Indian J Physiol Pharmacol 2014; 58(4) : 423–436

Original Article

Modification of mercury-induced biochemical alterations by *Triticum Aestivum* Linn in rats

B.V.S. Lakshmi*, M. Sudhakar and G. Nireesha

Department of Pharmacology,
Malla Reddy College of Pharmacy,
Dhulapally (via Hakimpet), Maisammaguda,
Secunderabad – 500 014, Andhra Pradesh, India

Abstract

The present investigation has been undertaken to evaluate role of Wheat grass extract in modifying mercury-induced biochemical alterations in albino rats. Mercuric chloride 5 mg/kg body weight i.p. was given on 11, 13 & 15th day of the experiment. Wheat grass extract (400 mg/kg) and Quercetin (10 mg/kg) were administered 10 days before mercuric chloride administration and continued up to 30 days after mercuric chloride administration. The animals were sacrificed on 1, 15 and 30 days, the activity of serum alkaline and acid phosphatase and the iron, calcium, BUN, creatinine, SGPT, SGOT, total bilirubin, total protein levels were measured. Tissue lipid peroxidation content, glutathione (GSH) level, anti-oxidant enzymes- CAT and GR were measured. Hematological indices were also estimated. Mercury intoxication causes significant increase ($P<0.001$) in calcium level, acid phosphatase, BUN, creatinine, SGOT, SGPT, total bilirubin, lipid peroxidation content and significant decrease in iron level, alkaline phosphatase, total protein, and CAT, GR and glutathione level. Wheat grass extract pre- and post-treatment ameliorated mercury-induced alterations in terms of biochemical and hematological parameters. Concomitant treatment of Wheatgrass extract with Mercury showed prominent recovery and normal architecture with mild residual degeneration in the tissues. Thus from present investigation, it can be concluded that Wheat grass extract pre- and post-treatment with $HgCl_2$ significantly modulate or modify mercury-induced biochemical alteration in albino rats.

Introduction

The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO (1). In all the heavy metal, mercury is known to be extremely toxic. It is being widely used in the industrial, medical, agriculture and other fields (2). Inorganic mercury present in the environment is a well-established toxicant to human health (3). Mercury promotes the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhances the subsequent iron and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical (4, 5, 6). These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell.

*Corresponding author :
Dr. B.V.S. Lakshmi, Malla Reddy College of Pharmacy, Department of Pharmacology, Dhulapally (via Hakimpet), Maisammaguda, Secunderabad-500014, Andhra Pradesh, India, Tel.: 09885324334, Fax No.: 040-23792154
E-Mail: adithya.neha@gmail.com
(Received on May 16, 2014)

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 (7) and dimercaprol (also known as BAL or British anti-lewisite) (8) 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.

Shoot of *Triticum aestivum* Linn. (Hindi Name- gehun, kanak, and Sanskrit name- godhuma) is called as a Wheat grass extract, belonging to family: Gramineae, which possess high chlorophyll content and essential vitamins, minerals, vital enzymes, amino acids, dietary fibers (9) Wheat grass extract has been shown to possess anti-cancer activity (10) anti-ulcer activity (11), antioxidant activity (12), anti-arthritic activity (13), and blood building activity in Thalassemia Major (14). It has been argued that Wheat grass extract helps blood flow, digestion and general detoxification of the body. Wheatgrass extract contains minerals and trace elements including calcium, iodine, magnesium, selenium, zinc, chromium, antioxidants like vitamin C, vitamin E, β-carotene, vitamin B_1, antianemic factors like vitamin B_{12}, iron, folic acid, pyridoxine, abscissic acid, ferulic acid, and vanilic acid the concentrations of which increase with the germination period. The major clinical utility of Wheat grass extract in diseased conditions might be due to the presence of biologically active compounds and minerals in it and due to its antioxidant potential which is derived from its high content of bioflavonoids such as apigenin, quercitin, luteoline. Furthermore, indole compounds namely choline and laetrile present in it might be also responsible for its therapeutic potential. The presence of 70% chlorophyll, which is almost chemically identical to hemoglobin, in Wheat grass extract makes it more useful in various clinical conditions involving hemoglobin deficiency and other chronic disorders (14).

Considering the rich antioxidant and vitamin contents of wheatgrass, this study investigated status of wheatgrass in modifying the mercury-induced biochemical alterations in blood of albino rats.

Material and Methods

Animals

Male Wistar Albino rats weighing 150-250g were obtained from National Institute of Nutrition, Hyderabad. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum*. All the pharmacological experimental protocols were approved by the Institutional Animal Ethics Committee (Reg no: MRCP/CPCSEA/IAEC/2012-13/MPCOL/08).

Growing of Wheat grass extract

The grass of *T. aestivum* used in this study was grown under indoor conditions. Over-night soaked *T. aestivum* seeds were used to cultivate. Little quantities of water were sprinkled evenly over soil and 3-4 hours indirect sunlight was allowed daily for growth of grass. On the Seventh day, grass is harvested and used for further studies.

Preparation of plant extract

The harvested wheatgrass (*T. aestivum) is* washed dried at room temperature. The dried grass was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. This powder was packed into soxhlet apparatus and made to extract using ethanol. The extract was then filtered and concentrated under reduced pressure using a rotator evaporator at 40°C until the solvent completely dried. The yield of the ethanolic extract was 30%. The extract obtained was then dissolved in 2% Gum acacia for the pharmacological studies. It was suspended in 1% gum acacia. Acute toxicity studies

were performed according to the OECD Guideline no. 423. No mortality was observed till the dose of 2000 mg/kg. Hence 1/5 of the dose 2000 mg/kg i.e.; 400 mg/kg has been fixed as ED_{50}.

Mercuric chloride

Mercury in the form of inorganic mercury (mercuric chloride) was used for the present study. It was dissolved in 0.9% NaCl at 5.0 mg/kg body weight dose and administered intraperitoneally at once.

Experimental design

Thirty Wistar Albino male rats of weight 150-250 g were selected for this study. Animals were divided into five groups of six animals each.

Group 1 : Control group (1% gum acacia 1 ml)

Group 2 : Mercuric chloride group (Intraperitoneal injection 5 mg/kg body weight)

Group 3 : Wheatgrass 400 mg/kg body weight p.o.

Group 4 : Wheatgrass 400 mg/kg body weight p.o. + Mercuric chloride 5 mg/kg body weight i.p.

Group 5 : Quercetin 10 mg/kg body weight p.o. + Mercuric chloride 5 mg/kg body weight i.p.

Animals were grouped into five groups as explained above. The control group animals were given 1% gum acacia 1ml for 30 days. Group 2 animals were given 1% gum acacia until 30th day and single dose of Mercuric chloride 5 mg/kg body weight i.p. was given on 11, 13 & 15th day of the experiment. Group 3 animals were given wheatgrass 400 mg/kg body weight p.o. until 30th day. Group 4 animals were given wheatgrass 400 mg/kg body weight p.o. until 30th day and single dose of Mercuric chloride 5 mg/kg body weight i.p. on 11, 13 & 15th day of the experiment. Group 5 animals were given Quercetin 10 mg/kg body weight p.o. until 30th day and single dose of Mercuric chloride 5 mg/kg body weight i.p. was given on 11, 13 & 15th day of the experiment (15).

The animals were sacrificed on 1, 15 & 30th day using ether anesthesia, blood was collected by cardiac puncture method. Blood was centrifuged using Remi cool centrifuge at 4000 rpm for 15 minutes. Serum was separated for the estimation of various biochemical parameters like serum iron, serum calcium, serum acid phosphatase, alkaline phosphatase, SGPT, SGOT (16), total protein, total bilirubin (17), blood urea nitrogen and serum creatinine (18) as per the standard procedures.

Serum calcium

The serum calcium was estimated by Connerty and Biggs (1966), as modified by Baginski et al., (1973), cited from Practical Clinical Biochemistry, Varley, 5th ed., pp. 4649 method by using commercially available arsenazo reagent set, manufactured by Pointe Scientific Inc., USA (19). The method is based on the principle that the calcium reacts with arsenazo-III in a slightly alkaline medium to form a purple coloured complex, which absorbs at 650 nm. The intensity of the colour is proportional to the calcium concentration and represented as mg/dL.

Serum iron

Serum iron was estimated by Henry (1964) (20) as modified by Young (1991) (21) by using commercially available Iron TIBC reagent kit, manufactured by Raichem, Division of Hemagen Diagnostics Inc., San Diego, CA. The method is based on the principle that iron is accomplished by releasing the protein bound iron from its carrier protein transferrin. The acidic pH of buffered reagent releases iron in the ferric form from the protein. The released ferric iron is then reduced in ferrous form by hydroxylamine. The ferrous iron reacts with ferene to produce coloured complex. The absorbance of this coloured complex read at 595 nm. It is proportional to the concentration of iron and represented as mg/dL.

Serum acid phosphatase

Serum acid phosphatase activity was measured by King and Jagatheesan, (1959) (22) using commercially accessible kits (Span Diagnostics Ltd., Surat, India). Acid phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol, at

pH4.9; phenol so formed reacts in alkaline medium with 4-aminoantipyrine in the presence of an oxidizing agent potassium ferricyanide, and forms an orange-red coloured complex, which is measured colorimetrically or spectrophotometrically at 510 nm. The colour intensity is proportional to the enzyme activity, which is expressed as Kings and Armstrong unit (KAU).

Serum alkaline phosphatase

Alkaline phosphatase from serum converts phenyl phosphate to inorganic phosphate and phenol, at pH 10.0; phenol so formed reacts in alkaline medium with 4-aminoantipyrine in the presence of an oxidizing agent, potassium ferricyanide, and forms an orange-red coloured complex, which can be measured colorimetrically or spectrophotometrically at 510 nm. The colour intensity is proportional to the enzyme activity, which is expressed as Kings and Armstrong unit (KAU) (23).

Tissue sample preparation

At the end of the experiment, animals were sacrificed with light ether anesthesia. Liver and kidney tissues were separated and washed with phosphate buffer saline (0.05M, P^H7.4). The liver and kidney later were taken and minced into small pieces and homogenized in ice cold phosphate buffer saline (0.05M, P^H7.4) using tissue homogenizer to obtain 1:9 (w/v) (10%) whole homogenate. A part of the liver and kidney homogenate was taken and mixed with equal volume of 10% Trichloroacetic acid (TCA) for the estimation of malondialdehyde. Homogenate was centrifuged using Remi cool centrifuge at 8000 rpm for 30 mins. The supernatant was separated and used for estimation of anti-oxidant levels of different enzymes i.e. Catalase, glutathione reductase and reduced glutathione in the tissues-liver and kidney.

Lipid peroxidation (LPO)

LPO was estimated colorimetrically by measuring malondialdehyde (MDA) formation as described by Nwanjo and Ojiako, 2005 (24). In brief, 0.1 ml of homogenate was treated with 2 ml of a 1:1:1 ratio of TBATCAHCl (TBA 0.37%, TCA 15%, HCl 0.25 N) and placed in water bath at 65°C for 15 min, cooled, and centrifuged at 5,000 rpm for 10 min at room temperature. The optical density of the clear supernatant was measured at 535 nm against a reference blank. The MDA formed was calculated by using the molar extinction coefficient of thiobarbituric acid reactants (TBARS; 1.56×10^5l/mole cm^{-1}). The product of LPO was expressed as nmol of MDA formed per g of tissue.

Catalase (CAT)

Catalase (CAT) activity was estimated following the method of Aebi, 1993 (25). The homogenate (100 µl) was treated with ethanol (10 µl) and placed on an ice bath for 30 min. To this, 10 µl of 25% triton X-100 was added and again kept for 30 min on ice. To 200 µl phosphate buffer (0.1 M), 50 µl of treated liver and kidney homogenate and 250 µl of 0.066 M H_2O_2 (prepared in 0.1 M phosphate buffer, pH 7.0) were added in a cuvette. The decrease in optical density was measured at 240 nm for 60 s. The molar extinction coefficient of 43.6 cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2degraded/min/mg protein.

Glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman, 1959 (26). In brief, 1 ml of supernatant was taken after precipitating 0.5 ml of liver and kidney homogenate with 2 ml of 5% TCA. To this, 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (1 M, pH 8.0) was added. The color developed was read at 412 nm. Reduced GSH concentration is measured by using a drawn standard curve and was expressed as mg/g of tissue.

Glutathione reductase (GR)

Glutathione Reductase is also called GSR; it is an enzyme that reduces Glutathione Disulfide (GSSG) to sulfhydryl form GSH, which is an important cellular antioxidant. The enzyme activity was quantitated at room temperature by measuring the disappearance of NADPH at 340 nm and was calculated as U/ml of enzyme. To 350 µl of Potassium Phosphate buffer

(100mM. P^H 7.5), 50 µl of NADPH, 500 µl of Oxidized Glutathione and 100 µl of the homogenate was added and made the volume was made up to 1 ml with distilled water. The reaction is started by the addition of NADPH solution. Mix by inversion. Place the cuvette in Spectrophotometer and start the program. The concentration of the enzyme can be calculated using the formula :

$$= \frac{(\text{DA Sample DA blank}) \times \text{Dilution Factor}}{\epsilon^{mM} \times \text{Volume of Sample in ml}}$$

For NADPH $\epsilon^{mM} = 6.22\ m\ M^{-1}\ Cm^{-1}$

Hematological parameters

Hemoglobin content (Hb %), Red blood cell count and Total leukocyte count were studied for hematological investigation. Immediately after collection of blood, blood was transferred to sterile test tube containing anticoagulant at a ratio of 1: 10. The collected blood was used for different hematological parameters within two hours of collection. The hematological parameters were determined as per method cited by Lamberg and Rothstein (1977) (27). The result was read in daylight by observing the high of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in g%.

Histopathological studies

At the end of the experimental period, the rats were sacrificed and liver and kidneys were removed. The tissue sample from each group was selected and stored in 10% buffered formalin solution and further embedded in paraffin with wax. The blocks were processed for sectioning; the sections were then stained with haematoxylin and eosin as nuclear and cytoplasmic stains, respectively to assess the activity. Pathological changes, if any, were viewed under light microscope and recorded.

Statistical analysis

The experimental results were expressed as the Mean±SEM with six rats in each group. The intergroup variation between various groups were analyzed statistically using one-way analysis of variance (ANOVA) using the Graph Pad Prism version 5.0, followed by Dunnett's multiple comparison test (DMCT). Results were considered statistically significant when $P<0.05$.

Results

Effect of wheatgrass extract on serum calcium

A significant elevation was recorded in serum calcium level in Hg toxicity group at each autopsy intervals. Wheatgrass alone treatment did not show any significant alteration in calcium level at all autopsy intervals. Whereas, treatment with wheatgrass during mercuric chloride intoxication significantly decreased level of calcium as compared to mercury treated animals (Fig. 1).

Effect of wheatgrass extract on serum iron

A significant decline in serum iron level in mercuric chloride treated rats was observed during the entire period of study as compared to control animals. Only wheatgrass treatment did not show any significant alteration in serum iron level at all autopsy intervals. However, in combined treatment of wheatgrass with mercuric chloride, a significant elevation was observed at all autopsy intervals (Fig. 2).

Effect of wheatgrass extract on acid phosphatase

A significant elevation in acid phosphatase activity was observed in Hg induced toxicity group throughout whole experimentation period. Only wheatgrass treatment did not show any significant alteration in acid phosphatase activity at all autopsy intervals. However, in combined treatment of wheatgrass and mercury results in gradual recovery in acid phosphatase activity. Significant decline in acid phosphatase activity was noticed with respect to $Hgcl_2$ treated animals (Fig. 4).

Effect of wheatgrass extract on blood urea nitrogen, creatinine, SGOT, SGPT, total bilirubin, alkaline phosphatase and total protein.

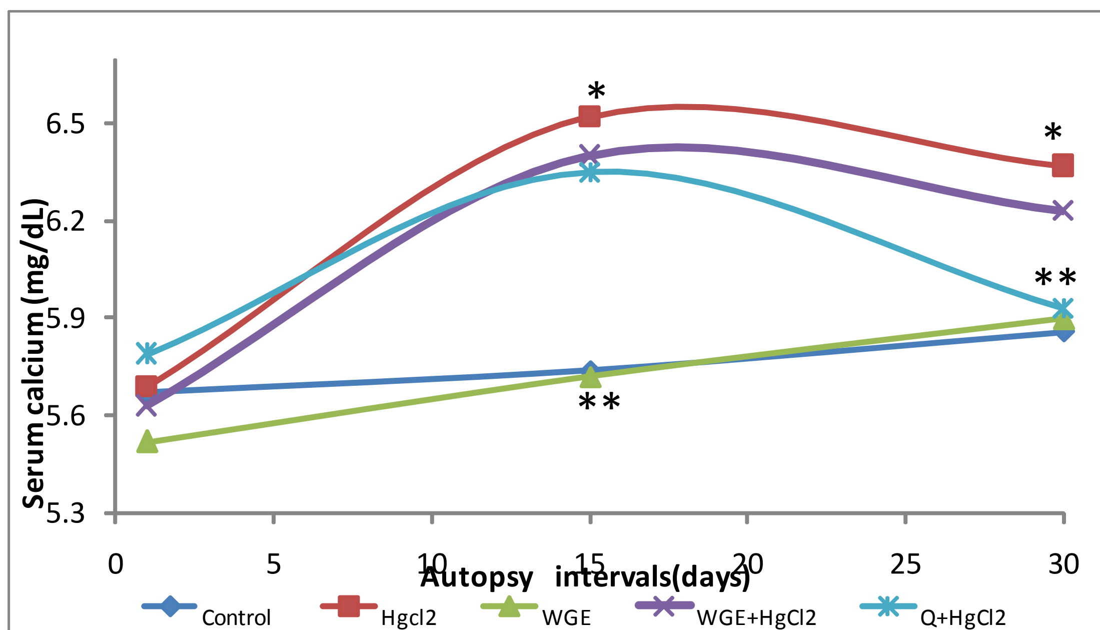

Fig. 1: Effect of Wheatgrass extract on Serum Calcium in mercury intoxicated rats. Values are expressed as mean±SEM, n=6. **P<0.0001 significant as compared with mercuric group. *P<0.0001 vs. Control group.

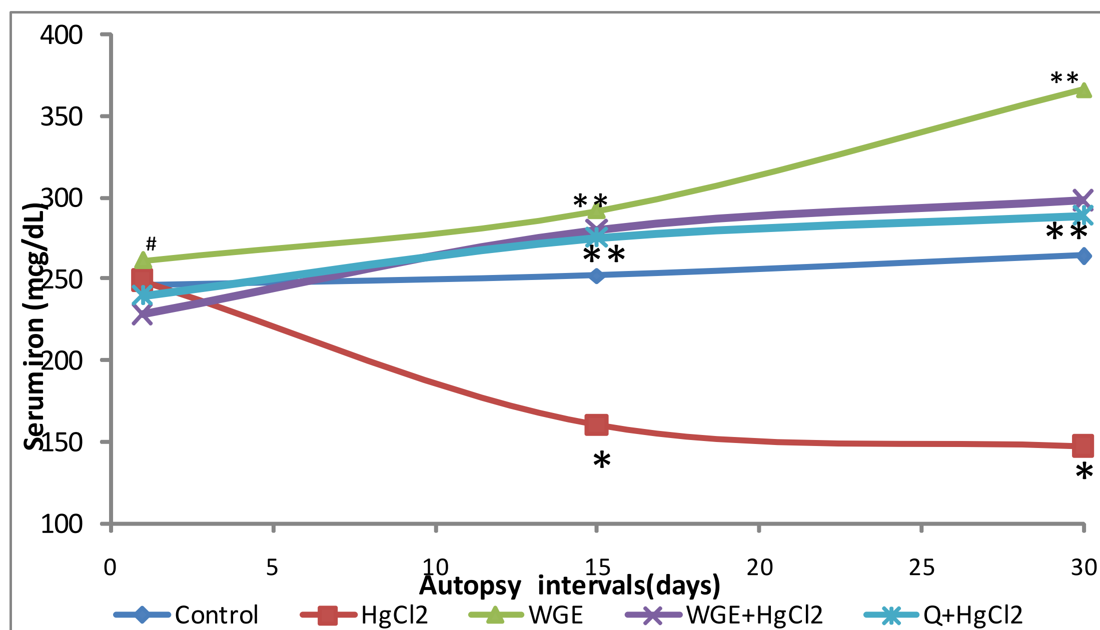

Fig. 2: Effect of Wheatgrass extract on Serum Iron in mercury intoxicated rats. Values are expressed as mean±SEM, n=6. **P<0.0001 vs mercuric group; *P<0.0001 vs control group, # P<0.05 vs. mercuric group, ®P<0.001 vs. mercuric group.

In $HgCl_2$ treated group a significant elevation of blood urea nitrogen, creatinine SGOT, SGPT, total bilirubin and a significant inhibition was noticed in alkaline phosphatase activity and Total protein, at all autopsy intervals with respect to their control animals. Wheatgrass alone treatment did not show any significant alteration at all autopsy intervals. Whereas, combined treatment of wheatgrass with mercury ameliorated the above biochemical changes (Fig. 3, Tables – I, II, III, IV, V and VI).

Effect of wheatgrass extract on renal and hepatic antioxidant parameters

The levels of MDA were significantly (P<0.001)

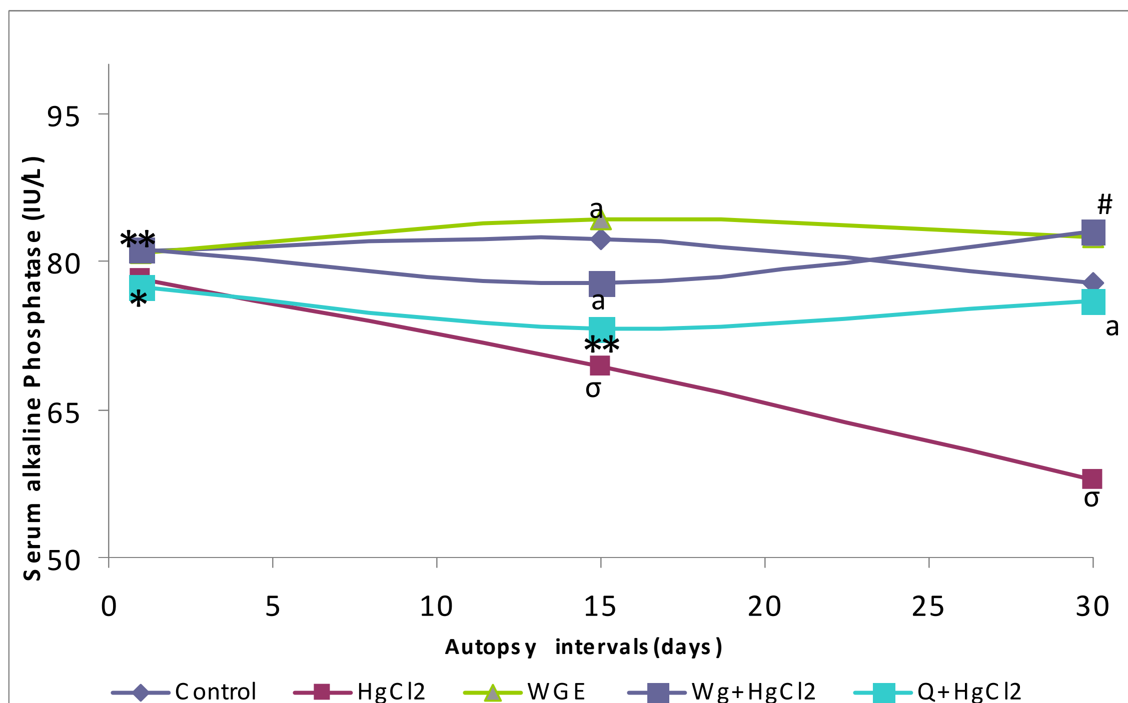

Fig. 3 : Effect of Wheatgrass extract on Serum Alkaline Phosphatase in mercury intoxicated rats. Values are expressed as mean±SEM n=6; #P<0.0001 vs mercuric group; *P<0.05 vs control group; **P<0.05 vs mercuric group; aP<0.001 vs mercuric group; σP<0.001 vs control group.

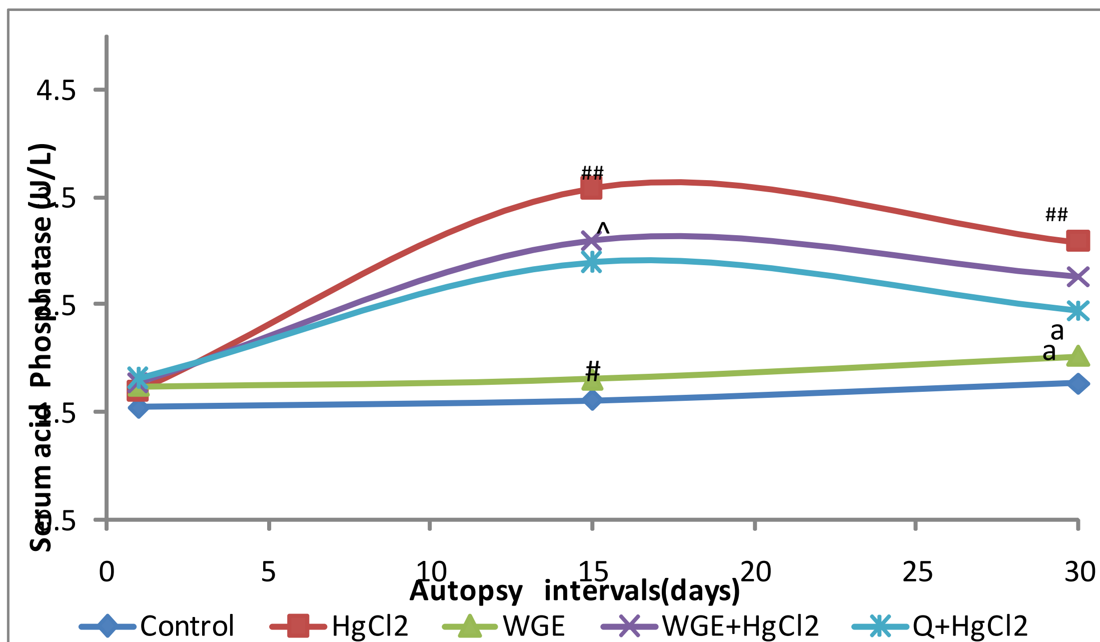

Fig. 4 : Effect of Wheatgrass extract on Serum Acid Phosphatase in mercury intoxicated rats. Values are expressed as mean±SEM, n=6. #P<0.0001 vs mercuric group: ##P<0.0001 vs control group: aP<0.001 vs mercuric group; ^P<0.001 vs mercuric group.

increased, levels of reduced glutathione significantly depleted, significant reductions in CAT and GR activities in mercuric chloride group animals when compared to control group. Wheatgrass alone treatment did not show any significant alteration when compared to control group. Treatment with wheatgrass during mercuric chloride intoxication significantly (P<0.001) ameliorated the above changes (Tables – VII, VIII).

TABLE I : Effect of Wheatgrass extract on Blood Urea Nitrogen in mercury intoxicated rats.

Groups (N=6)	*Treatment*	*Blood urea nitrogen (mg/dl)*		
		1st day	*15th day*	*30th day*
Control	Vehicle	21.57±0.64	23.2±0.7	26.4±0.4
$Hgcl_2$	5 mg/kg	23.5±0.28	28.3±0.6#	34.2±0.4#
WGE	400 mg/kg	22.5±0.67	24.2±0.4	25.7±0.7^
WGE+$Hgcl_2$	400 mg/kg + 5 mg/kg	21.4±0.45	25.9±0.9	30.2±1.1**
Q+$Hgcl_2$	10 mg/kg + 5 mg/kg	20.5±0.41	22.5±0.69*	28.7±0.9**

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #P<0.01 vs normal control; **P<0.05 vs mercuric group; *P<0.01 vs mercuric group; ^P<0.001 vs mercuric group.

TABLE II : Effect of Wheatgrass extract on serum creatinine in mercury intoxicated rats.

Groups (N=6)	*Treatment*	*Creatinine (mg/dl)*		
		1st day	*15th day*	*30th day*
Control	Vehicle	3.38±0.03	3.71±0.06	3.78±0.03
$Hgcl_2$	5 mg/kg	3.36±0.03	4.6±0.05*	5.35±0.03**
WGE	400 mg/kg	3.52±0.04	3.61±0.07	3.82±0.04^
WGE+$Hgcl_2$	400 mg/kg + 5 mg/kg	3.35±0.04	3.4±0.04^	4.11±0.06^
Q+$Hgcl_2$	10 mg/kg + 5 mg/kg	3.56±0.07	3.65±0.06[a]	4.21±0.06[a]

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. ^P<0.001 vs mercuric group; [a]P<0.0001 vs mercuric group; *P<0.0001 vs control group; **P<0.001 vs control group.

TABLE III : Effect of Wheat grass extract on SGOT in mercury intoxicated rats.

Groups (N=6)	*Treatment*	*SGOT (IU/DL)*		
		1st day	*15th day*	*30th day*
Control	Vehicle	18.9±0.15	19.92±0.38	20.1±0.16
$Hgcl_2$	5 mg/kg	17.6±0.46	26.4±0.49#	39.4±0.52**
WGE	400 mg/kg	19.3±0.45[a]	18.5±0.4	20.5±0.53*
WGE+$Hgcl_2$	400 mg/kg + 5 mg/kg	18.4±0.62	21.3±0.5	27.3±0.55#
Q+$Hgcl_2$	10 mg/kg + 5 mg/kg	17.8±0.45	20.5±0.45	26.3±0.5#

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. [a]P<0.05 vs mercuric group; #P<0.001 vs control group; **P<0.0001 vs control group; *P<0.0001 vs mercuric group.

TABLE IV : Effect of Wheat grass extract on SGPT in mercury intoxicated rats.

Groups (N=6)	*Treatment*	*SGOT (IU/DL)*		
		1st day	*15th day*	*30th day*
Control	Vehicle	30.17±0.57	33.9±0.3	31.5±0.3
$Hgcl_2$	5 mg/kg	32.4±0.46	40.7±0.52**	43.2±0.2**
WGE	400 mg/kg	32.6±0.46	33.2±0.48*	32.4±0.52*
WGE+$Hgcl_2$	400 mg/kg + 5 mg/kg	30.6±0.43[a]	32.6±0.6	38.4±0.24[a]
Q+$Hgcl_2$	10 mg/kg + 5 mg/kg	31.3±0.52	35.5±0.52	37.2±0.63[a]

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. [a]P<0.05 vs mercuric group; **P<0.0001 vs control group; *P<0.0001 vs mercuric group.

TABLE V : Effect of Wheatgrass extract on Total protein in mercury intoxicated rats.

Groups (N=6)	*Treatment*	*Total protein (g/dl)*		
		1st day	*15th day*	*30th day*
Control	Vehicle	6.89±0.04	7.08±0.03	7.31±0.03
$Hgcl_2$	5 mg/kg	6.72±0.06	5.18±0.04	3.74±0.04#
WGE	400 mg/kg	6.95±0.09	7.46±0.03	8.34±0.04*
WGE+$Hgcl_2$	400 mg/kg + 5 mg/kg	6.8±0.05	6.9±0.08*	6.57±0.04**
Q+$Hgcl_2$	10 mg/kg + 5 mg/kg	7.1±0.07	6.6±0.04*	6.38±0.07**

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. #P<0.0001 vs control group; *P<0.0001 vs mercuric group; **P<0.05 vs mercuric group.

TABLE VI : Effect of Wheatgrass extract on Total bilirubin in mercury intoxicated rats.

Groups (N=6)	*Treatment*	*Total bilirubin (mg/dl)*		
		1st day	*15th day*	*30th day*
Control	Vehicle	1.02±0.01	1.31±0.03	1.81±0.02
$Hgcl_2$	5 mg/kg	1.14±0.04	2.3±0.03	3.61±0.11##
WGE	400 mg/kg	1.08±0.02	1.84±0.05	2.18±0.04
WGE+$Hgcl_2$	400 mg/kg + 5 mg/kg	0.95±0.06^	1.36±0.04	2.89±0.05
Q+$Hgcl_2$	10 mg/kg + 5 mg/kg	1.23±0.05	1.56±0.04x	2.42±0.05x

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. ##P<0.001 vs control group; ^P<0.01 vs mercuric group; xP<0.001 vs mecuric group.

TABLE VII : Effect of Wheatgrass extract on MDA and CAT in mercury intoxicated rats.

Groups (N=6)	*MDA (nm/g)*		*Catalase (k/min)*	
	Liver	*Kidney*	*Liver*	*Kidney*
Control	37.6±0.51	40.7±0.55	35.15±0.5	37.5±0.5
$Hgcl_2$	51.4±0.41**	57.1±0.66*	24±0.45**	24.5±0.72**
WGE	27.5±0.58*	33.4±0.66	39.3±0.89*	38.5±0.43
WGE+$Hgcl_2$	38.6±1.0	45.2±0.44	30.5±058	28.6±0.51^
Q+$Hgcl_2$	32.6±0.46*	41.±0.43	31.3±0.58	30.6±0.46

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. *P<0.0001 vs mercuric group; **P<0.0001 vs control group:; ^P<0.05 vs mercuric group.

TABLE VIII : Effect of Wheatgrass extract on GSH and GR in mercury intoxicated rats.

Groups (N=6)	*GR*		*GSH (mcg/ml)*	
	Liver	*Kidney*	*Liver*	*Kidney*
Control	14.82±0.44	28.2±0.35	74.13±0.8	51.33±0.69
$Hgcl_2$	21.6±0.42**	37.3±0.51**	36.3±0.15a	19.9±0.3a
WGE	42.56±0.54	45.33±0.66*	66.4±0.9*	40.26±0.3*
WGE+$Hgcl_2$	34.3±0.69#	33.51±0.54*	63.4±0.7*	42.1±0.5*
Q+$Hgcl_2$	18.2±0.577	48.24±1.07^	64.2±0.6*	45.20.6

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. **P<0.001 vs control group; *P<0.0001 vs mercuric group; #P<0.02 vs mercuric group; ^P<0.01 vs mercuric group; aP<0.0001 vs control group.

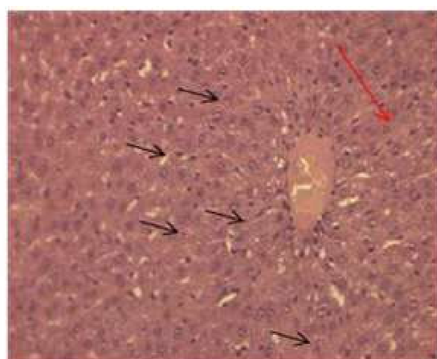

Fig.6.(a)Control Liver-Black arrow indicates that individual hepatocytes appeared normal Portal(red arrow), per portal region appeared normal. Sinusoidal spaces appeared normal. Degenerative and inflammatory changes were not observed. Tissue is stained with Haematoxylin and Eosin at magnification 80X.

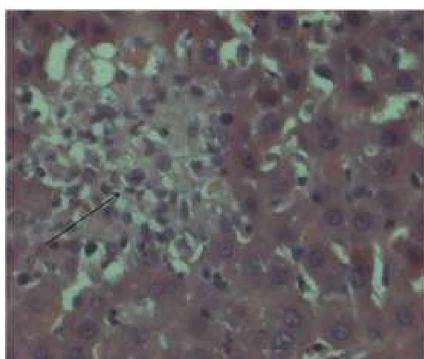

Fig.6.(b) Hgcl$_2$– Liver- Multiple foci of necrosis observed in the periportal region of liver (arrow). There is invasion of inflammatory cells in the necrosed region. Tissue is stained with Haematoxylin and Eosin at magnification 100X

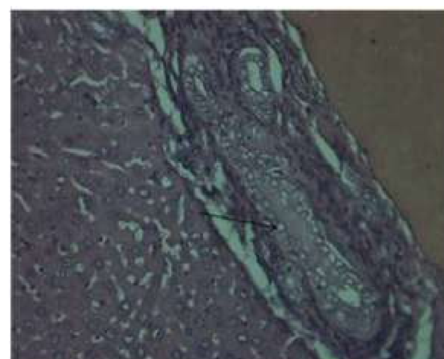

Fig.6.(c) WGE – Liver Also bile duct hyperplasia / bile duct proliferation were observed in the portal region (arrow). Tissue is stained with Haematoxylin and Eosin at magnification 800X

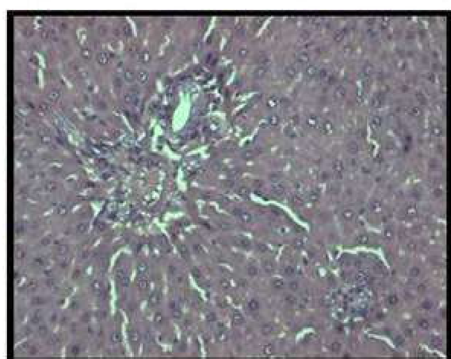

Fig.6.(d).WGE+ Hgcl2- liver: A mild foci of inflammation was observed in the periportal region. Sinusoidal spaces appeared normal. Tissue is stained with Haematoxylin and Eosin at magnification 100X

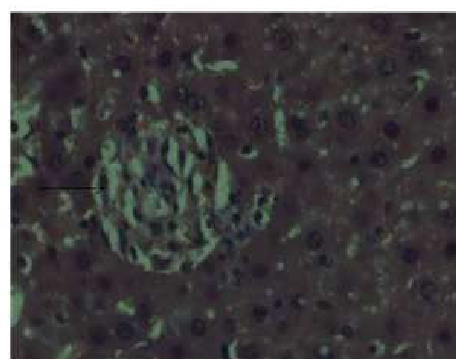

Fig.6.(e).Q+Hgcl$_2$ Liver:Multiple foci of necrosis observed in the periportal region of liver. (Arrow). No inflammatory cells in these necrotic foci. Tissue is stained with Haematoxylin and Eosin at magnification 400X

Hematological parameters

Parameters of toxin control rats were found to be significantly altered compared to those of the normal control group. The leucocytes (WBC) count was found to be increased; RBC and hemoglobin count was significantly decreased in toxin control animals significantly when compared to the normal control group. Treatment with wheatgrass extract produced a significant increase in Hb level, RBC count. No alteration in the WBC count was observed. Whereas, in mercuric chloride intoxicated animals' treatment with wheatgrass prevented the hemolysis and significantly increased the Hb content and RBC count. WBC count was also brought back towards the normal levels when compared to toxin control animals. The effect of wheatgrass was comparable to those of Quercetin treated animals (Table IX).

TABLE IX : Effect of Wheatgrass extract on Hematological Parameters in mercury intoxicated rats.

Groups	*Hemoglobin (Percentage)*	*RBC count (Million/Cmm)*	*WBC count (Cells/Cmm)*
Control	9.5±0.30	6.89±0.02	5,300
Hgcl$_2$	6.86±0.07*	5.31±0.08	12,400*
WGE	14.4±0.44**	7.7±0.27**	5,600**
WGE+Hgcl$_2$	8.46±0.03	6.84±0.04	8,900**
Q+Hgcl$_2$	8.92±0.11#	7.13±0.03#	9,300#

Values are expressed as mean±SEM, n=6. Data analyzed by one way ANOVA followed by Dunnet's Multiple Comparison Test. *P<0.001 vs control group; **P<0.001 vs mercuric group; #P<0.0001 vs mercuric group.

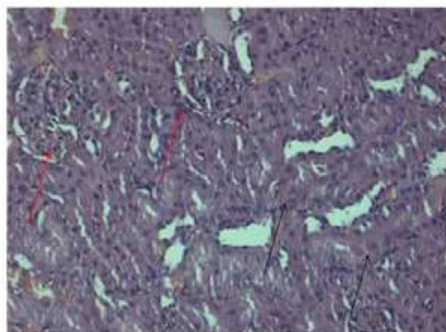

Fig.5.(a).Control Kidney showed normal Glomerulus and tubular structures. No reactive changes like degeneration, Inflammation in the kidney were observed. Red arrow indicates Glomerulus, Black arrow indicates Tubules. Tissue is stained with Haematoxylin and Eosin at magnification 40X.

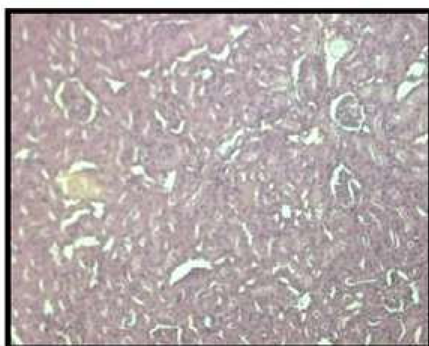

Fig.5.(b).WGE–Kidney:Glomerulus region appeared normal. Inflammation was not observed on the kidney. Tissue is stained with Haematoxylin and Eosin at magnification 100X.

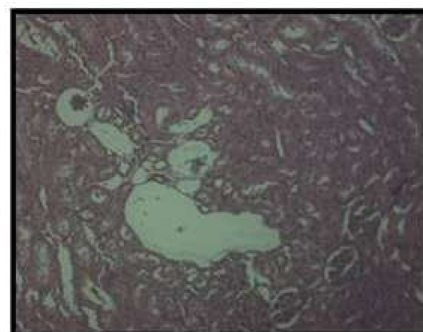

Fig.5.(c). Hgcl2-Kidney: Glomerulus region appeared normal. But mild cystic degeneration was observed in the tubular region accumulated with fluids and RBC (arrow) Tissue is stained with Haematoxylin and Eosin at magnification 400X.

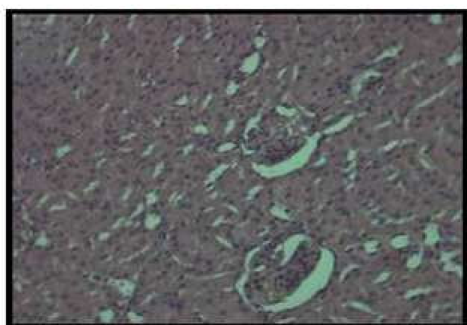

Fig.5.(d).WGE+Hgcl2: Glomerulus and tubular structure appeared normal. No degenerative, Necrotic and inflammatory changes Were observed in the kidney. Glomerulus indicates red arrow. Tubules indicates black arrow Tissue is stained with Haematoxylin and Eosin at magnification 80X.

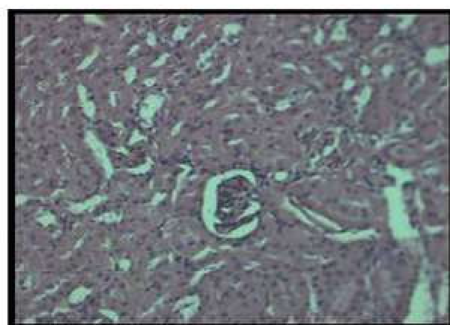

Fig.5.(e).Q+Hgcl2– kidney: Kidney showed normal Glomerulus and tubular structures. No reactive changes like degeneration, Inflammation in the kidney were observed. Tissue is with Haematoxylin and Eosin at magnification

Discussion

The present study was focused to evaluate that the Wheat grass extract protects hepatic and renal cells against mercury induced damage via the scavenging of reactive oxygen species. The results of the present study revealed that mercury intoxication causes significant increase in calcium level, acid phosphatase, blood urea nitrogen, creatinine, SGOT, SGPT, Total bilirubin and lipid peroxidation content and significant decrease in serum iron level, alkaline phosphatase, Total protein and blood glutathione level.

The divalent calcium cation (Ca^{2+}) has a unique position among cellular ions. In the soluble form, it is the major constituent of bones, teeth and plays an important role such as membrane stabilizer, cofactor for proteins and electric charge carrier. In the present investigation highly significant increase in the serum calcium level was observed in Hg intoxicated rats. Present findings are in agreement with the findings of Shenker et al (1993) (28). They observed an increase in calcium level in white blood corpuscles (WBC) due to mercuric chloride exposure. An increase in intracellular calcium may play a role in the cytotoxicity in terms of apoptosis and necrosis (29).

There was significant increase in serum acid phosphatase activity after $Hgcl_2$ exposure. Similar reports have also been reported by Mehra and Kanwar (1986) following $Hgcl_2$ administration (30). Acid phosphatase activity is localized in cellular lysosomes. An enhanced peroxidation of lysosomal membranes due to $Hgcl_2$ intoxication causes lysis of

membrane and oozing out of the enzyme hence results in an increased acid phosphatase activity.

Further, there was a significant (P<0.001) decrease in serum iron level and alkaline phosphatase activity recorded following mercury intoxication. The mercuric chloride treatment significantly decreases the red blood corpuscles (RBC) count (31). Erythropoiesis is controlled by kidney producing hormone erythropoietin. Mercury causes severe renal damage. So due to renal damage, erythropoietin cannot be synthesized and process of red blood corpuscles (RBC) formation is disturbed. So, decrease in RBC count may be correlated with the decrease in serum iron level.

In the liver, enzyme alkaline phosphatase is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra hepatic or intra hepatic leads to decrease in serum alkaline phosphatase activity (32). Present findings are in agreement with the findings of El-Demerdash (2001) (33) who showed that $Hgcl_2$ (5.0 mg/kg b.w.) intoxication significantly decreases the alkaline phosphatase activity in rats.

Mercury intoxication showed a significant increase in transaminases (SGOT and SGPT) activities. The increase in SGOT and SGPT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminases in the blood stream. The increase in serum bilirubin concentrations in toxin control rats was observed. It was found that increase in serum bilirubin is associated with free radical production (34). Significant decrease in serum total protein was recorded in mercury induced rats. This decrease might be due to alterations in protein synthesis and/or metabolism.

The blood lipid peroxidation level showed a highly significant elevation and GSH level, CAT level showed a highly significant depletion following Hg exposure. Lipid peroxidation is considered as a molecular mechanism of oxidation of cellular lipid based macromolecules. Overproduction of ROS enhances the lipid peroxidation and subsequently increases the lipid peroxidation products like malondialdehyde (MDA) and other TBARS levels which lead to degradation of cellular macromolecules.

GSH is the major thiol, which binds electrophilic molecular species and free radical intermediates. It plays a central role in the antioxidant defence system, metabolism and detoxification of exogenous and endogenous substances (35). Mercury has high affinity for GSH and causes the irreversible excretion of, up to two GSH tripeptides (36). The metalGSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. However, it depletes the GSH from the cell and thus decreases the antioxidant potential. Hydrogen peroxide subsequently converts into non toxic water and oxygen molecules by the action of catalase.

Chlorophyll: A 70-83% increase in red blood cells and hemoglobin concentration was noted within 10-16 days of regular administration of chlorophyll derivatives. It was reported that chlorophyll enhanced the formation of blood cells in anemic animals (37). Chlorophyll is soluble in fat particles, which are absorbed directly into blood via the lymphatic system. Chlorophyll present in wheatgrass can protect us from carcinogens; it strengthens the cells, detoxifies the liver and blood stream, and chemically neutralizes the polluting elements. It has superoxide dismutase and Cytochrome oxidase, which neutralize free radicals and prevent them from causing the cell damage.

Because it is also high in saponins, wheat grass extract helps to detoxify the body by increasing the elimination of hardened mucous, crystallized acids and solidified, decaying fecal matter and thus, provides an optimum nutritional environment inside the body system. Histopathological changes also support the above results. Histological sections of liver in control and Wheatgrass treated rats showed the normal hepatocytes, and central vein. Mercury intoxication produced various pathological lesions in the liver such as cytoplasmic vacuolization, and centrilobular necrosis. Concomitant treatment of Wheatgrass with Mercury showed prominent recovery and normal architecture with mild residual degeneration. Histological sections of kidney in

control and Wheatgrass treated rats showed the normal glomerulus and tubular structure. Mercury intoxication causes cystic degeneration in the tubular region accumulated with fluids and RBC. Concomitant treatment of Wheatgrass with Mercury showed prominent recovery and normal architecture with mild residual degeneration

To conclude, the present study demonstrated that deleterious reactive oxygen species or lipid peroxides responsible for Hg induced toxicity may be alleviated by several active components found in Wheatgrass extract, which in turn is reflected by nearly normal level of several biochemical parameters in blood of albino rats. Wheatgrass extract can be given as a dietary supplement to human populations exposed to environmental toxicants and can provide protection against toxic effects without being appreciably harmful itself.

Acknowledgements

The authors are thankful to the authorities of Malla Reddy College of Pharmacy, Secunderabad, for providing support to this study.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflicts of Interest Statement

The authors declare that there are no conflicts of interest.

References

1. World Health Organization (WHO). Environmental Health Criteria 118. Inorganic mercury-environmental aspects. WHO, Geneva, Switzerland, 1991, pp. 115–119.
2. Jarup L. Hazards of heavy metal contamination. *Br Med Bull* 2003; 68: 167–182.
3. Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological profile for mercury. ATSDR/US. Public Health Service. 1989.
4. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Claredon Press, Oxford, UK. 1989.
5. Miller OM, Lund BO, Woods JS. Reactivity of Hg (II) with super oxide. Evidence for the catalytic dismutation of superoxide by Hg (II). *J Biochem Toxicol* 1991; 6: 293.
6. Hussain S, Atkinson A, Thompson SJ, Khan AT. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver and kidneys of mice. *J Environ Sci Health B* 1999; 34(4): 645–660.
7. Tripathi N, Flora SJ. Effects of some thiol chelators on enzymatic activities in blood, liver and kidneys of acute arsenic(III) exposed mice. *Biomed Environ Sci* 1998; 11 (1): 38–45.
8. Wentz PW. Chelation therapy: conventional treatments. In: Advance Magazines/Administrators of the Laboratory. LabCorp, Burlington, NC, King of Prussia, PA, Merion. 2000.
9. Swati P, Sushma D, Indira R, Alka G, Mamta D. Multitude potential of Wheat grass extract Juice (Green Blood): An overview. *Chronicles of Young Scientist* 2010; 1(2): 23–28.
10. Lai CN, Dabney B, Shaw C. Inhibition of In vitro metabolic activation of carcinogens by wheat sprout extracts. *Nutrition & Cancer* 1978; 1(1): 27–30.
11. Ben-Arye E, Goldin E, Wengrower D, Stamper A, Kohn R, Berry E. Wheat grass extract Juice in the Treatment of Active Distal Ulcerative Colitis: A Randomized Double-blind Placebo-controlled Trial. 2002; 37(4): 444–449.
12. Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam TP, Reddy AV. Evaluation of the antioxidant activity of wheatgrass (Triticum aestivum L.) as a function of growth under different conditions. *Phytother Res* 2006; 20(3): 218–227.
13. Nenonen MT, Helve TA, Rauma AL, Hanninen OO. Uncooked, Lactobacilli-rich, Vegan Food and Rheumatoid Arthritis. *British Journal of Rheumatology* 1998; 37: 274–281.
14. Marwaha RK, Bansal D, Kaur S, Trehan A. Wheat grass extract Juice Reduces Transfusion Requirement in Patients with Thalassemia Major: A Pilot Study. *Indian Pediatrics* 2004; 41: 716–720.
15. Mukesh Kumar Sharma, Reena Patni, Madhu Kumara, Ashok Kumara. Modification of mercury-induced biochemical alterations in blood of Swiss albino mice by *Spirulina fusiformis Environ Toxicol Pharmacol* 2005; 20: 289–296.
16. Reitman S, Frankel SA. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28: 56–63.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 1979; 95(1): 351–358.
18. Bowers LD. *Clinical Chemistry* 1980; 26: 665.
19. Varley H. Practical Clinical Biochemistry, vol. 1, fifth ed. William and Hieneman, Medical Books Ltd., London. 1980.
20. Henry RH. Clinical Chemistry, Principles, and Techniques. Harper and Row, New York, 1964; p. 684.

21. Young DS. Effects of Drugs on Clinical Laboratory Test. AACC Press, Washington, DC, 1991; pp. 390–391.
22. King EJ, Jagatheesan KA. *J Clin Pathol* 1959; 12: 86.
23. Fiske CH, Subbarow Y. The colorimetric determination of phosphatase. *J Biol Chem* 1925; 66: 375–400.
24. Nwanjo HU, Ojiako OA. Effect of vitamins E and C on exercise induced oxidative stress. Global *J Pure Appl Sci* 2005; 12: 199–202.
25. Aebi HE. Catalase. In: Bergmeyer, H.U., Bergmeyer, J., Grabl, M., editors. Methods of enzymatic analysis. Vol. 3. Weinheim: Velag Chemie Gmbh. 1993; pp. 273–286.
26. Ellman GC. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70–77.
27. Lamberg SL, Rothstein R. 1977. Laboratory Manual of Hematology and Urinalysis. Avi. Publishing Company. Inc, West Port Connecticut. U.S.S.R.
28. Shenker BJ, Berthold P, Rooney C, Vitale L, Debolt K, Shapiro IM. Immunotoxic effects of mercuric compounds on human lymphocytes and monocytes. III. Alterations in B-cell function and viability. *J Immunopharmacol Immunotoxicol* 1993; 15(1): 87–112.
29. Kim SH, Sharma RP. Mercury-induced apoptosis and necrosis in murine macrophages: role of calcium-induced reactive oxygen species and p38 mitogen-activated protein kinase signaling. *Toxicol Appl Pharmacol* 2004; 196(1): 47–57.
30. Mehra MO, Kanwar KC. Enzyme changes in the brain, liver and kidney following repeated administration of mercuric chloride. *J Environ Pathol Toxicol Oncol* 1986; 7(1/2): 65–71.
31. Patni R, Sharma MK, Kumar M. Modulation of certain hematological parameters in mercuric toxicity by *Spirulina fusiformis* in Swiss albino mice. *Indian J Environ Toxicol* 2001; 11(1): 32–34.
32. Vandenberghe J. Hepatotoxicology: mechanisms of liver toxicity and methodological aspects. In: Niesink, J.M., Vries, J.D., Hollinger, M.A. (Eds.), Toxicology: Principle and Applications. CRC Press, Boca Raton, 1995; p. 718.
33. El-Demerdash FM. Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver and blood of rats. *J Environ Sci Health B* 2001; 36: 489.
34. Thomas W, Sedlak MD, Solomon H, Snyder MD. Bilirubin benefits: cellular protection by a biliverdin reductasecycle. *Pediatrics* 2004; 113: 1776–1782.
35. Zalups RK, Lash LH. Interactions between glutathione and mercury in the kidney, liver and blood. In: Chang, L.W. (Ed.), Toxicology of Metals. CRC Press, Boca Raton, 1996; pp. 145–163.
36. Patrick L. Mercury toxicity and antioxidants. Part 1. Role of glutathione and alpha-lipoic acid in the treatment of mercury toxicity. *Altern Med Rev* 2002; 7(6): 456–471.
37. Singh N, Verma P, Pandey BR. Therapeutic Potential of Organic Triticum aestivum Linn. (Wheat grass extract) in Prevention and Treatment of Chronic Diseases: An Overview. *Int J Pharm Sci Drug Res* 2012; 4(1): 10–14.