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# *Panax* ginseng Extract as Protectant in Mercuric Chloride Induced Alterations in Protein Biochemistry in the Serum of Albino Rats

**K. Mahour[1], Prabhu N. Saxena[1#] and Ashok Kumar[2]**

[1]*Toxicology Laboratory, Department of Zoology, School of Life Sciences, Khandari Campus, DR. B.R. Ambedkar Univeristy, Agra-282002 (U.P.), India*
[2]*Cancer and Radiation Biology Laboratory Department of Zoology University of Rajasthan, Jaipur, Rajasthan, India*
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**Abstract :** Adverse changes in individual's biochemistry under heavy metal stress are directly linked with its metabolic activity and health status. The present investigation highlights the differences in protecting role of *Panax* ginseng extract against mercuric chloride induced alterations in serum proteins. The assessment was based on dividing fifty albino rats into two sets, one for acute and the other for sub-acute study. All the sets had five groups with five albino rats in each i.e. control group, mercuric chloride treated group, *Panax* ginseng extract treated group, mercuric chloride followed by *Panax* ginseng extract treated group and *Panax* ginseng extract followed by mercuric chloride treated group. Mercuric chloride was given orally 0.926 mg/kg body weight for acute set and 0.044 mg/kg body weight for sub-acute set after LD50 (9.26 mg/kg body weight) determination by probitt analysis. 10 mg/kg body weight *Panax* ginseng extract was given in both acute and sub-acute sets after incorporating safety trials. The control group received tween-20 and distilled water only. The result exhibited significantly reduction ($P<0.01$) in serum protein, albumin and globulin following mercuric chloride intoxication whereas significant ($P<0.01$) enhancement in other groups with *Panax* ginseng extract as an ingredient confirming its protective role. All serum samples were also electrophoresed in 10% SDS with standard marker using discontinuous buffering system. Gradual disappearance of alpha-2 and beta-1 globulin bands from electrophoretic pattern was observed, while a single sharp band was observed between beta-2 and gamma globulin in serum protein pattern of acutely mercuric chloride treated rats. However, this band could not be visualized in sub-acute studies. *Panax* ginseng extract exhibits a better protection after acute intoxication.

**Key words:** *Panax* ginseng extract, mercuric chloride, electrophoresis

## INTRODUCTION

Physiology of an individual is the symbol of health status which mainly consists of enzymological functions, biochemical functions and haematological functions. Any change in any part of these physiological functions can cause adverse effect. However, biochemical functions have an edge over others. Biochemical functions include protein formation, albumin formation, glucose formation, cholesterol formation, triglyceride formation and their status maintenance on one hand and on the other the consideration of protein as the basic unit of life on account of its involvement in morpho-histological set up of every tissue and organ. Any change in proteins level can cause malfunctioning of a specific type of cell or tissue and may be a reason for delay in cellular, biochemical events of particular tissue or organ.

The emphasis has been laid to observe the changes after acute and sub-acute intoxication induced by mercuric chloride. In the present investigation the toxic effect of mercuric chloride on serum proteins and its respective components has been taken into consideration. The protective action of *Panax* ginseng extract in *Rattus norvegicus* after acute and sub-acute treatment signifies its importance towards the xenobiotics substance, the mercuric chloride.

## MATERIAL AND METHODS

### Preparation of plant root extract

The coarse powder (100 gm) of the shade dried root of

[#]To whom correspondence should be addressed.
(Tel) +91-562-252-6432; (Fax) +91-562-252-6432
(E-mail) prof_pnsaxena@yahoo.co.in

the *Panax* ginseng extract was exhaustively successively extracted using D.W. in soxhlet extractor for a period of 22 hrs. The extract was concentrated under reduced pressure by rotatory evaporator to a syrupy consistency after which it was evaporated to dryness (yield: 10 gm).

**Experimental animals**

*Rattus norvegicus* weighing 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, Bombay) and water was provided *ad libitum.*

**Experimental compound**

Experimental compound (mercuric chloride) was obtained from Bayer India Ltd. Bombay. The acute oral $LD_{50}$ was determined on albino rats. The mercuric chloride was dissolved in distilled water of pharmaceutical quality and introduced by gavage tube. The data were analyzed by probit analysis[4] for $LD_{50}$ determination (Table 1). Rats from the control set were given distilled water only.

Animals were divided into 5 groups of 5 rats each. Group I (normal) received 1 ml of distilled water and 10 µl tween-20, group II received *Panax* ginseng extract (10 mg/kg b. wt), group III received mercuric chloride after $LD_{50}$ (9.26 mg/kg b. wt.) determination for acute (0.926 mg/kg b. wt.) and sub-acute (0.044 mg/kg b. wt.) sets, group IV received *Panax* ginseng extract followed by mercuric chloride while group V received mercuric chloride followed by *Panax* ginseng extract. The details of group and treatment are given below. Animals were sacrificed 24 hrs after the last treatment. The blood was collected directly from ventricle of heart with a sterilized syringe. Serum was collected for different biochemical analyses. Total serum proteins determination was done by the Biuret method (Gornal *et al.* 1949)[2] that gives purple colouration when peptide bonds react with cupric ions ($Cu^{2+}$) at alkaline pH which can be read at 549 nm. Serum

**Table 1.** Toxicity evaluation of Mercuric Chloride in albino rat, *Rattus norvegicus* Specifying fiducial limits

| Experimental individual | Compound | Regression equation | $LD_{50}$ (mg/kg b.w.) | Variance | Fiducial limits |
|---|---|---|---|---|---|
| Rattus norvegicus | Mercuric Chloride | Y=5.146+3.410 (x-1.009) | 9.26 mg | 0.006 | $m_1$=(±)0.972<br>$m_2$=(−)0.960 |

**Table 2.** Total protein (g/dl) in albino rat

| Treatment duration | Treatment sets | Control | Mercuric chloride treated | *Panax* ginseng extract treated | Mercuric chloride treated followed by *Panax* ginseng extract | *Panax* ginseng extract treated followed by mercuric chloride |
|---|---|---|---|---|---|---|
| 6 hrs | | 5.07±0.57* | 3.53±0.03$^d$ | 4.33±0.06$^b$ | 4.05±0.02$^d$ | 5.86±0.03$^a$ |
| 12 hrs | Acute | 5.15±0.59* | 3.56±0.03$^d$ | 4.33±0.06$^a$ | 4.18±0.01$^d$ | 5.84±0.02$^b$ |
| 24 hrs | | 5.12±0.61* | 3.46±0.14$^d$ | 4.63±0.21$^a$ | 4.27±0.01$^d$ | 5.79±0.02$^c$ |
| 7 days | | 5.53±0.34* | 2.66±0.23$^d$ | 5.83±0.06$^c$ | 4.34±0.02$^d$ | 5.75±0.01$^d$ |
| 14 days | Sub-acute | 5.96±0.176* | 2.76±0.13$^d$ | 6.60±0.25$^a$ | 4.49±0.01$^d$ | 5.70±0.00$^d$ |
| 28 days | | 5.70±0.43* | 2.40±0.10$^d$ | 6.83±0.76$^a$ | 4.71±0.01$^d$ | 5.62±0.01$^d$ |

*Mean±S.Em.
Significance level; a=>0.05, b=<0.05, c=<0.01, d=<0.001

**Table 3.** Albumin (g/dl) in albino rat

| Treatment duration | Treatment sets | Control | Mercuric chloride treated | *Panax* ginseng extract treated | Mercuric chloride treated followed by *Panax* ginseng extract | *Panax* ginseng extract treated followed by mercuric chloride |
|---|---|---|---|---|---|---|
| 6 hrs | | 2.88±0.32* | 2.03±0.03$^b$ | 2.60±0.05$^a$ | 2.28±0.01$^d$ | 2.90±0.01$^d$ |
| 12 hrs | Acute | 2.91±0.30* | 2.13±0.03$^b$ | 2.63±0.03$^b$ | 2.31±0.00$^d$ | 2.87±0.01$^d$ |
| 24 hrs | | 2.93±0.29* | 2.16±0.03$^a$ | 2.80±0.05$^c$ | 2.35±0.01$^d$ | 2.78±0.01$^d$ |
| 7 days | | 3.16±0.16* | 2.16±0.06$^d$ | 3.43±0.18$^a$ | 2.42±0.01$^d$ | 2.72±0.01$^d$ |
| 14 days | Sub-acute | 3.21±0.12* | 1.90±0.05$^d$ | 3.60±0.20$^a$ | 2.45±0.02$^d$ | 2.65±0.03$^d$ |
| 28 days | | 3.21±0.10* | 1.91±0.04$^d$ | 3.93±0.29$^b$ | 2.48±0.01$^d$ | 2.60±0.01$^d$ |

**Table 4.** Globulin (g/dl) in albino rat

| Treatment duration | Treatment sets | Control | Mercuric chloride treated | *Panax* ginseng extract treated | Mercuric chloride treated followed by *Panax* ginseng extract | *Panax* ginseng extract treated followed by mercuric chloride |
|---|---|---|---|---|---|---|
| 6 hrs | | 2.19±0.32* | 1.5±0.00$^c$ | 1.73±0.01 | 1.77±0.01 | 2.96±0.02$^d$ |
| 12 hrs | Acute | 2.24±0.29* | 1.43±0.00$^c$ | 1.70±0.03 | 1.87±0.01 | 2.97±0.01$^d$ |
| 24 hrs | | 2.19±0.32* | 1.30±0.00$^c$ | 1.83±0.16 | 1.92±0.00 | 3.01±0.01$^d$ |
| 7 days | | 2.36±0.18* | 0.50±0.11$^d$ | 2.40±0.11 | 1.92±0.01 | 3.03±0.00$^d$ |
| 14 days | Sub-acute | 2.75±0.05* | 0.86±0.17$^d$ | 3.00±0.05 | 2.04±0.01 | 3.05±0.03$^d$ |
| 28 days | | 2.49±0.33* | 0.49±0.06$^d$ | 2.90±0.47 | 2.23±0.00 | 3.02±0.00$^d$ |

*Mean±S.Em.
Significance level; a=>0.05, b=<0.05, c=<0.01, d=<0.001

albumin was determined by modification of the Bromocresol-green method of Mepherson and Everald (1972)[3]. The concentration of albumin can be measured at 632 nm, while serum globulin was calculated with the help of total protein and albumin concentrations. SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis.) Technique[4] was carried out with discontinuous buffering system. To achieve this, 10 μl of diluted serum from four groups along with control animals were applied to a 10% gel containing 1% SDS and electrophoresed with discontinuous buffering system for 3 hours. All the chemicals were obtained from Sigma chemical company, Germany.

Statistically significant values between experimental and control values were calculated according to Fisher's student't' test[5].

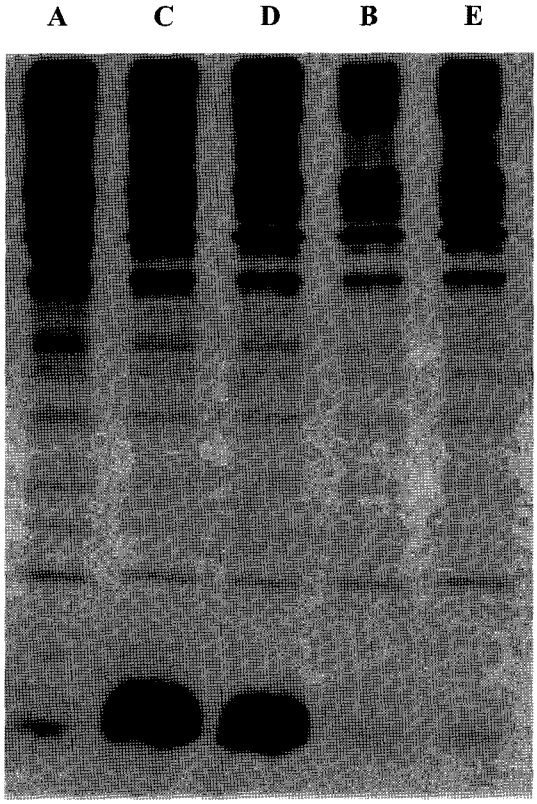
## RESULTS & DISCUSSION

### Biochemical analysis

Initially, varying amounts of 0.044 and 0.926 mg mercuric chloride as per kg body wt. were administered acutely and sub-acutely for 28 days *vide supra.* Total serum proteins, protein pattern, serum albumin and serum globulin so determined have been presented. (Table 2-4)

Serum protein, albumin and globulin represent important and most abundant constituent of blood plasma[6]. It serves as a storage protein and also functions in the transport of fatty acid from blood [7,8 & 9].

Total serum protein, albumin and globulin were significantly decreased in acutely and sub-acutely mercuric chloride treated sets. Decrease in total serum protein, albumin and globulin is probably due to the ionic interaction between mercury ions and the oxygen, nitrogen atoms of serum protein which lead to rearrangement of serum protein molecules around the mercury ions and thus decreasing levels of serum proteins, albumin and globulin[10].




A= control
C= Mercuric chloride
B= *Panax* ginseng extract
D= Mercuric chloride followed by *Panax* ginseng extract
E= *Panax* ginseng extract followed by mercuric chloride

**Fig. 1.** Showing banding pattern of serum protein after mercuric chloride, *Panax* ginseng extract, mercuric chloride followed by *Panax* ginseng extract and *Panax* ginseng extract followed by mercuric chloride treatment.

Total serum protein, albumin and globulin were significantly increased after acute and subacute treatment of

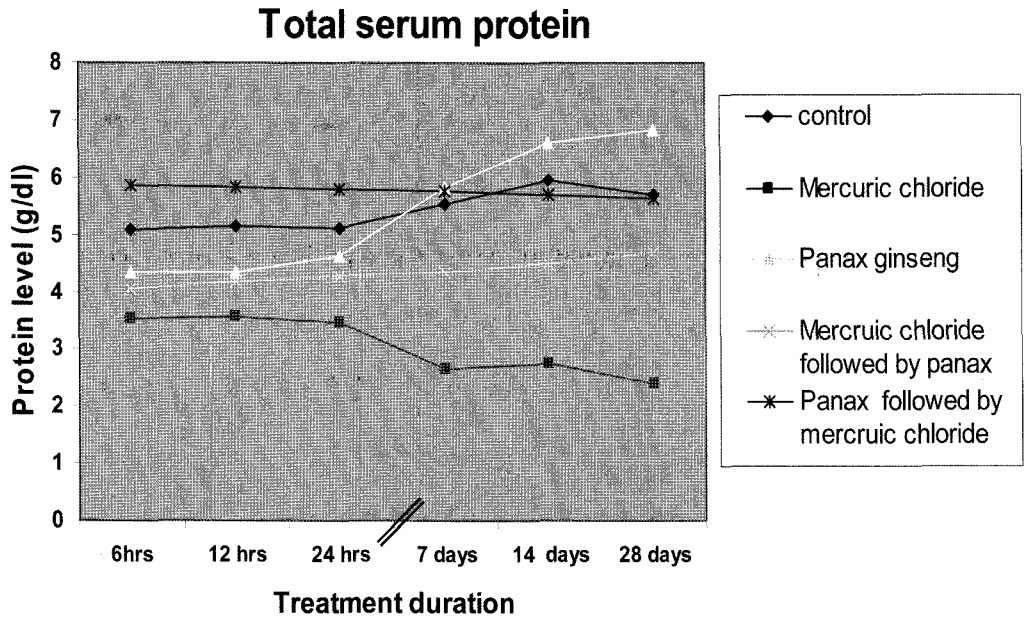



**Fig. 2.** Showing serum total protein level after mercuric chloride, *Panax* ginseng extract, mercuric chloride followed by *Panax* ginseng extract and *Panax* ginseng extract followed by mercuric chloride treatment.

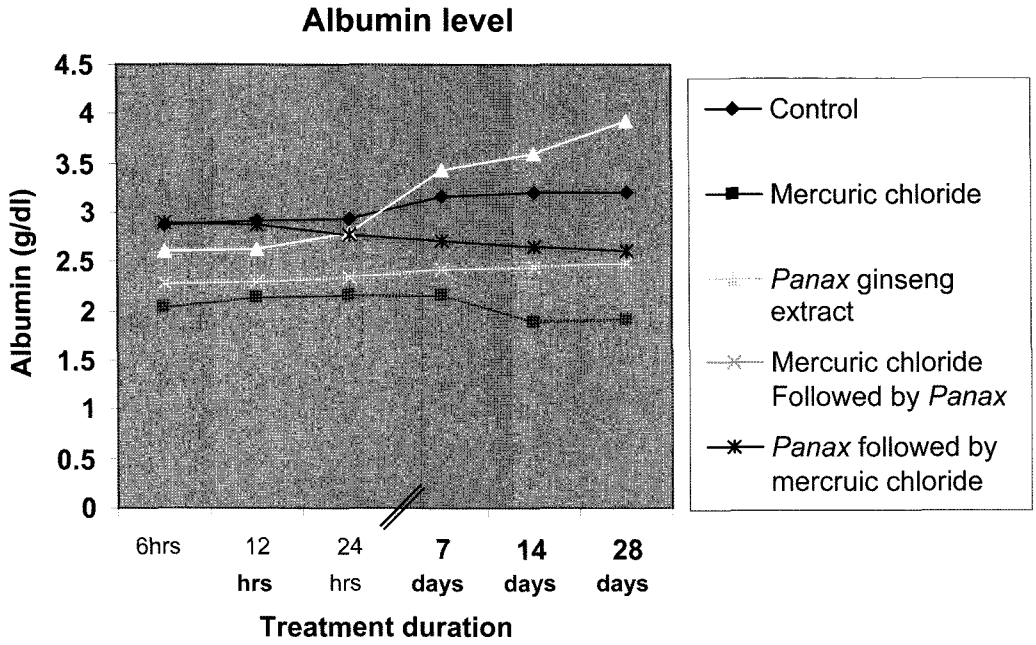



**Fig. 3.** Showing serum albumin level after mercuric chloride, *Panax* ginseng extract, mercuric chloride followed by *Panax* ginseng extract and *Panax* ginseng extract followed by mercuric chloride treatment.

*Panax* due to their antioxidant activity. The antioxidant compounds have been known to protect cells from various oxidative damage and there is considerable evidence that the natural antioxidants are anticarcinogens (Ames, 1983)[11]. Therefore, it is important to elucidate the effect of any of these antioxidants in mercuric chloride induced toxicity.

In the present study, administration of mercuric chloride followed by *Panax* ginseng extract to rats resulted in significant improvement in total serum protein, albumin and globulin, however, not up to the normal levels. Moreover, *Panax* ginseng extract followed by mercuric chloride has

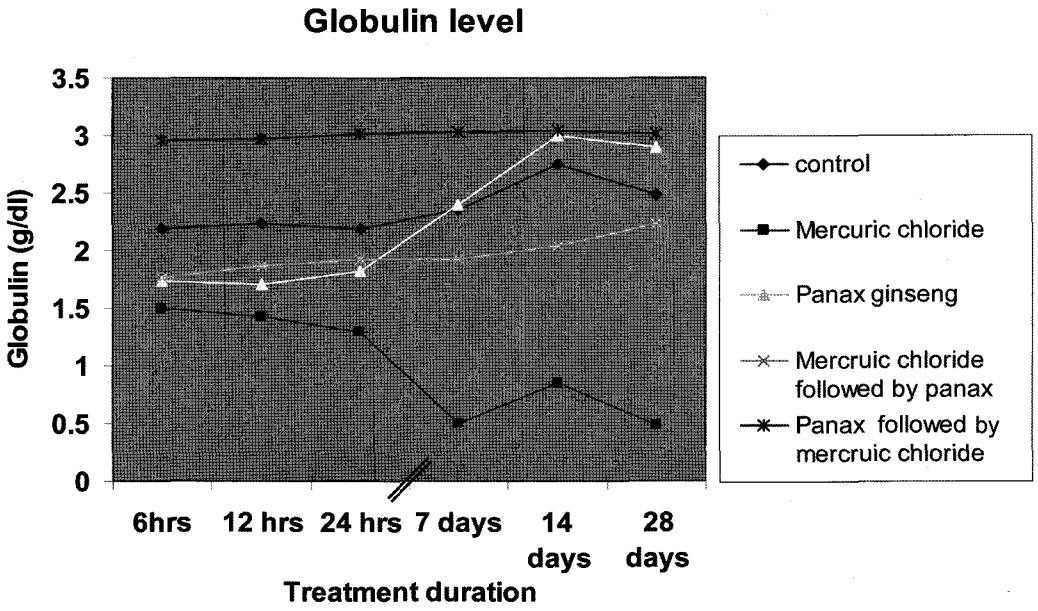



**Fig. 4.** Showing serum globulin level after mercuric chloride, *Panax* ginseng extract, mercuric chloride followed by *Panax* ginseng extract and *Panax* ginseng extract followed by mercuric chloride treatment.

revealed better protective action as compared the mercuric chloride followed by *Panax* ginseng extract action. It is of interest to mention that *Panax* exhibited a pronounced protection against mercuric chloride intoxication.

*Panax* ginseng extract followed by mercuric chloride resulted in significant concomitant increase in total serum protein, albumin and globulin contents. This result was in conformity with those reported with Yokozawa et al. (1994)[12].

Lastly, the effects of mercuric chloride, *Panax*, *Panax* followed by mercuric chloride and mercuric chloride followed by *Panax* on serum protein patterns were studied electrophoretically. Comparison of the serum protein from different groups with control showed that gradual disappearance of alpha-2 and beta-1 globulin bands from electrophoretic pattern was observed, while a single sharp band was observed between beta-2 and gamma globulin in serum proteins pattern in acute mercuric chloride treatment (Fig. 1). However, this band could not be visualized in control as well as *Panax* ginseng extract treated group. On the other hand these bands are lightly marked in mercuric chloride followed by *Panax* ginseng extract treated group but absent in *Panax* ginseng extract followed by mercuric chloride treated group (Fig. 1). These variations in proteins pattern was in affirmation to earlier reports[16,17]. From SDS-PAGE analysis it has cleared that *Panax* ginseng extract exhibited a protective phenomenon against mercuric chloride, which detoxified the toxicity of mercuric chloride and stop the degradation of proteins pattern.

Hence on the basis of biochemical and electrophoresis proteins pattern we can say that *Panax* ginseng extract is a protective remedy against mercuric chloride intoxication in albino rats.

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