

## Original Research Article

# Ameliorative Effect of *Bryophyllum pinnatum* against Induced Mercuric Chloride Toxicity in Rat: A Haemato-Biochemical Study

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## ABSTRACT

The present investigation was undertaken to evaluate ameliorative effect of *Bryophyllum pinnatum* against induced mercuric chloride toxicity in rat. Thirty six albino rats (Wistar strain) of 7-9 weeks age weighing around 130-140 g. All rats were divided in four groups *i.e.*, Groups II, III and IV were reared with uniform managemental conditions for entire study. The Group I without any treatment was kept as control, otherwise Groups 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) treated respectively. Haematological and biochemical parameters of each group were assessed. Haematological observations revealed significant ( $P < 0.05$ ) decrease in Hb, PCV, TEC, TLC, MCH and MCHC in the mercuric chloride treated group II as compared to control. Group IV showed increased values as compared to group II. Differential leucocyte count in mercury (II) chloride treated groups was characterized by significant ( $P < 0.05$ ) decrease in lymphocyte count with significant ( $P < 0.05$ ) increase in the neutrophil count as compared to control. Biochemical alterations observed in mercuric chloride induced toxicity caused increased levels of AST, ALT, ALP, LDH, BUN, creatinine, and decreased levels of serum total protein and serum albumin and there was improvement in these altered levels 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 protection against the toxic effects caused by mercury in rats. It showed potent antioxidant activity, hepatoprotective and nephroprotective activity against mercury induced toxicity.

## Keywords

Mercuric chloride,  
*Bryophyllum pinnatum*, rat,  
haemato-  
biochemical

## Introduction

Mercury is one of the most common heavy metals, used for more than 3000 years in medicines (as disinfectants, vaccines), industries (fluorescent lamps, batteries, thermostats, thermometers), gold mining and therapeutically as a cathartic, diuretic, anti-

inflammatory and in dental amalgams (Clarkson *et al.*, 2003). Mercury (Hg) is a highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders (Rishe and Amler,

2005). The leaves of *Bryophyllum pinnatum* plant have been reported to possess antimicrobial, antifungal, anti-ulcer (Akinpelu, 2000), anti-inflammatory and analgesic (Pal and Nag, 1992), antihypertensive (Ojewole, 2002), antidiabetic (Ojewole, 2005), hepatoprotective and antimutagenic activities. The study was conducted to explore the ameliorative effect of *Bryophyllum pinnatum* on mercuric chloride induced haematological and haemato-biochemical changes in rats.

### **Materials and Methods**

The programme, on approval by IAEC (Institution Animal Ethical Committee), Albino rats (Wistar strain) of 7-9 weeks age weighing around 130-140 g were procured from certified enterprise. The animals were housed in polypropylene cages at a population density of four per cage. Animals were acclimatized for two weeks with normal light and dark cycle. Commercial standard rat pellet diet and water were provided ad libitum. The *Bryophyllum pinnatum* leaves obtained from Purba Medinipur District of West Bengal, 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 37°C. A brownish colour residue weighing 28.5 g was obtained. This was kept in air tight bottle in a refrigerator until use. Mercuric chloride was obtained from Merck India Ltd. (Mumbai, India), and dissolved in distilled water and administered per OS route. Serum biochemical kits were obtained from Erba Transasia bio-medicals Ltd. A total of thirty six albino rats of Wistar

strain were used in this study and were divided into four groups. Each group contained nine animals. At the commencement of the study the weight variation of animals was minimized and did not exceed  $\pm 10$  per cent of mean weight of each group. The experiment was conducted for a period of 90 days. (Table.1)

Blood was collected from rats on the day of sacrifice from retro-orbital plexus with the help of capillary tube as described by Sorg and Buckner (1964). Blood was collected in sterilized vials containing 4.0 % potassium Ethylene Diamine Tetra Acetic acid (EDTA) as an anticoagulant for estimation of haematological parameters and a smear was prepared from freshly collected blood. Haemoglobin (Hb) estimation, Total Erythrocytes Count (TEC), Packed Cell Volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC) were estimated as per method describe by Schalm *et al.*, (1975).

Samples of blood collected from rats on day of sacrifice were allowed to clot and centrifuged at 1500 rpm for 30 min to separate the sera for estimation of Serum biochemical parameters. Tests were performed with the help of standard biochemical kits (Erba Transasia bio-medicals Ltd.) by using Erba Semi-autoanalyzer. Serum alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) were estimated International Federation of Clinical Chemistry (IFCC) method (Bradley *et al.*, 1972). Serum alkaline phosphatase (ALP) was estimated by IFCC method (Burtis and Ashwood, 2001). Serum lactate dehydrogenase (LDH) was estimated by DGKC kinetic method (Burtis *et al.*, 2012),

serum urea by glutamate dehydrogenase (GLDH) method (Tiffany *et al.*, 1972) and creatinine by Jaffe's kinetic method (Bowers, 1980). Total protein and albumin were estimated by Biuret and Duma's method (Tietz, 1986). The data collected from the experimental observations were analyzed by two ways ANOVA as per methods described by Snedecor and Cochran, (1994).

## Results and Discussion

The Hb, TEC and MCHC values (table.2) on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day were significantly reduced in group II and Group IV as compared to control (Group I), whereas higher but non-significant variation was observed in Group III ( $P < 0.05$ ). Within the groups there was significant low level of Hb concentration and TEC in group II and group IV on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. On the other hand no significant time dependant variation in level of Hb concentration was observed in control and group III. However, in group III the TEC values on 60<sup>th</sup> and 90<sup>th</sup> day significantly increased as compared to 30<sup>th</sup> day. Within the groups MCHC values in group IV significantly decreased on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. However, MCHC values in group III was significant less on 60<sup>th</sup> day but non-significant variation was observed on 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. On the other hand no significant time dependant variation in level of MCHC was observed in group I and group II.

A significantly decreased the PCV values on 30<sup>th</sup> day in group II and group III was observed as compared to control, whereas lower but non-significant variation was observed in group IV ( $P < 0.05$ ). On 60<sup>th</sup> day the PCV values differed significantly amongst all the treatment groups. However,

on 90<sup>th</sup> day group II and IV revealed significant decreased PCV values as compared with that in control group whereas lower but non-significant variation was observed in group III. Within the groups there was significant lower level of PCV in group II on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. However, in group IV the PCV values on 60<sup>th</sup> day significantly reduce as compared to 30<sup>th</sup> day but non-significant with higher PCV value was recorded on 90<sup>th</sup> day. Whereas as significant increase in PCV values were observed in group I and III on 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment.

On 30<sup>th</sup> and 90<sup>th</sup> day the MCV values significantly increase in group II and group IV as compared to control, whereas lower but non-significant variation was observed in group III. On 60<sup>th</sup> day the MCV values significantly increase amongst all the treatment groups as compared to control. Within the groups there was a significant high level of MCV in group II, III and IV on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. On the other hand no significant time dependant variation in values of MCV was observed in Group I.

The MCH and TLC values on 30<sup>th</sup> day significantly decreased in Group II as compared to control, where as non-significant variation was observed in Group III and IV. On 60<sup>th</sup> day the MCH value was significantly increased in group IV. However, MCH values did not differ significantly among groups at 90<sup>th</sup> day. Within the groups MCH values in group II was significant increase on 60<sup>th</sup> day but comparable at 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. However, MCH values in group IV was significant increase on 60<sup>th</sup> day but non-significant variations was observed on 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. On 60<sup>th</sup> and 90<sup>th</sup> day the

TLC values significantly decreased in group II and IV as compared to control (group I), whereas higher but no significant variation was observed in group III. Within the groups TLC values in group II and group IV on 30<sup>th</sup> and 60<sup>th</sup> day were comparable but significantly decreased at 90<sup>th</sup> day of experiment. Whereas no significant time dependant variation in level of MCH and TLC was observed in group I and group III.

Lymphocyte and Neutrophil percent on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day the was found to be significantly decreased and increased respectively in group II and IV as compared to control, whereas a non-significant variation was observed in group III. Within the groups lymphocyte percent in group II and group IV on 30<sup>th</sup> and 60<sup>th</sup> day were comparable but significantly decreased on 90<sup>th</sup> day of experiment.

On the other hand no significant time dependant variation in lymphocyte and neutrophil percent was observed in group I and group III. It was observed that there was no significant variation in monocyte and eosinophil percent between different groups and within different groups at 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day of experiment. However, the highest monocyte percent was recorded in group II and followed by group IV and group III as compared to control on 90<sup>th</sup> day.

Significant higher level of AST, ALT and ALP and LDH (Table.3) was recorded on 30<sup>th</sup> 60<sup>th</sup> and 90<sup>th</sup> day in group II and group IV as compared to control (Group I,  $P < 0.05$ ), whereas lower but non-significant variation was observed in group III.

Within the groups there was significant higher level of AST ALT and LDH in group II and group IV on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. Within the groups there was significant higher level

of ALP in group IV on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. Significantly lower level of ALP on 60<sup>th</sup> day but on 90<sup>th</sup> day non-significant variation was observed in group II as compared to 30<sup>th</sup> day of experiment. On the other hand no significant time dependant variation in level of AST, ALT ALP and LDH was observed in group I and group III.

It was observed that there was no significant variation in level of serum total protein and serum albumin between different groups and within different groups at 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day of experiment however, the lowest value of serum total protein was recorded in group II and followed by group IV as compared to other two other experimental groups.

On 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day significantly higher level of Urea was recorded in group II and group IV as compared to control (group I), whereas lower but non-significant variation was observed in group III. Within the groups there was significant higher level of Urea in group II and group IV on 60<sup>th</sup> and 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment. A non-significant time dependant variation in level of Urea was observed in group I and group III.

A significant higher level of Serum Creatinine on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day of experiment was recorded in group II as compared to control (group I), whereas higher but non-significant variation was observed in group IV.

Within the groups there was significantly higher level of Serum Creatinine in group II on 90<sup>th</sup> day as compared to 30<sup>th</sup> day of experiment whereas higher but non-significant variation was observed on 60<sup>th</sup> day. In group I, III and IV, no significant time dependant variation in level of Serum Creatinine was observed ( $P < 0.05$ ).

**Table.1**

Group I	Group II	Group III	Group IV
Control, maintained on commercial rat pellet diet and water	Mercuric chloride (2mg/kg bw) orally (Gavage)	Bryophyllum pinnatum (200 mg/kg bw) orally (Gavage)	Mercuric chloride + Bryophyllum pinnatum (2mg/kg bw + 200mg/kg bw respectively) orally (Gavage)

**Table.2** Haematological parameters in rat

Parameter	Group	Period		
		30 Days	60 Days	90 Days
Hb(g%)	I	15.56±0.27 <sup>C</sup>	15.46±0.17 <sup>C</sup>	15.86±0.17 <sup>C</sup>
	II	11.13±0.14 <sup>Ac</sup>	10.40±0.11 <sup>Ab</sup>	9.60±0.11 <sup>Aa</sup>
	III	15.73±0.23 <sup>C</sup>	15.33±0.17 <sup>C</sup>	15.86±0.17 <sup>C</sup>
	IV	13.20±0.05 <sup>Bc</sup>	12.40±0.11 <sup>Bb</sup>	11.46±0.06 <sup>Ba</sup>
PCV(%)	I	47.26±0.43 <sup>Ba</sup>	48.14±0.12 <sup>Ca</sup>	50.56±0.48 <sup>Cb</sup>
	II	46.16±0.08 <sup>Ac</sup>	45.26±0.24 <sup>Ab</sup>	40.19±0.12 <sup>Aa</sup>
	III	48.13±0.12 <sup>Ca</sup>	49.36±0.17 <sup>Db</sup>	50.24±0.08 <sup>Cc</sup>
	IV	46.76±0.06 <sup>Ab</sup>	46.03±0.12 <sup>Ba</sup>	47.43±0.31 <sup>Bb</sup>
TEC(× 106/μl)	I	8.54±0.21 <sup>C</sup>	8.66±0.07 <sup>D</sup>	8.75±0.11 <sup>C</sup>
	II	6.86±0.16 <sup>Ab</sup>	5.78±0.06 <sup>Aa</sup>	5.54±0.09 <sup>Aa</sup>
	III	8.36±0.03 <sup>Ca</sup>	8.48±0.04 <sup>Cb</sup>	8.61±0.02 <sup>Cb</sup>
	IV	7.48±0.18 <sup>Bb</sup>	6.46±0.01 <sup>Ba</sup>	6.55±0.18 <sup>Ba</sup>
MCV(fl)	I	55.35±1.12 <sup>A</sup>	55.59±0.41 <sup>A</sup>	57.79±1.22 <sup>A</sup>
	II	67.34±1.56 <sup>Ca</sup>	78.34±1.13 <sup>Dc</sup>	72.57±0.97 <sup>Bb</sup>
	III	57.53±0.11 <sup>Aa</sup>	58.19±0.32 <sup>Bab</sup>	58.35±0.14 <sup>Ab</sup>
	IV	62.60±1.55 <sup>Ba</sup>	71.22±0.17 <sup>Cb</sup>	72.52±1.88 <sup>Bb</sup>
MCH(pg)	I	18.23±0.55 <sup>B</sup>	17.86±0.22 <sup>A</sup>	18.13±0.29
	II	16.24±0.47 <sup>Aa</sup>	17.99±0.02 <sup>Ab</sup>	17.34±0.46 <sup>ab</sup>
	III	18.80±0.24 <sup>B</sup>	18.07±0.18 <sup>A</sup>	18.43±0.25
	IV	17.66±0.39 <sup>Ba</sup>	19.18±0.17 <sup>Bb</sup>	17.53±0.22 <sup>a</sup>
MCHC(g%)	I	32.93±0.39 <sup>C</sup>	32.13±0.43 <sup>C</sup>	31.38±0.57 <sup>B</sup>
	II	24.11±0.29 <sup>A</sup>	22.98±0.35 <sup>A</sup>	23.89±0.32 <sup>A</sup>
	III	32.68±0.42 <sup>Cb</sup>	31.06±0.44 <sup>Ca</sup>	31.58±0.34 <sup>Bab</sup>
	IV	28.22±0.09 <sup>Bc</sup>	26.93±0.30 <sup>Bb</sup>	24.17±0.16 <sup>Aa</sup>
TLC (× 103/μl)	I	8.17±0.24 <sup>B</sup>	8.05±0.21 <sup>B</sup>	8.10±0.25 <sup>B</sup>
	II	7.27±0.17 <sup>Ab</sup>	6.82±0.18 <sup>Aab</sup>	6.34±0.08 <sup>Aa</sup>
	III	8.22±0.34 <sup>B</sup>	8.32±0.17 <sup>B</sup>	8.42±0.09 <sup>B</sup>
	IV	7.60±0.07 <sup>ABb</sup>	7.04±0.24 <sup>Aab</sup>	6.78±0.20 <sup>Aa</sup>
Lymphocyte (%)	I	75.67±1.20 <sup>C</sup>	76.00±0.58 <sup>C</sup>	76.67±1.20 <sup>B</sup>
	II	63.67±1.45 <sup>Ab</sup>	59.00±2.08 <sup>Aab</sup>	55.34±2.02 <sup>Aa</sup>
	III	76.67±0.67 <sup>C</sup>	76.67±1.20 <sup>C</sup>	76.34±0.34 <sup>B</sup>
	IV	69.00±1.52 <sup>Bb</sup>	66.00±3.05 <sup>Bab</sup>	58.34±2.72 <sup>Aa</sup>
Neutrophil (%)	I	21.33±1.20 <sup>A</sup>	20.34±0.34 <sup>A</sup>	20.00±1.00 <sup>A</sup>
	II	32.67±1.45 <sup>Ca</sup>	36.34±1.85 <sup>Bab</sup>	39.67±0.88 <sup>Bb</sup>
	III	19.00±0.58 <sup>A</sup>	22.34±2.40 <sup>A</sup>	19.00±0.57 <sup>A</sup>
	IV	27.00±2.08 <sup>Ba</sup>	30.67±2.72 <sup>Bab</sup>	37.00±2.51 <sup>Bb</sup>
Monocyte (%)	I	1.67±0.34	2.00±0.88	1.67±0.34
	II	2.00±0.58	2.67±0.34	3.34±0.89
	III	2.00±0.58	2.34±0.34	2.34±0.34
	IV	2.34±0.34	2.00±0.58	2.67±0.34
Eosinophil (%)	I	1.34±0.34	1.34±0.34	1.67±0.67
	II	1.34±0.34	2.00±0.00	1.67±0.34
	III	2.34±0.34	2.00±0.58	2.34±0.34
	IV	1.67±0.34	1.34±0.34	2.00±0.38

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.3** Biochemical parameters in rats

Parameters	Groups	Period		
		30 Days	60 Days	90 Days
AST (IU/L)	I	53.14±1.11 <sup>A</sup>	53.72±0.83 <sup>A</sup>	53.61±0.28 <sup>B</sup>
	II	79.66±0.85 <sup>Ca</sup>	87.35±0.40 <sup>Cb</sup>	109.25±0.23 <sup>Dc</sup>
	III	52.32±0.23 <sup>A</sup>	53.16±0.56 <sup>A</sup>	52.09±0.27 <sup>A</sup>
	IV	60.81±0.17 <sup>Ba</sup>	71.29±0.46 <sup>Bb</sup>	86.34±0.47 <sup>Cc</sup>
ALT (IU/L)	I	52.91±5.72 <sup>A</sup>	47.03±0.63 <sup>A</sup>	45.75±0.40 <sup>A</sup>
	II	67.03±0.21 <sup>Ba</sup>	79.25±0.12 <sup>Cb</sup>	102.96±0.13 <sup>Cc</sup>
	III	45.76±0.55 <sup>A</sup>	46.05±0.62 <sup>A</sup>	45.98±1.12 <sup>A</sup>
	IV	54.96±0.13 <sup>Aa</sup>	62.97±0.63 <sup>Bb</sup>	86.37±0.62 <sup>Bc</sup>
ALP (IU/L)	I	113.20±0.69 <sup>A</sup>	112.69±0.24 <sup>A</sup>	112.98±1.60 <sup>A</sup>
	II	152.69±0.92 <sup>Cb</sup>	146.14±0.73 <sup>Ca</sup>	152.96±0.66 <sup>Cb</sup>
	III	113.06±0.69 <sup>A</sup>	112.12±0.59 <sup>A</sup>	112.03±0.50 <sup>A</sup>
	IV	126.55±0.94 <sup>Ba</sup>	137.32±0.97 <sup>Bb</sup>	144.76±0.77 <sup>Bc</sup>
LDH (IU/L)	I	66.14±0.65 <sup>A</sup>	68.19±0.59 <sup>A</sup>	65.62±0.95 <sup>A</sup>
	II	105.27±0.83 <sup>Ca</sup>	121.69±1.09 <sup>Cb</sup>	140.86±0.93 <sup>Cc</sup>
	III	66.01±0.69 <sup>A</sup>	67.92±0.66 <sup>A</sup>	66.13±0.66 <sup>A</sup>
	IV	92.56±1.68 <sup>Ba</sup>	108.11±2.27 <sup>Bb</sup>	124.70±3.73 <sup>Bc</sup>
Serum Total Protein (g/dl)	I	6.16±0.62	6.27±0.89	6.30±0.55
	II	4.70±0.35	4.39±0.99	4.09±0.22
	III	6.21±0.95	6.29±0.82	6.31±0.85
	IV	5.19±0.80	5.48±0.55	5.72±0.83
Serum Albumin (g/dl)	I	3.81±0.33	3.84±0.16	3.82±0.26
	II	3.09±0.40	2.96±0.99	2.87±0.27
	III	3.77±0.12	3.83±0.08	3.55±0.59
	IV	3.38±0.75	3.07±0.61	3.22±0.43
Serum Urea (mg/dl)	I	20.92±0.13 <sup>A</sup>	21.42±0.29 <sup>A</sup>	22.71±0.24 <sup>A</sup>
	II	34.61±1.33 <sup>Ca</sup>	39.21±0.34 <sup>Cb</sup>	51.02±0.17 <sup>Cc</sup>
	III	20.76±1.12 <sup>A</sup>	21.13±0.12 <sup>A</sup>	21.96±1.22 <sup>A</sup>
	IV	28.44±1.33 <sup>Ba</sup>	34.02±0.27 <sup>Bb</sup>	46.24±0.89 <sup>Bc</sup>
Serum Creatinine (mg/dl)	I	0.77±0.33 <sup>A</sup>	0.76±0.07 <sup>A</sup>	0.79±0.09 <sup>A</sup>
	II	1.05±0.06 <sup>Ba</sup>	1.17±0.03 <sup>Bab</sup>	1.42±0.22 <sup>Bb</sup>
	III	0.76±0.12 <sup>A</sup>	0.76±0.03 <sup>A</sup>	0.77±0.09 <sup>A</sup>
	IV	0.89±0.06 <sup>AB</sup>	1.03±0.06 <sup>AB</sup>	1.21±0.23 <sup>AB</sup>

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).

R.B.C. counts were significantly decreased due to the cytotoxic effect of mercuric chloride on the erythropoietic tissue, bone marrow etc. Such a disturbance in bone marrow leads to alteration of cell cycle and reduction in erythropoiesis (Sharma *et al.*, 2007). In present findings, there was also

significant decrease in PCV in mercuric chloride treated groups on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day as compared to its respective control. These findings were in agreement with previous reports where significant decrease in PCV was observed by Sheikh *et al.*, (2013). There was significant improvement

in Hb, TEC and PCV due to simultaneous administration of mercuric chloride and *Bryophyllum pinnatum*. The result pattern indicates that some of the phytochemical constituents of the crude ethanolic leaf extract of *B.pinnatum* may have stimulatory effect on the bone marrow for haemoglobin synthesis and erythropoiesis. This observed effect may be as a result of the tannin, ascorbic acid and phenol content of the plant. Other phytochemical constituents of *B.pinnatum* which might have likely affected the haematological parameters in this study include flavonoid, zinc, riboflavin and niacin (Ufelle *et al.*, 2011), which needs to further elucidated. There was significant increase MCV in mercuric chloride treated groups on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day as compared to its respective control, whereas MCH decreased significantly on 30<sup>th</sup> day. However, MCHC decreased significantly in mercuric chloride treated groups on 30<sup>th</sup>, 60<sup>th</sup> and 90<sup>th</sup> day as compared to its respective control. The result suggested anaemia which was macrocytic hypochromic type which supports the mechanism discussed above with respect to anaemia caused by mercuric chloride during this study.

The decrease in TLC indicated the toxic effect of orally given mercuric chloride. Lysis and depletion of lymphocytes in spleen also responsible for decrease in total leucocyte count. There was improvement in TLC values after simultaneous administration of mercuric chloride and *Bryophyllum pinnatum*. The result pattern indicates that some of the phytochemical constituents of the crude ethanolic leaf extract of *B.pinnatum* may have stimulatory effect on the bone marrow for leucocyte production. This ameliorative property may be lying with the tannin, ascorbic acid and phenol content, may chemical constituent of the plant.

Decrease in lymphocyte count along with decrease in total leucocyte count in the present study suggest it to be due to toxic effect of mercury on lymphocytopoiesis as well as their cytolysis which was authenticated by histopathological examination of spleen. Moreover, increase in neutrophils count as compared to control might be either a relative increase or due to deposition of mercury metal in visceral organs which initiated the inflammatory cascade.

Increased level of serum ALT and AST has also been recorded in case of mercuric chloride toxicity in rats (Oriquat *et al.*, 2012). In the present study, there was significant decreased ALT AST levels in mercuric chloride along with *Bryophyllum pinnatum* treated group indicating this plant to possess potent hepatoprotective activity (Yadav and Dixit, 2003 and Ozolua *et al.*, 2010). The decrease in ALT and AST level may be due to hepatocellular membrane stability and prevention of cellular leakage and increasing hepatic regeneration. Elevated level of serum ALP has also been reported in case of mercuric chloride toxicity in rats (Oda and El-Ashmawy, 2012). In the present study, there was significant decrease ALP levels in mercuric chloride along with *Bryophyllum pinnatum* treated group which may be due to potent hepatoprotective activity (Yadav and Dixit, 2003 and Ozolua *et al.*, 2010) of *Bryophyllum pinnatum*. Afzal *et al.*, (2013) also found very effective hepatoprotective as it significantly lowers the enzyme ALP.

Our finding is in accordance with the report of Oriquat *et al.*, (2012) who observed elevated serum LDH in rats exposed to sub-chronic toxicity with mercuric chloride. Raised level of LDH was also recorded in rats suffering from mercuric chloride toxicity (Venktatesan *et al.*, 2010). The

simultaneous administration of mercuric chloride and *Bryophyllum pinnatum* showed significant decrease in LDH as compared with group II. The effect of *Bryophyllum pinnatum* against HgCl<sub>2</sub> related toxicity suppressed can be attributed to its free radical scavenging capacity and antioxidant activity.

The reduction in serum total protein and albumin levels may be due to decline in protein synthesis by hepatic cells reflecting hepatic dysfunction (Sankar *et al.*, 2009) and by increased rate of excretion due to renal damage (Said *et al.*, 2008) that accompanied by mercury treatment. The increase in total serum protein and serum albumin in mercuric chloride plus *Bryophyllum pinnatum* treated group might be due to protective effect on liver and kidney

Our finding support the report of Oriquat *et al.*, (2012) who observed elevated serum urea in rats exposed to sub- chronic toxicity with mercuric chloride. The present finding was also supported by Said *et al.*, (2008) in rats. In the present study, there was significant decrease in urea levels in mercuric chloride along with *Bryophyllum pinnatum* treated group may be due to potent nephroprotective activity (Harlalka and Patil, 2007) of *Bryophyllum pinnatum*. Jain and Argal, (2012) also found it to be very effective nephroprotective as it significantly lowers BUN.

The higher levels of creatinine clearly reflected progressing renal insufficiency in rats treated with mercuric chloride. The rise in creatinine level might be due to damage produce in kidney tubules by mercury (Sheikh *et al.*, 2013). In the present study there was significant decreased creatinine levels in mercuric chloride along with *Bryophyllum pinnatum* treated group may be due to potent nephroprotective activity

(Harlalka and Patil, 2007) of *Bryophyllum pinnatum*. Jain and Argal, (2012) was found very effective nephroprotective as it significantly lowers the enzyme serum creatinine.

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