* root extract.

Increased generation of oxidative free radicals or impaired antioxidant defense mechanisms, have been implicated in the ageing process. This is only possible by some specific natural free radical scavenging enzymes in biotransformation process. *Panax ginseng* demonstrates a wide variety of pharmacologic effects, likely due to its structural diversity. Phytopanaxadiols, a group of ginsenosides are containing two glucose moieties on the C - 3 positions, although differing between glucose and arabinose on C - 20. Further, ginsenosides as a whole appear to have free radical scavenging and metal ion chelating abilities, different fractions of phytopanaxadiols appear to exert their antioxidant function via different mechanisms. Although *Panax ginseng* has long been reported to be a scavenger of free radicals, most of the evidenceshavecome through in vitro studies.

The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. GSH offers protection against derived free radicals and cellular lethality following exposure to heavy metals. The results show increased activity of GST in mercuric chloride treated albino rat. GST is a prevalent enzyme of the cytosol and is considered to play a role in detoxification process. The activity of Glutathione - s - transferse (GST) significantly ($p<0.05$) increases in mercuric chloride treated group because glutathione performs non enzymatic addition reaction with free radicals and reactive intermediates of mercuric chloride, while significantly ($p<0.05$) decreases in *Panax ginseng* treated group. It has also been found to be decreased in *Panax ginseng* followed by mercuric chloride and mercuric chloride followed by *Panax ginseng* treated group. However, the reduction has been observed to be more in *Panax ginseng* followed by mercuric chloride (18,19). The activity of hepatic GST gradually increases in mercuric chloride treated rats as compared to untreated rats. Elevated total GST activity has also been shown in viral hepatitis, fulminant hepatitis, acute liver injury caused by chemicals, drugs and chronic alcohalics (20). In the present investigation in mercuric chloride treated albino rat liver GST activity showed significant increase, which is indicative of increased stress due to toxin in the liver tissue. Treatment with *Panax ginseng* is to decrease the total hepatic GST activity. This probably is due to induction of GST - Ya gene which inhibits xenobiotic metabolizing enzymes (Table 3).

The activity of glutathione peroxidase (GPx) was significantly ($p<0.01$) increased in mercuric chloride treated group, while significantly decreased ($p<0.001$) in *Panax ginseng* treated group. The GPx activity gradually decreases in mercuric chloride followed by *Panax ginseng* and *Panax ginseng* followed by mercuric chloride treatment but it was significantly ($p<0.001$) decreased in *Panax ginseng* followed by mercuric chloride treated group (18,19).Administration of *Panax ginseng* promotes the conversion of GSR into GSH by the reactivation of hepatic GSR reductase enzyme in mercuric chloride treated animals. The availability of a sufficient amount of GSH thus increases the detoxification of active metabolites of mercuric chloride through the involvement of GST and GP_X (Table 4).

The activity of glutathione reductase (GR) was significantly ($p<0.001$) increased in mercuric chloride treated group, while significantly ($p<0.05$) decreased in *Panax ginseng* treated group. The GR activity gradually decreases in mercuric chloride followed by *Panax ginseng* and *Panax ginseng* followed by mercuric chloride treated group. *Panax ginseng* significantly depicted GR levels with that of normal. The depiction of GR level in the *Panax ginseng* treated animals could be due to initial higher availability of free radicals, which increases antioxidant ability to cope with the free radicals produced by stress (21) (Table 5).

The activity of glutathione (GSH) was significantly ($p<0.01$) decreased in mercuric chloride treated group. Reduction in GSH levels indicated its role in toxic state. Role of - SH groups in heavy metal toxicity is

well known(22). The depletion of GSH in metal fed animals has also been reported by many investigators(23). It was significantly increased in *Panax ginseng* treated sets, while it was almost similar to control group in mercuric chloride followed by *Panax ginseng* and *Panax ginseng* followed by mercuric chloride treated groups (Table 6).

Table 1, Toxicity evaluation of Mercuric Chloride in Rattus norvegicus specifying fiducial limits

Experimental individual	Compound	Regression equation	LD_{50} (mg/kg body weight)	Variance	Fiducial limits
Rattus norvegicus	Mercuric Chloride	Y = 5.146+3.410 (X - 1.009)	9.26 mg	0.006	m_1 = (+)0.972 m_2 = (-)0.960

Table 2, Liver weight (gm) after successive treatment of mercuric chloride, Panax ginseng and their combinations

Treatment duration	Treatment sets	Control	Mercuric chloride treated	*Panax ginseng* treated	Mercuric chloride followed by *Panax ginseng*	*Panax ginseng* followed by mercuric chloride
6 hrs	Acute	3.30±0.051*	3.38±0.008[b]	3.19±0.005[c]	3.34±0.003[a]	3.29±0.003[a]
12 hrs		3.21±0.02*	3.21±0.005[a]	3.20±0.01[c]	3.19±0.00[a]	3.17±0.01[a]
24 hrs		3.19±0.02*	3.28±0.008[c]	3.10±0.008[c]	3.21±0.01[a]	3.18±0.00[a]
7 days	Sub acute	2.52±0.02*	2.85±0.261d	3.21±0.00[c]	3.22±0.01[a]	3.17±0.01[a]
14 days		3.02±0.18*	3.12±0.008[d]	2.96±0.008[c]	3.30±0.003[b]	3.00±0.008[a]
28 days		3.06±0.16*	3.58±0.01[d]	2.13±0.008[c]	3.30±0.003[a]	3.02±0.008[b]

Abbreviation used
**=Mean ± S.Em. , Student't'*
a=>0.05
b=<0.05
c=<0.01
d=<0.001

Table 3, Glutathione - s - transferase (µ mole conjugate formed/min/g liver) after successive treatment of mercuric chloride, Panax ginseng and their combinations

Treatment duration	Treatment sets	Control	Mercuric chloride treated	*Panax ginseng* treated	Mercuric chloride followed by *Panax ginseng*	*Panax ginseng* followed by mercuric chloride
6 hrs	Acute	81.545±0.17*	87.68±0.78^{a}	80.96±0.40^{a}	82.11±0.08^{b}	80.68±0.27^{a}
12 hrs		81.56±0.19*	88.67±0.30^{b}	80.56±0.31^{b}	83.01±0.04^{c}	79.78±0.29^{b}
24 hrs		81.895±0.15*	90.83±0.19^{c}	79.12±0.13^{b}	82.74±0.32^{a}	81.05±0.08^{b}
7 days	Sub acute	81.66±0.20*	91.48±0.23^{d}	78.41±0.24^{a}	83.03±0.09^{b}	81.13±0.26^{a}
14 days		81.7125±0.19*	93.98±0.06^{d}	77.93±0.10^{b}	83.03±0.03^{b}	80.60±0.17^{b}
28 days		81.8225±0.13*	94.92±0.11^{d}	77.01±0.13^{b}	83.90±0.10^{d}	80.54±0.13^{b}

Table 4, Glutathione peroxidase (µ mole GSH oxidized/min/g liver) after successive treatment of mercuric chloride, Panax ginseng and their combinations

Treatment duration	Treatment sets	Control	Mercuric chloride treated	*Panax ginseng* treated	Mercuric chloride followed by *Panax ginseng*	*Panax ginseng* followed by mercuric chloride
6 hrs	Acute	33.0925±0.22*	33.49±0.20^{a}	31.13±0.08^{d}	32.75±0.37^{a}	28.73±0.13^{d}
12 hrs		33.4375±0.17*	34.36±0.22^{a}	30.43±0.26^{d}	31.73±0.13^{d}	28.95±0.07^{d}
24 hrs		33.355±0.10*	33.93±0.10^{b}	29.95±0.08^{d}	31.14±0.07^{d}	28.98±0.06^{d}
7 days	Sub acute	33.2375±0.17*	35.43±0.16^{d}	29.08±0.12^{d}	32.06±0.06^{b}	29.29±0.09^{d}
14 days		33.2175±0.11*	36.61±0.07^{d}	28.20±0.17^{d}	32.22±0.03^{b}	29.99±0.07^{d}
28 days		33.68±0.14*	38.60±0.07^{d}	27.06±0.17^{d}	32.34±0.04^{c}	30.40±0.27^{d}

Table 5, Glutathione reductase (µ mole GSH oxidized/min/g liver) after successive treatment of mercuric chloride, Panax ginseng and their combinations

Treatment duration	Treatment sets	Control	Mercuric chloride treated	*Panax ginseng* treated	Mercuric chloride followed by *Panax ginseng*	*Panax ginseng* followed by mercuric chloride
6 hrs	Acute	133.15±0.95*	150.0±0.02^{c}	132.0±0.01^{a}	132.34±0.19^{a}	132.95±0.25^{c}
12 hrs		133.16±0.63*	183.0±0.05^{c}	130.0±0.00^{a}	132.23±0.02^{b}	133.09±0.09^{a}
24 hrs		133.23±0.44*	204.0±0.03^{d}	129.0±0.00^{b}	132.00±0.10^{a}	133.29±0.11^{b}
7 days	Sub acute	132.86±0.71*	218.0±0.01^{d}	127.0±0.01^{b}	131.34±0.15^{b}	132.90±0.20^{b}
14 days		130.12±2.38*	235.0±0.00^{d}	126.0±0.01^{b}	131.11±0.07^{d}	133.23±0.06^{b}
28 days		130.85±1.28*	238.0±0.00^{d}	126.0±0.00^{b}	131.21±0.15^{b}	133.21±0.09^{b}

Table 6, Glutathione (µg/100 mg liver weight) after successive treatment of mercuric chloride, Panax ginseng and their combinations

Treatment duration	Treatment sets	Control	Mercuric chloride treated	*Panax ginseng* treated	Mercuric chloride followed by *Panax ginseng*	*Panax ginseng* followed by mercuric chloride
6 hrs	Acute	217±3.25*	180±2.36^{c}	199±1.36^{b}	198±3.12^{b}	200±3.16^{b}
12 hrs		216±4.27*	176±2.26^{c}	208±5.27^{b}	198±2.36^{b}	201±1.72^{b}
24 hrs		218±2.64*	150±3.67^{c}	209±4.34^{b}	199±3.78^{b}	201±1.96^{b}
7 days	Sub acute	218±4.25*	130±2.36^{c}	211±2.36^{b}	200±3.19^{b}	204±2.38^{b}
14 days		218±5.27*	106±1.36^{c}	212±2.67^{b}	201±3.54^{c}	209±1.28^{b}
28 days		217±4.64*	98±2.61^{d}	215±3.16^{b}	202±5.02^{c}	210±5.07^{b}

DISCUSSION

Alteration in hepatic oxidative damage induced by mercuric chloride has been explained on the basis of percentage of biotransformation potential by Panax ginseng with ratio analysis as stress is leads to enhance the generation of free radicals. These free radicals mediate damage of polysaturated fatty acids leading to the destruction of cell membrane and cell organelles. Panax ginseng increases the degree of lipid peroxidation in the time dependent manner. Comparing these data it can be surprised that percentage biotransformed Panax ginseng followed by mercuric chloride is better than the percentage biotransformed mercuric chloride followed by Panax ginseng treatment probably may be is due to the antioxidant and lipid peroxidation activity of Panax ginseng. The significance of the present study reveals that Panax ginseng supplementation could decrease intracellular reactive oxygen species (ROS) generation in the liver which promotes the phase - II reaction enzymes of hepto-biotransformation following mercuric chloride intoxication.

CONCLUSION

A modulating role of this root powder extract against raised enzyme levels and liver weight induced by mercuric chloride in albino rat.

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