



The Antioxidant Efficacy of Wheatgrass (*Triticum aestivum*) on Mercuric Chloride (HgCl₂) -Induced Oxidative Stress in Rat Model

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Abstract

Mercury is a harmful toxic pollutant, which has hepato-nephrotoxic, hematotoxic, genotoxic and neurotoxic effects. The aim of the study was to evaluate the protective efficacy of wheatgrass on mercuric chloride (HgCl₂) induced oxidative stress and associated complications in rat model. Albino rats were divided into four groups (three rats per group). Group I normal control group. Group II oxidative stressed group received mercuric chloride (0.5 mg/kg/day). Group III only received wheatgrass extract (100 mg/kg/day), whereas Group IV received wheatgrass (100 mg/kg/day) after one hour, followed by mercuric chloride (0.5 mg/kg/day) for 30 days. The results of the study showed that wheatgrass supplementation significantly decreased the HgCl₂ induced elevated oxidative stress parameters Plasma Malondialdehyde (MDA) content, Plasma membrane redox system (PMRS), Advanced oxidation protein products (AOPP), simultaneously elevated lipid profile (Total Cholesterol, Triglycerides, Low-density lipoprotein (LDL), liver enzymes as, Plasma Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT), Serum Urea, and Creatinine levels in rats. In addition, wheatgrass treatment improved the antioxidant status in terms of intracellular Reduced Glutathione (GSH), Ferric reducing antioxidant power (FRAP) and 2, 2- diphenyl -1- picrylhydrazyl (DPPH). Therefore it can be concluded that wheatgrass has great potential to diminish the stress-mediated complications and improve the antioxidant status.



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Introduction

Exposure of mercury pulled attention of whole world due to its harmful effects. It is a heavy metal and harmful toxic pollutant which has multiple adverse effects. It is commonly used in battery monometer, dental amalgamation, electrical switches, as a catalyst in chemical manufacturing, wood preservative, photography industry, limited use in gold mining and in leather industry although. Furthermore it is heavily used in many industries household product, pharma industry and agriculture industry,¹ which leads to high dispersion in environment. Bringing in notice over there fatal effect on human health and the environment.²⁻⁶ Its effects are nephrotoxic,^{7, 8} hematotoxic,⁶ hepatotoxic,^{8, 9} genotoxic,¹⁰ neurotoxic¹¹ and reduced reproductive strength.^{12, 13}

Mercury affects antioxidant mechanisms and increase in oxidative stress through the alteration in various mechanism, is possibly resulted in the reduction in Adenosine triphosphate (ATP) content, decrease of antioxidant enzyme activity, depletion of cellular cysteine thiols, mercury has ability to react with Glutathione (GSH, thiol antioxidant)¹⁴ and creates a complex formulation which reduced the antioxidant GSH level in body and results to oxidative stress.^{15, 16} Oxidative stress leads to impaired transport mechanism, lipid peroxidation, denaturation of protein and DNA.¹⁷⁻²⁰

Various previous studies reported that plant and plant based products are good source of natural antioxidant that can be used as herbal natural remedy for protection from oxidative stress, plant based treatments has no side effects and has been used since long time.²¹ Among the various medicinal plants wheatgrass is one which is good source of antioxidant and used globally in various perspectives. Wheatgrass (*Triticum aestivum*) is a young grass (seven to nine days) of wheat plant. Wheat (*Triticum*) species is an annual and biennial cereal grass of the (Poaceae) family, is the world's largest edible grain cereal grass crop which is widely cultivated almost all over the world. Almost more than twenty five cultivars of wheat are identified in world of which *Triticum aestivum* is the foremost Indian cultivar of wheatgrass.²² Wheatgrass is a versatile medicinal plant and had been used as a natural medicinal plant from the ancient period of time. In traditional Indian ayurvedic system wheatgrass is

an excellent source of vitamins (A, C, E), minerals (iron, calcium, phosphorus), proteins, enzymes, chlorophyll and other bioactive compounds (Rutin, Chlorophyllin, Apigenin, Quercetin).²³ These nutrients and bioactive compounds increased clinical utility of wheatgrass and makes it a medicinal plant for the treatment of various diseases and life threatening conditions. It has been shown various pharmacological potentials such as anti-cancer activity,²⁴ anti-ulcer activity,²⁵ anti-diabetic activity,²⁶ antioxidant activity,²⁷ anti-thalassemic activity²⁸ anti-arthritis activity, anti-inflammatory and anti-aging activity.²⁹ These pharmacological potentials were due to presence of various phenolic, flavonoid compounds, vitamins and minerals that makes wheatgrass a sturdy natural therapeutic agent for the prevention of oxidative stress and allied complications with mercuric chloride toxicity. Therefore, the purpose of the experiment was to assess the protective effect of wheatgrass on mercuric chloride induced oxidative stress in rats.¹

Materials and Methods

Chemicals and Reagents

Mercuric chloride (HgCl_2 , 99.5% purity) was purchased from Sigma-Aldrich Chemical Company India and other required chemicals of analytical grade were procured, Merck India and HIMEDIA Labs, India.

Cultivation of the Wheatgrass

Wheatgrass seeds (cultivar sharbati) were purchased from local market of Allahabad, India. For experimental work wheatgrass was grown at laboratory of Centre of Food Technology, University of Allahabad, U.P. India, by using tray method in indoor conditions. Plastic trays were filled with soil contains three parts of soil and one part of organic fertilizer. One night soaked wheat grains were then evenly spread on the surface of the soil and further covered with a thin layer of soil. Spray some water evenly over soil and three to four hours morning sunlight was allowed daily for growth of grass. On the seventh day, grass was harvested. At this stage, wheatgrass is at its nutritional peak.³⁰

Preparation of Plant Extract

The harvested wheatgrass was shorted and cleaned with water, and then dried in a cabinet tray dryer (Chemida, Mumbai, India) at $55 \pm 2^\circ\text{C}$ for six hours. After then dried grass was subjected to making

fine powder by using a high speed electronic mixer grinder (Sumeet Domestic Plus, M/s. Sumeet, Nashik, India), and passed through fine sieve no. 40. 10g wheatgrass powder was suspended in 100 ml of ethanol using 250 ml conical flask and kept on orbital shaker for 48 h at 37°C. After 48h, the supernatant was filtered through Whatman filter paper no.1 and lyophilized the sample for drying. The filtrate was then reduced to 1/10th of its initial volume. Obtained lyophilized dried sample was then dissolved in drinking water for dosing and stored at 4°C for further analysis.^{31, 32}

Experimental Animals

The experiment was carried out with 12 male albino rats (4 to 5 months old) with body weight between 157 ± 51g (IAEC/AU/2017(1)/008). They were housed in a temperature controlled facility (25 ± 5°C) with 12 h light–dark cycle for one week, at laboratory of Department of Biochemistry, University of Allahabad, U.P. India. All rats were fed with normal laboratory diet nutrient rich pellets, and had free access to drinking water.

Animal Model and Study Protocol

After the one week of stabilization period, the rats were randomized and grouping three rats were placed in each group, and were given the following dosing:

Group I

Normal control (NC), received no treatment/ supplementation.

Group II

Oxidative stressed group (Hg), received HgCl₂ (0.5 mg/kg).

Group III

(Hg+WG) group received HgCl₂ 0.5 mg/kg body weight and wheatgrass extract 100 mg/kg body weight.

Group IV

Normal control group treated with only wheatgrass extract (N+WG) 100 mg/kg body weight.

One hour before treatment with wheatgrass, group III received HgCl₂. All the administrations were performed once daily through oral gavage for 30 days. Oxidative stress was induced by

administration of mercuric chloride in drinking water at a dose of 0.5mg/kg body weight. The doses of HgCl₂ and wheatgrass were selected based on previous studies.^{33, 34}

Biochemical Assays

After 24 hours of last administration. The rats were sacrificed under light anaesthesia. Blood samples were collected by cardiac puncture into 10 units / ml heparin rinsed anticoagulant syringes. Plasma was obtained from blood sample after centrifugation (1500×g for 10 min) and stored at 4°C for analysis. and then red blood cells were pelleted by centrifugation at 800 g for 10 min at 4°C. After the removal of plasma (immediately frozen at -80°C until use for biochemical assays), buffy coat, and the upper 15% of packed red blood cells (PRBCs), the RBCs were washed twice with cold phosphate buffered saline (PBS) (0.9% NaCl and 10 mmolL⁻¹ Na₂HPO₄; pH 7.4) and then used for further experiment.³⁵

Oxidative Stress Markers

Erythrocyte Malondialdehyde (MDA) was measured using the thiobarbituric acid (TBA) method.³⁶ Estimation of Advanced oxidation protein products (AOPP) levels in plasma was performed by spectrophotometric detection method.^{37, 38} The activity of the erythrocyte Plasma membrane redox system (PMRS) was measured by the reduction of ferricyanide the method.³⁹

Antioxidant Status

Glutathione (GSH) was estimated by 5,5'-dithiobis (2- nitrobenzoic acid) (DTNB) method it is based on the ability of the SH group to reduce DTNB and form a yellow coloured anionic product whose optical density is measured at 412 nm.^{40, 41} The Ferric reducing antioxidant activity of the plasma samples was determined using Ferric reducing antioxidant power (FRAP) assay.^{42, 43} Radical scavenging capacity of plasma samples were estimated by 2, 2- diphenyl -1- picrylhydrazyl (DPPH) reduction assay.^{44, 45}

Lipid Profile, Liver and Kidney Functions Tests

Lipid profiles (Total cholesterol, triglyceride and LDL-Cholesterol), liver function parameters such as (Plasma Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were measured in serum

using kits from (Erba Diagnostics, Mannheim, Germany). Kidney dysfunction test (Serum Urea and Creatinine) were assayed using the kit from (Span Diagnostic Ltd.Surat, India).^{46, 47}

Statistical Analysis

Statistical analyses were performed using one way ANOVA followed by Tukey's Multiple Comparison Test. $P < 0.05$ was considered to be statistically significant with Graph Pad Software, San Diego California USA version 5.01 for Windows. Results were expressed as the mean \pm S.D.

Results and Discussion

Mercuric chloride is the one of the most harmful toxic pollutant of the world, excess accumulation of mercuric chloride in human body poses various adverse changes. It leads to formation of free radicals that cause oxidative stress and other associated complications such as hepato-nephrotoxicity,⁴⁸⁻⁵⁰ mental retardation⁵¹ and heart problems.⁵²

In this study, protective role of wheatgrass extract was investigated in rat model against oxidative stress induced by $HgCl_2$. Wheatgrass aqueous extract act as a quencher of free radicals and neutralizing those.^{14, 53} Therefore, it is concluded that the wheatgrass reduces the adverse consequences of $HgCl_2$ induced oxidative stress through countering the excessive radicals of all major types. The present study demonstrated that after 30 days of oral administration of wheatgrass extract, the antioxidant

status (GSH, FRAP, DPPH) of stressed rats were significantly elevated. On the other hand reduction in lipid profiles (TC,TG,LDL), kidney function tests (urea, creatinine), liver function tests (ALP, AST, ALT) and oxidative stress markers (MDA, AOPP, PMRS) was observed in the $HgCl_2$ -induced oxidative stressed rats. The results of the study revealed that wheatgrass protects the antioxidant defense system by activating antioxidant enzymes and reduced mercury-induced oxidative stress by scavenging free radicals *in-vivo* conditions.

Evaluation of the plasma antioxidant capacity (GSH, FRAP and DPPH) is the primary step in the prophecy of oxidative stress in various pathological conditions such as heart diseases, hepato- nephrotoxicity, cancer, Alzheimer disease and hyperglycemia.⁵⁴ The result of the experiment shows that, the reduced glutathione (GSH), FRAP and DPPH levels significantly decreased in $HgCl_2$ -treated rats in comparison to the normal control rats ($P < 0.001$) Figure 1,2,3. Conversely finding of the study showed that oral supplementation with wheatgrass extract significantly increased ($P < 0.001$) the antioxidant status (GSH, FRAP and DPPH) level as noticed in the Hg+WG treated groups in comparison to $HgCl_2$ -treated rats and this might be as a result of the wheatgrass extract containing polyphenols, flavonoids, chlorophyll, vitamin A, vitamin C, and vitamin E, all these bioactive compounds poses good antioxidant capacity and makes wheatgrass a strong antioxidant agent.^{27,55}

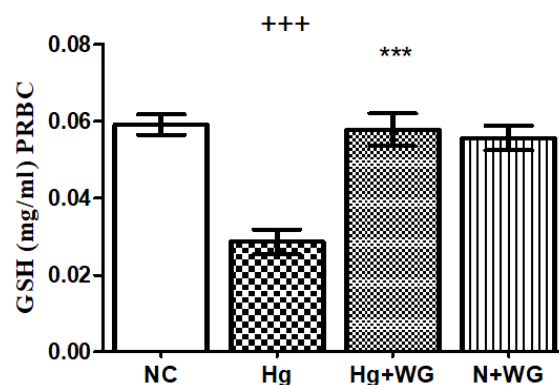


Fig.1: Effect of wheatgrass treatment on GSH level of $HgCl_2$ induced oxidative stressed rats. Concentration of GSH is expressed as mg/ml PRBC. Values are presented as means \pm SD. +++ $P < 0.001$ compared with oxidative stressed group vs. control. * $P < 0.001$ compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)**

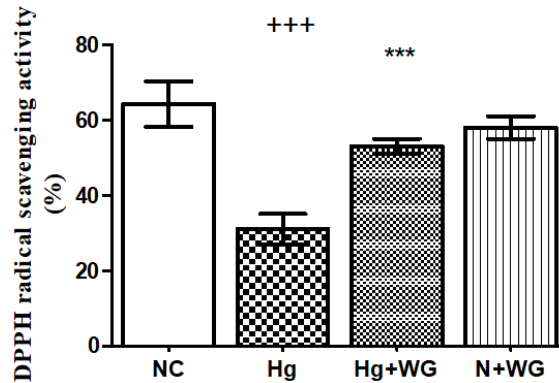


Fig. 2: Effect of wheatgrass treatment on DPPH % inhibition of HgCl₂ induced oxidative stressed rats. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

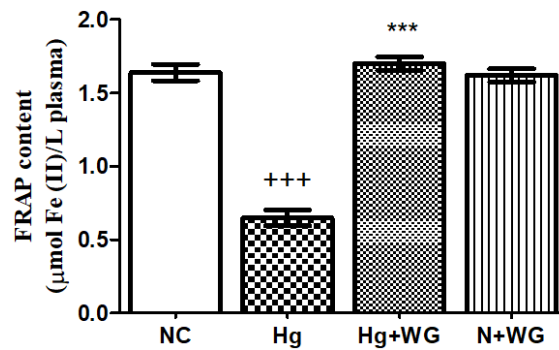


Fig. 3: Effect of wheatgrass treatment on FRAP level of HgCl₂ induced oxidative stressed rats. FRAP value is expressed in µmolFe(II)/l plasma. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

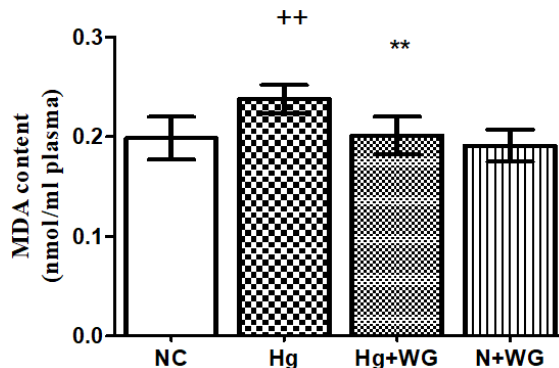


Fig. 4: Effect of wheatgrass treatment on plasma malondialdehyde (MDA) content of HgCl₂ induced oxidative stressed rats. Concentration of MDA is expressed as nmol/ml of plasma. Values are presented as means ± SD. ++P<0.01 compared with oxidative stressed group vs. control. **P<0.01 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

HgCl₂ causes the generation of free radicals, reactive oxygen species (ROS) are highly responsible for the lipid peroxidation of biological membranes, which involves decomposition of fatty acids especially (PUFA), production of 4-hydroxynonenal (4HNE) and malondialdehyde (MDA). Lipid oxidation products, such as MDA reacts with proteins, DNA, phospholipids modify RNA, and other biomolecules, another product 4HNE, is hepatotoxic, cytotoxic, genotoxic, mutagenic and induce dysfunction of immune systems. MDA are mostly used parameter for the estimation of the lipid peroxidation by reactive oxygen species (ROS).⁵⁶ The result of the study demonstrated that HgCl₂ administrated group significantly ($P < 0.01$) enhance in MDA level in comparison with the control group. After wheatgrass treatment MDA level was significantly ($P < 0.01$) decrease in Hg+WG group when compare with HgCl₂ treated group⁵⁷ Figure 4.

Plasma membrane redox system (PMRS) of Red blood cells (RBCs) transfers electrons from

intracellular substrates to extracellular electron acceptors⁴⁰ neutralization of oxidative stress, recycling of ascorbic acid and regulating of normal energy metabolism.⁵⁸⁻⁶⁰ It has been observing in various clinical studies that elevated level of PMRS was found in the erythrocytes during oxidative stress and associated condition such as diabetic nephropathy, type 2 diabetes mellitus and during aging in humans.⁶¹ The result of the present study revealed that HgCl₂ administration significantly ($P < 0.001$) increased level of erythrocyte PMRS in HgCl₂ treated rats when compare to control group. However, after wheatgrass supplementation the PMRS activity was significantly decreased in Hg+WG treated group as compare to HgCl₂-treated group ($P < 0.001$) Figure 5. Elevated level in erythrocyte PMRS activity in HgCl₂ treated rats is the indication of excess production of free radicals. Wheatgrass supplementation restored PMRS activity near to normal level, through the scavenging of free radicals and improving the antioxidant system.⁵⁵

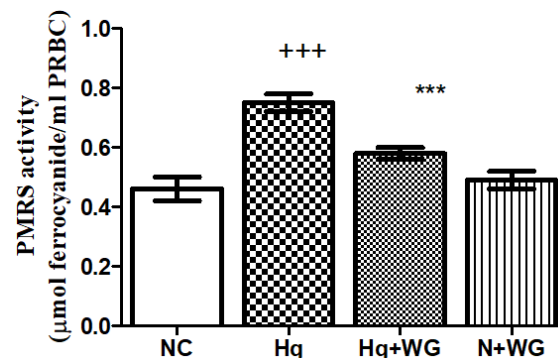


Fig. 5: Effect of wheatgrass treatment on PMRS activity of HgCl₂ induced oxidative stressed rats. Concentration of PMRS is expressed as µmol ferrocyanide/ml PRBC. Values are presented as means ± SD. +++ $P < 0.001$ compared with oxidative stressed group vs. control. * $P < 0.001$ compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)**

AOPPs are the biomarkers to estimate the degree of oxidative modifications of proteins.³² Various pathological conditions such as oxidative stress, hepatotoxicity, kidney disease, diabetes, mental retardation, and muscular dystrophy are associated with excess accumulation of the protein products (AOPPs).^{62, 63} In this study researcher observed a significantly ($P < 0.001$) increased formation of AOPP and protein oxidation in mercuric chloride

administered rats as compared to control rats. Present study revealed that after the wheatgrass extract supplementation protein oxidation level was significantly decreased ($P < 0.001$) in Hg+WG rats in comparison with HgCl₂-treated rats, Figure 6. Therefore, it could be conclude that wheatgrass demonstrated considerable antioxidant efficacy against protein oxidation influence by oxidative stress.

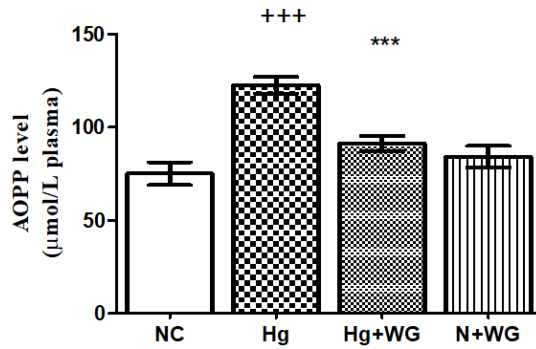


Fig. 6: Effect of wheatgrass treatment on AOPP level of HgCl₂ induced oxidative stressed rats. Concentration of AOPP level is expressed as µmol/l of plasma. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

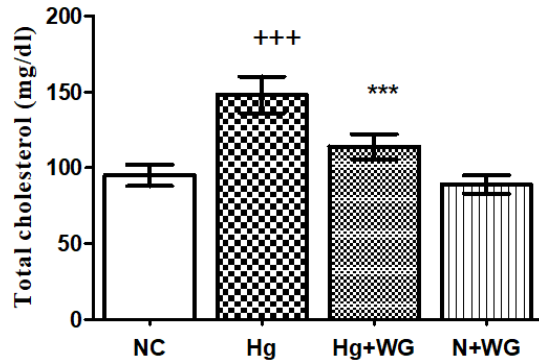


Fig. 7: Effect of wheatgrass treatment on total cholesterol level of HgCl₂ induced oxidative stressed rats. Concentration of total cholesterol is expressed as mg/dl. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

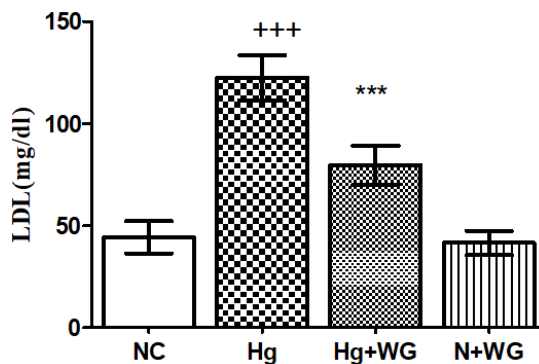


Fig. 8: Effect of wheatgrass treatment on LDL level of HgCl₂ induced oxidative stressed rats. Concentration of LDL is expressed as mg/dl. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

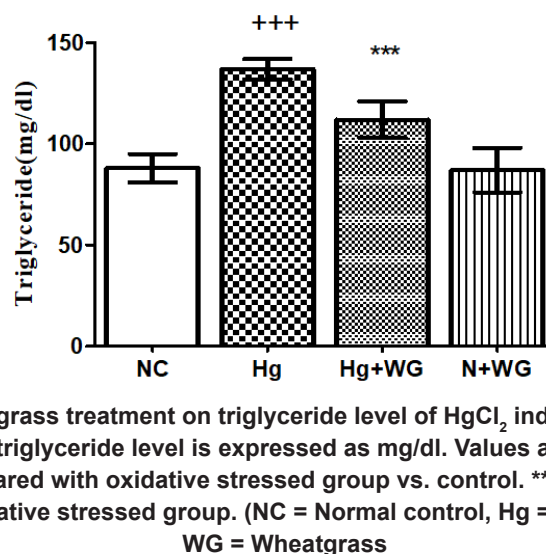


Fig. 9: Effect of wheatgrass treatment on triglyceride level of HgCl_2 induced oxidative stressed rats. Concentration of triglyceride level is expressed as mg/dl. Values are presented as means \pm SD. +++ $P < 0.001$ compared with oxidative stressed group vs. control. * $P < 0.001$ compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)**

Oxidative stress is generally associated with increased formation of free radicals, responsible for the lipid peroxidation; lipid peroxidation makes possible occurrence of cardiac necrosis and accrual of lipids, which leads to injure of cardiac tissues. Cardiac tissue damage induced by HgCl_2 in rats was indicated by elevated level of the lipid profile. Elevated level of lipid profile (plasma triglycerides, total cholesterol, LDL-S) in the HgCl_2 treated group demonstrated that HgCl_2 may be interfering with metabolism or biosynthesis of lipids.^{64,65}

Results showed that HgCl_2 caused adverse alterations in lipid profiles. Mercuric chloride significantly increased ($p < 0.001$) cholesterol, LDL and triglycerides levels in HgCl_2 treated rats as compared to control group. Administration of wheatgrass extract has significantly reduced ($p < 0.001$) elevated TC, LDL and triglycerides levels in Hg+WG treated group as comparison to HgCl_2 -treated group, Figure 7,8,9. These findings are in parallel with the other experimental studies.^{66,67} These changes in lipid profile after wheatgrass treatment may be the presence of its bioactive compounds, flavonoids triterpenoids and tannins, which are reported to lipid lowering effect in many more scientific studies. Wheatgrass supplementation was proficient to condense the HgCl_2 induced cardiotoxicity, shows by various ways. Gamma sitosterol an active component of wheatgrass has been reported to persuade cholesterol synthesis in liver and intestine.⁵⁴ Studies revealed that anti-

platelet aggregation was shown by caryophyllene and its oxides, are active biological components of wheatgrass.⁶⁸ Another wheatgrass compounds alpha and beta amyryns are the two biologically active penta cyclic triterpenes, have anti-hyperglycemic effect and hypolipidemic effect and suggesting that this compound is a potential candidate for diabetes and atherosclerosis.⁶⁹ This proves that wheatgrass is very efficient in lowering the lipid levels in rats. To conclude, the present result suggests that wheatgrass extract can be used as a lipid lowering agent and as a primary therapy in treating cardiovascular diseases.

Serum urea and creatinine tests are basic parameters of kidney dysfunctions test.¹⁴ By the previous studies it was proved that that kidneys functions are the badly altered by the induction of HgCl_2 , because Hg accumulates more in kidneys.⁷⁰ In the present study, enhanced levels of serum creatinine and urea in HgCl_2 -treated group indicate the renal dysfunction and nephrotoxicity.⁷¹ This elevation in serum creatinine and urea might be due to damage in renal tissues and alteration in renal functions. Similar findings have stated by the other previous studies.^{10,15,72,73} The results demonstrate that increased serum urea and creatinine level was observed in HgCl_2 - induced stressed rats in comparison with the control rats, ($p < 0.001$), ($p < 0.01$) respectively. On the other hand, treatment with wheatgrass extract at dose 100 mg/kg body weight showed a significant reduction ($p < 0.05$) in

the levels of serum urea and creatinine in Hg+WG treated group as compared to the HgCl₂-treated group¹⁴ Figure 10,11. Findings of the study showed that wheatgrass extract improved HgCl₂-induced nephrotoxicity; this is due to the antioxidant potential of wheatgrass to quenching free radicals. The antioxidant attributes of wheatgrass was

proved by the previous studies, which showed that wheatgrass has a great efficiency for scavenging of ROS as metal chelating agent, and inhibits lipid peroxidation.¹⁵ The antioxidant efficacies of aqueous extract of wheatgrass and suggest that it may be used as a therapeutic agent in the prevention of renal toxicity caused by Hg.⁷⁴

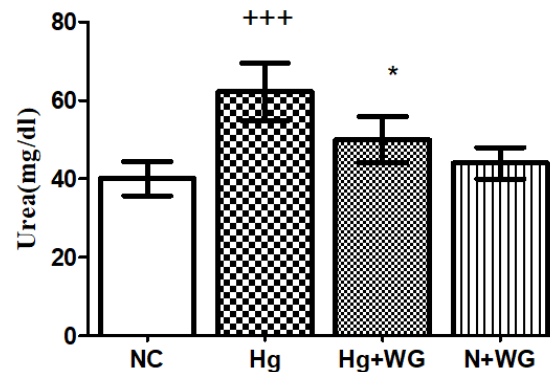


Fig. 10: Effect of wheatgrass treatment on urea level of HgCl₂ induced oxidative stressed rats. Concentration of urea is expressed as mg/dl of serum. Values are presented as means \pm SD. +++P<0.001 compared with oxidative stressed group vs. control. *P<0.05 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

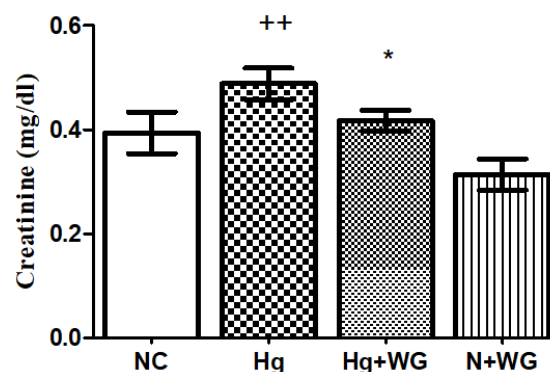


Fig. 11: Effect of wheatgrass treatment on serum creatinine level of HgCl₂ induced oxidative stressed rats. Concentration of creatinine is expressed as mg/dl of serum. Values are presented as means \pm SD. ++P<0.01 compared with oxidative stressed group vs. control. *P<0.05 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

ALP, AST and ALT are the enzymes produced by the liver and all body tissues including muscles, heart, kidney and brain with higher amounts in the liver. Raised levels of these enzymes are the reasonably sensitive indicators of liver damage and tissue injury, where damaged liver cells liberate these enzymes in to the blood circulation and their levels

get increased. Elevated levels of these enzymes in stressed conditions, is the initial identification of oxidative stress induced by mercury, which supports the results of this study, our results demonstrated that, liver enzymes (ALP, AST and ALT) levels were augmented in the stressed induced group that confirms occurrence of hepatotoxicity.⁷⁵ Therefore

it is concluded that Hg causes hepatotoxicity.^{10,76} To estimate the efficacy of wheatgrass extract on HgCl₂ -induced hepatic toxicity, liver function tests were measured after wheatgrass supplementation in the serum of HgCl₂ treated rats. The finding of the study demonstrated that, HgCl₂ administration caused increases ($p < 0.001$) in liver enzymes; ALP, AST and ALT levels, when compare to control rats, while after the supplementation with wheatgrass extract remarkably inhibited HgCl₂ -induced liver damage, improved liver functions in Hg+WG treated group when compare to HgCl₂-treated

group as evidenced by significantly decreased activities of liver enzymes and restored the almost normal serum ALP, AST and ALT levels ($p < 0.001$), ($p < 0.05$), and ($p < 0.001$) respectively, Figure 12,13,14. Findings of the study were in agreement with previous studies, demonstrated hepatoprotective potential of wheatgrass.^{77, 78} Wheatgrass and its phytochemicals such as flavonoids, phenols, phytol, sitosterol, squalene, especially alpha and beta amyryns (penta cyclic triterpenes) might be responsible for hepatoprotective potential that improves liver function activities.^{79, 80}

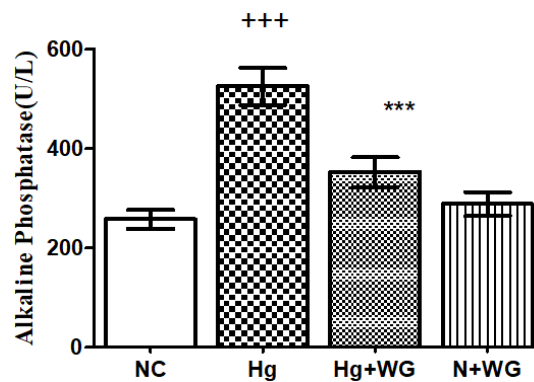


Fig. 12: Effect of wheatgrass treatment on alkaline phosphatase level of HgCl₂ induced oxidative stressed rats. Concentration of alkaline phosphatase is expressed as U/L of serum. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. *P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)**

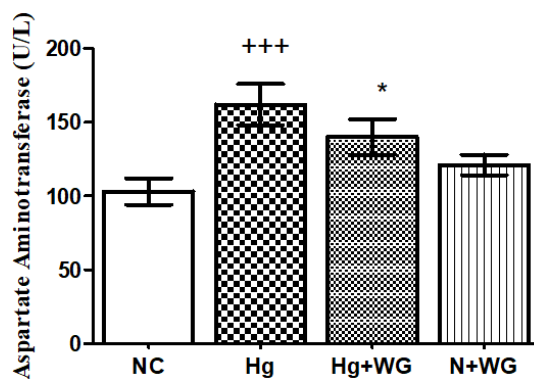


Fig. 13: Effect of wheatgrass treatment on AST level of HgCl₂ induced oxidative stressed rats. Concentration of AST is expressed as U/L of serum. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. *P<0.05 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

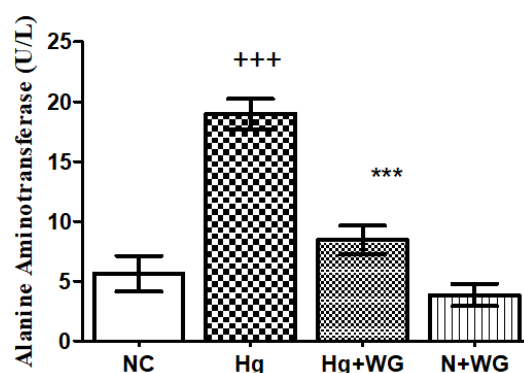


Fig. 14: Effect of wheatgrass treatment on ALT level of HgCl_2 induced oxidative stressed rats. Concentration of ALT level is expressed as U/L of serum. Values are presented as means \pm SD. +++ $P < 0.001$ compared with oxidative stressed group vs. control. * $P < 0.001$ compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)**

Conclusion

The results of the present study concludes that wheatgrass act as an efficient antioxidant agent which was confirmed by the findings of the study. Administration of HgCl_2 caused oxidative stress was proved by the elevated levels of PMRS, MDA, AOPP, ALP, AST, ALT serum urea, creatinine, TC, TG, LDL simultaneously treatment with wheatgrass significantly improved the antioxidant status-DPPH, FRAP, GHS, hence wheatgrass extract has great potential to improve the antioxidant status and provide protection against mercuric chloride induced oxidative stress and associated complications.

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Conflict of Interest

No conflicts of interest to disclose.

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