

EFFICACY OF *TRITICUM AESTIVUM* (WHEATGRASS) AGAINST ARSENIC INDUCED HEPATIC DAMAGES

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ABSTRACT

Objective: Arsenic is a metalloid element that is one of the most important global environmental toxicants and is found in both organic and inorganic forms. The present study was designed to find out the preventive role of *Triticum aestivum* against arsenic-induced oxidative stress in the liver of Swiss albino mice

Methods: The protective role of *T. aestivum* (Wheatgrass) against arsenic-induced hepatic damages was investigated in adult Swiss albino mice. The animals were divided into four groups: (i) Control group – only vehicle (double distilled water), (ii) *T. aestivum* treated group-20 ml/kg body weight (b.wt.), orally (iii) NaAsO₂ treated group- 4.0 mg/kg b.wt., and orally (iv) combination group – *T. aestivum* leaves extract (20 ml/kg b.wt.) and NaAsO₂ (4.0 mg/kg/b.wt.). Bodyweight and liver weight were measured in the process. Activities of marker enzymes such as alkaline phosphatase (ALP), Glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase were measured in serum. Activities of lipid peroxidation (LPO), glutathione (GSH), and Lactate dehydrogenase (LDH) were measured in the liver.

Results: The results indicated that arsenic intoxication caused a decrease in b.wt. and liver weight. Arsenic intoxication significantly increased hepatic LPO, Serum Glutamate Oxaloacetate Transaminase (SGOT), and Serum Glutamate Pyruvate Transaminase (SGPT) activities whereas significantly decreased hepatic GSH, hepatic LDH, and serum ALP activities. Combined treatment of *T. aestivum* and NaAsO₂ showed: (i) An increase in body and liver weight, (ii) a significant decrease in LPO, SGOT, and SGPT activities, (iii) an elevation in GSH content, LDH, and serum ALP activities, as compared to NaAsO₂ treated group.

Conclusion: Thus, *T. aestivum* was found to be protective against arsenic-induced hepatic damages.

Keywords: Arsenic, *Triticum aestivum*, Lipid peroxidation, Glutathione, Serum glutamate oxaloacetate transaminase, Lactate dehydrogenase.

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INTRODUCTION

Arsenic, a highly poisonous metalloid, is one of the natural constituents of the earth's crust. It is found in various concentrations in all ecosystems. Arsenic can enter the body through inhalation or consuming arsenic-contaminated food and drinking water or through skin contact [1]. Arsenic ingested with contaminated food and drinking water is mainly absorbed through the small intestine. Arsenic exerts its toxicity by inactivating up to 200 enzymes, especially those involved in cellular energy pathways and DNA synthesis and repair [2]. Exposure to arsenic may cause severe pathological problems such as arsenicosis, cardiovascular diseases, cerebrovascular diseases, and congenital malformations in offsprings as well as cancers of different organs [3,4]. Arsenic exposure stimulates the release of iron from ferritin and the resulting free iron is considered as a potent inducer of the formation of reactive oxygen species (ROS) through Fenton-type reaction [5]. Arsenic exposure produces a large amount of ROS that can impair the cellular antioxidant defense system and simultaneously damage the cellular ingredients such as lipids, proteins, and DNA [6].

Natural antioxidants are beneficial to mitigate chemical-induced oxidative damages [7,8] and are prominent immune boosters [9]. The young grass (7–9 days old) of *Triticum aestivum* commonly called Wheatgrass (Family: Gramineae) is called superfood as it provides overall nourishment to the body [10]. Phytochemical investigation of wheatgrass has shown that it is a rich source of carbohydrates, amino acids, secondary metabolites, chlorophyll, and Vitamin C [11].

It is rich in chlorophyll content and essential vitamins, minerals, vital enzymes, amino acids, and dietary fiber [12]. Runjala and Murthy [12] have also stated that the medicinal value of *T. aestivum* might be due to the presence of biologically active compounds and minerals in it and its antioxidant potential, which is attributed to its high bioflavonoid content. Medicinal properties of *T. aestivum* are due to its rich content of chlorophyll, antioxidants, minerals, and phytochemicals [13]. Qualitative analysis of phytochemicals confirms the presence of alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides in *T. aestivum* extract [14]. Ferruzzi *et al.* [15] have demonstrated that chlorophyll derivatives present in dietary supplements have antioxidant and antimutagenic activities. Chlorophyll is found to have potential anti-inflammatory, anticancer, antioxidant, anti-aging, and anti-mutagenic properties [16,17]. The present investigation has been undertaken to elucidate the biochemical mechanism associated with the hepatoprotective role of *T. aestivum* against arsenic-induced toxicity in the liver of Swiss albino mice.

METHODS

Animals

Random bred, male Swiss albino mice (*Mus musculus*), 6–8 weeks old (average body weight [b.wt.] 22±2 g) were used for experiments, according to animal ethical committee approval. Animals were maintained according to the standards of the animal ethical committee. These animals were maintained in the animal house (Department of Zoology, University of Rajasthan) at temperatures of 24±3°C and light of 14:10 h of light and dark. These animals were housed in polypropylene

cages and fed standard mice feed on Hindustan Limited, Delhi. Tap water was provided to the animals ad libitum, and tetracycline was given monthly to the animals against infections.

Plant material

T. aestivum (RUBL No. 20597) was grown in an earthen pot under controlled conditions of temperature and light. Fresh leaves of wheatgrass were cut from 7-day old plants. The juice was extracted from its leaves after washing, air drying, and homogenizing in a pestle mortar and finally filtered through a sterile gauze cloth.

T. aestivum drug tolerance study and optimum dose selection

T. aestivum fresh leaves juice (without adding water) was given to animals at different dose levels (5, 10, 20, 40, and 80 ml/kg b.wt.) by oral gavage for 30 days, in the morning. The optimum dose selection of *T. aestivum* leaves extract was done by determining the level of LPO and GSH (Tables 1 and 2). Out of different doses, 20 ml/kg b.wt. was selected for the experimental protocol as maximum GSH* and minimum LPO* level was observed at this dose.

Determination of radical scavenging activity of *T. aestivum* using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay

Radical scavenging activity of leaves' extract against stable DPPH was determined spectrophotometrically. The DPPH assay was carried out as described by Cuendet *et al.*, 1997 [18]. In the present investigation, *T. aestivum* extract was observed to have good radical scavenging activity with the inhibition of 12% at a 1 ml/ml (concentration of the extract in methanol), as reported by Arya and Kumar [19].

Toxicant

Arsenic in the form of sodium arsenite, NaAsO₂ was used for the present study. It was obtained from HiMedia India Limited, Mumbai. It was dissolved in double distilled water (DDW) and administered orally 4 mg/kg b.wt./day for 30 days.

Experimental protocol (design of experiment)

Swiss albino mice were divided into the following groups.

Group I (n=30): The animals were administered only vehicle (DDW, orally, severed as a control for Groups II and III).

Group II (n=30): The animals were administered only *T. aestivum* leaves extract (20 ml/kg b.wt./day) orally by gavage once daily for 30 days.

Group III (n=30): The animals were administered NaAsO₂ (4 mg/kg b.wt.) dissolved in DDW orally by gavage once daily for 30 days.

Group IV (n=30): The animals were administered *T. aestivum* leaves extract (20 ml/kg b.wt., orally) once daily for 10 days. From day 11th animals were given NaAsO₂ (4.0 mg/kg b.wt., orally in DDW) by oral gavage once daily; after 1/2 h *T. aestivum* leaves extract was given once daily. This day was considered as day 0. NaAsO₂ and *T. aestivum* were given to the animals up to 30 days from day 0.

The animals were weighed and autopsied on 1, 3, 7, 15, and 30 days after the heavy metal or plant extract treatment. The liver was removed, rinsed in cold saline, blotted, weighed, and processed for various biochemical assays. Fresh unhemolysed serum was used for determining transaminases and alkaline phosphatase (ALP) activities.

Biochemical parameters

Reduced GSH

The GSH level in the liver was determined by the method of Moron *et al.* [20]. GSH was used as a standard to calculate micromole GSH/g tissue.

Lipid peroxidation (LPO) assay

LPO level in the liver was estimated by the method of Okhawa *et al.* [21] as thiobarbituric acid reactive substances (TBARS). The concentration of TBARS was expressed as n moles of malondialdehyde per mg of tissue using 1,1,3,3-tetramethoxypropane as the standard.

LDH

LDH activity in the liver was estimated by the "Wroblewski procedure" [22]. Optical Density was measured at 400–500 nm.

Serum ALP

Commercially accessible kits (Span Diagnostics Ltd., Surat, India) were used to measure serum ALP by Kind and King's method [23]. Optical density was measured in King Armstrong Unit at 510 nm.

Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT)

SGOT and SGPT activities were estimated by the method of Reitman and Frankel [24] using DNPH as a color reagent which can be measured colorimetrically.

Statistical analysis

Data are expressed as mean±S.E. Statistical significance of the difference between groups was determined by Student's t-test [25].

Table 1: Variation in hepatic LPO level (n mol of MDA/mg of tissue) at different dose levels of *Triticum aestivum* in Swiss albino mice

Experimental groups	Autopsy intervals in days				
	Day 1	Day 3	Day 7	Day 15	Day 30
Control (DDW)	3.89±0.20	3.93±0.04	3.82±0.22	3.85±0.18	3.73±0.12
<i>Triticum aestivum</i> (5 ml/kg b.wt.)	3.95±0.29	3.56±0.44	3.12±0.62	2.99±0.26	2.85±0.30
<i>Triticum aestivum</i> (10 ml/kg b.wt.)	2.92±0.19	2.70±0.23	2.63±0.17	2.48±0.21	2.32±0.29
<i>Triticum aestivum</i> (20 ml/kg b.wt.)	2.63±0.14	2.14±0.12	1.99±0.15	1.87±0.13	1.79±0.16*
<i>Triticum aestivum</i> (40 ml/kg b.wt.)	2.87±0.10	2.43±0.26	2.27±0.17	2.14±0.15	2.34±0.11
<i>Triticum aestivum</i> (80 ml/kg b.wt.)	2.60±0.14	2.51±0.20	2.28±0.13	2.20±0.12	2.44±0.19

Table 2: Variation in hepatic GSH content (μ mol/gm of tissue) at different dose levels of *Triticum aestivum* in Swiss albino mice

Experimental groups	Autopsy intervals in days				
	Day 1	Day 3	Day 7	Day 15	Day 30
Control (DDW)	7.87±0.53	7.36±0.76	7.97±0.63	7.90±0.69	8.37±0.80
<i>Triticum aestivum</i> (5 ml/kg b.wt.)	8.14±0.49	8.03±0.74	8.02±0.34	8.47±0.81	8.77±0.67
<i>Triticum aestivum</i> (10 ml/kg b.wt.)	8.42±1.15	8.46±0.84	9.10±0.75	9.02±1.00	8.86±0.69
<i>Triticum aestivum</i> (20 ml/kg b.wt.)	9.04±1.17	8.55±0.79	9.41±0.69	9.33±0.88	9.21±0.77*
<i>Triticum aestivum</i> (40 ml/kg b.wt.)	7.98±0.46	7.82±0.98	8.67±0.44	8.80±0.65	8.42±0.83
<i>Triticum aestivum</i> (80 ml/kg b.wt.)	7.92±0.38	7.86±0.81	8.40±0.24	8.72±0.55	8.33±0.62

RESULTS

B.Wt.

A non-significant increase in the b.wt. was observed in the *T. aestivum* treated group as compared to the control (DDW) group. In the NaAsO₂ treated group, a reduction in b.wt. was observed from day 1 to day 30 as compared to the control group. In the combination group, a significant increase was observed on days 3, 7, and 15 which became highly significant on day 30 as compared to the arsenic-treated group (Fig. 1a).

Liver weight

T. aestivum alone did not show any significant alterations in liver weight as compared to the control group. NaAsO₂ treated animals showed a

reduction in liver weight from day 1 to day 30 as compared to Group I. In the combination group a significant increase in liver weight was observed from day 3 to day 30 as compared to the arsenic-treated group (Fig. 1b).

Reduced GSH

In the NaAsO₂-treated group a significant ($p < 0.01$) decrease in GSH content was recorded from day 1 to day 30 as compared to the control (DDW) group. *T. aestivum* alone showed a non-significant increase in GSH level at all autopsy intervals as compared to the control group. The combined treatment of *T. aestivum* with arsenic caused a highly significant ($p < 0.001$) elevation in GSH level from day 1 to day 30 as compared to arsenic-treated group (Fig. 1c).

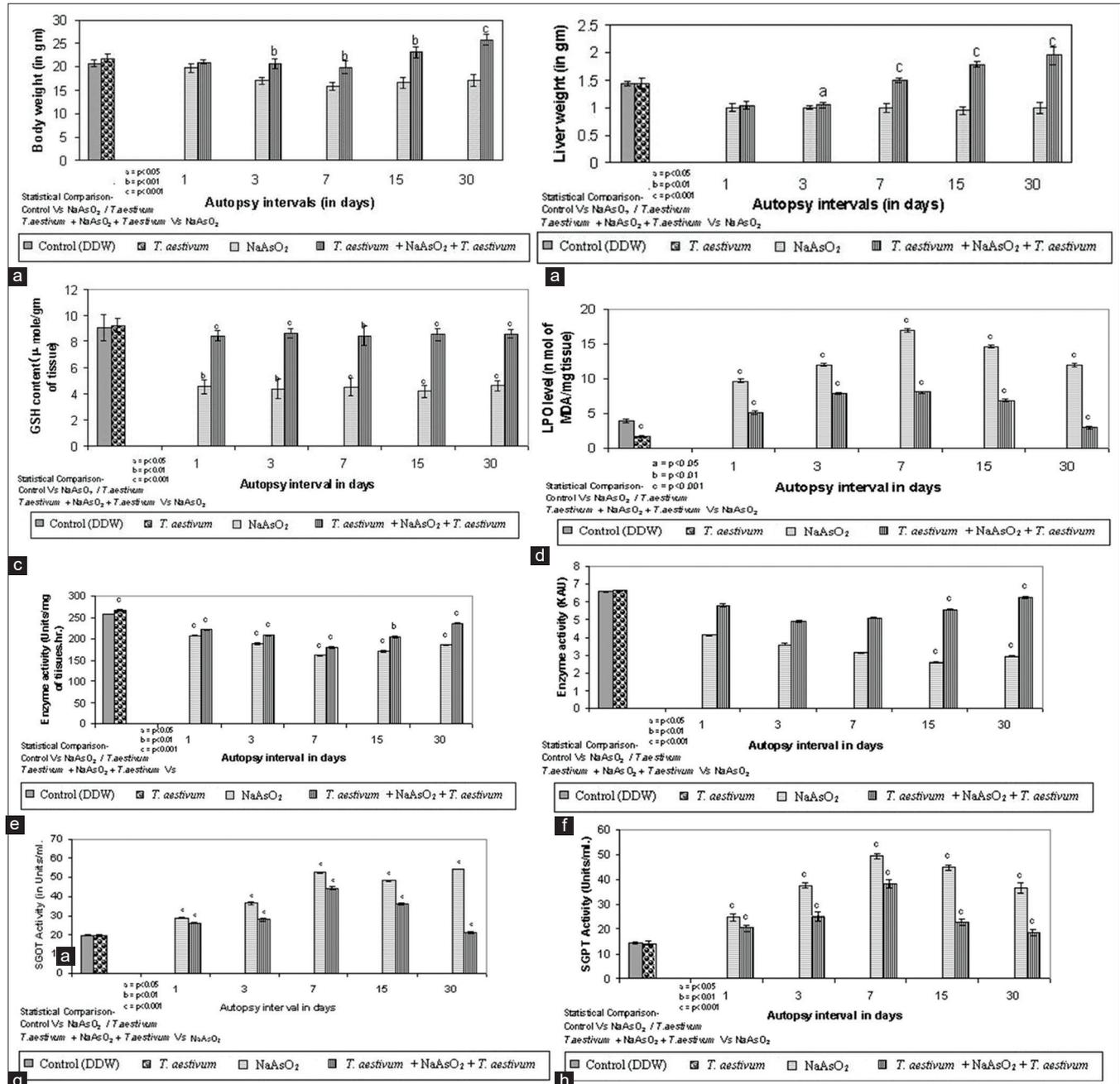


Fig. 1: (a) Variation in bodyweight in different experimental groups. (b) Variation in liver weight in different experimental groups. (c) Variation in glutathione in different experimental groups. (d) Variation in lipid peroxidation in different experimental groups. (e) Variation in lactate dehydrogenase in different experimental groups. (f) Variation in serum alkaline phosphatase in different experimental groups (g) Variation in serum glutamate oxaloacetate transaminase in different experimental groups. (h) Variation in serum glutamate pyruvate transaminase in different experimental groups

LPO

In the NaAsO₂-treated group, a highly significant ($p < 0.001$) increase in LPO level was noticed at all autopsy intervals when compared to the control (DDW) group. *T. aestivum* treated group showed a significant decline in hepatic LPO level as compared to control (DDW). The combined treatment of *T. aestivum* with sodium arsenite showed a highly significant decline ($p < 0.001$) in hepatic LPO level at all autopsy intervals for the arsenic-treated group (Fig. 1d).

LDH

A highly significant ($p < 0.001$) decline in LDH activity was observed in NaAsO₂-treated animals as compared to Group I. *T. aestivum* treated group showed a highly significant ($p < 0.001$) increase in liver LDH activity as compared to control (DDW). The pre- and post-treatment of *T. aestivum* with NaAsO₂ resulted in a highly significant ($p < 0.001$) increase in LDH activity throughout the whole experimental period as compared to arsenic-treated animals (Fig. 1e).

Serum ALP

In the NaAsO₂-treated group, a highly significant decline ($p < 0.001$) in serum ALP activity was observed throughout the whole experimental period. In the *T. aestivum* treated group, the enzymatic activities were found normal at all autopsy intervals. The combined treatment of *T. aestivum* with arsenic showed a highly significant elevation ($p < 0.001$) in serum ALP activity at all autopsy intervals for NaAsO₂-treated animals (Fig. 1f).

SGOT

A highly significant elevation ($p < 0.001$) in SGOT activity was noticed in animals throughout the experimental period in NaAsO₂-intoxicated mice. In *T. aestivum*-treated animals, there were no significant alterations in SGOT activity at all autopsy intervals. However, the combined treatment of *T. aestivum* with NaAsO₂ showed a highly significant decline ($p < 0.001$) in SGOT activity at all autopsy intervals as compared to the arsenic-treated mice (Fig. 1g).

SGPT

In NaAsO₂-treated animals, a highly significant increase ($P < 0.001$) in SGPT activity was noticed throughout the experimental period. Treatment with *T. aestivum* showed no alterations in enzymatic activities. The combined treatment of *T. aestivum* with arsenic showed a highly significant decline ($p < 0.001$) in SGPT activity from days 1 to 30 for arsenic-intoxicated mice (Fig. 1h).

DISCUSSION

Arsenic intoxication significantly decreased the b.wt. in mice throughout the whole experimental period as compared to control animals. Other studies have also reported similar results in mice and rats [26-28]. Arsenic intoxication increases permeability and damages intestinal lining which may be responsible for improper absorption of nutrients and loss of appetite in animals [29]. In a study subchronic exposure to low doses of arsenic through drinking water has been shown to alter the systematic physiology of male rats [30].

In the present study, arsenic intoxication produced a significant ($p < 0.001$) decrease in liver weight throughout the experimental period. Arsenic induces cell death (necrosis and apoptosis) in the liver [31]. Arsenic produces hepatocellular necrosis and liver degeneration [32]. Arsenic exposure causes a change in the balance between cell death and proliferation coupled with a robust loss of liver weight [33].

The liver is the major site for the accumulation of arsenic to toxic levels and therefore it is highly susceptible to arsenic damage which is reflected by changes in the activities of several liver enzymes and cellular damage in liver tissue of experimental animals [34]. The present investigation revealed a significant elevation in LPO level in terms of TBARS following NaAsO₂ exposure. An increase in the concentration of LPO products is an indication of the involvement of free radicals in particular pathological conditions [35]. These ROS attack the

polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such LPO reactions are especially dangerous for the viability of cells, even tissues [36]. The most significant effect of LPO in injured cells is the perturbation of membrane (cellular or organelle) structure and function. A radical is any species that contain one or more unpaired electrons present alone in atomic or molecular orbitals. The most reactive radical species to form in living organisms is hydroxyl radical, which can attack any biological molecule present next to them including cellular protein, DNA, and lipids. LPO diminishes membrane fluidity, increases non-specific permeability to ions (Ca²⁺), and inactivates membrane-bound enzymes [37]. An increase in the cytosolic free Ca²⁺ concentration because of increased permeability of plasma membrane has been proposed as a mechanism of oxidative stress-induced cell injury and cell death [38,39].

There is a positive relationship between arsenic exposure and oxidative damage. Arsenic exposure decreases the level of antioxidants present in the cell and increases the level of oxidants. Oxidative damage due to As³⁺ exposure is more significant than to As⁵⁺ [40]. Glutathione is well known for its pivotal role in the intrinsic antioxidant defense system. Arsenic is known to exert at least some of its toxic effects through interaction with sulfhydryl groups, and the non-protein sulfhydryl glutathione (GSH) appears to play an important role in the detoxification of arsenic [41]. GSH is required in the methylation of arsenic by stabilizing the reductive nature of the cell and methylation of arsenic can be a detoxification mechanism that does not permit the As(III) intermediates to accumulate [42]. The present investigation revealed that arsenic intoxication caused a significant decrease in reduced glutathione. Sodium arsenite-induced oxidative damage has been shown to elevate thiobarbituric acid-reactive substances (TBARS) in the liver of rats [43]. LPO is described as one of the mechanisms of arsenic toxicity associated with a concomitant decrease in cellular GSH level in the liver [27,44].

The present study showed a decrease in LDH activity following the exposure to sodium arsenite. Sodium arsenite-induced leakage of lactate dehydrogenase (LDH) has been used to assess the cytotoxicity of arsenic [45,46]. LDH release occurs only when cells have ruptured which is indicative of cell death [47,48]. An increase in arsenic concentration causes an increase in LDH leakage and a decrease in cell proliferation [45,49].

The results of the present investigation established that arsenic intoxication causes a significant decrease in serum ALP activity and a significant increase in SGOT and SGPT activity. ALP is an enzyme, or more precisely, a family of related enzymes, is produced in bile ducts by lipid membranes of the canalicular zone and sinusoidal membranes of the liver, so any interference in bile flow leads to a decrease in ALP activity [50,51]. The decrease in serum ALP activity in the present investigation may be due to damage to the cell membrane caused by NaAsO₂. The damage to the cell membrane due to LPO leads to the imbalance between synthesis and degradation of enzyme proteins [52]. Sharma *et al.* [27,45] also reported that sodium arsenite intoxication caused a significant decline in serum ALP activity in mice. An increase in both SGOT and SGPT is a potential marker of hepatocellular toxicity [53,54]. They leak out through the membrane into the peripheral blood where they can be measured. In the present study, a significant increase in SGOT and SGPT activity in serum indicates liver damage due to arsenic toxicity. Arsenic intoxication causes liver injury and these enzymes leak out of the liver in peripheral blood [46,55].

It has been observed that pre- and post-treatment with *T. aestivum* (Wheatgrass) protected against arsenic toxicity in Swiss albino mice effectively by exhibiting a significant decrease in hepatic MDA content, SGOT, SGPT, and serum ALP activity and a significant increase in b.wt., liver weight, GSH content, and LDH activity in the liver as compared with the arsenic-intoxicated mice.

The protective effect of *T. aestivum* (Wheatgrass) is attributed to its potential antioxidant properties and phytochemical profile. Wheatgrass has been proved to be an effective radical scavenger in different antioxidant assays [56,57]. Wheatgrass juice was found to have free-radical scavenging activity when evaluated by DPPH assay, nitric oxide, hydroxyl radical, and good antioxidant activity in mouse liver and brain as determined by FRAP assay and anti-LPO activity [58].

Wheatgrass juice is rich in secondary metabolites which are responsible for its antioxidant activity. Wheatgrass juice is found to have good antioxidant action in various models because it is rich in phenolic compounds, flavonoids, flavonols, and alkaloids [58]. Qualitative phytochemical analysis of wheatgrass leaves extract confirms the presence of various phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides [57]. Wheatgrass has good antioxidant and cytotoxic properties because of phenols and flavonoids present in it [59].

Wheatgrass improves overall nourishment. Experimental results have shown that wheatgrass is a good source of essential nutritional elements such as Zn, K, Na, Ca, Mg, Cl, and Fe in a bioavailable form with potential antioxidant activity [60-62]. It is rich in antioxidants which can overturn LPO and increase GSH activity. In a study done by Shakya *et al.* [63], wheatgrass has been found to protective against oxidative stress by decrease in LPO and increase in enzymatic and non-enzymatic antioxidants in male albino Wistar rats. Chlorophyll, flavonoids, and Vitamins C and E present in Wheatgrass (*T. aestivum*) are responsible for their benefits to immunological activity and oxidative stress [64]. Chlorophyll is one of the important pharmacologically active components in wheatgrass and it is found to have significant antioxidant activity which is comparable to ascorbic acid [57]. *T. aestivum* is potentially rich in chlorophyll, and vital antioxidant enzymes superoxide dismutase (SOD), cytochrome oxidase which converts dangerous free-radical ROS into hydrogen peroxide and an oxygen molecule [65]. In addition to antioxidant potential, indole compounds present in wheatgrass increase the activity of Phase I and Phase II xenobiotic metabolic enzymes in the liver and intestinal mucosa [66].

T. aestivum (Wheatgrass juice) has stimulatory effects on healthy tissue formation, tissue repair system, and healing system of the body [67,68]. Wheatgrass has been found to have wound healing activity in Wistar albino rats [69]. Wheatgrass derived polysaccharides protect against hepatic injury and improve the oxidative status of liver tissues in mice [70]. Medicinal plants with a high level of antioxidants provide effective therapy against hepatic damages [71]. Phenolic and polyphenolic compounds are among the most important antioxidants present in the diet and many plants of medicinal value [72,73]. The antioxidant activity of phenolic compounds is due to their ability to quench free radicals by donating a hydrogen atom or an electron to the free radical [74]. *T. aestivum* (Wheatgrass) has potential pharmacological properties and can be used for hepatoprotective treatment [75].

The protective role of *T. aestivum* (Wheatgrass) is demonstrated by its normalizing effects on various hepatic enzymes, which suggests that *T. aestivum* may show its modulatory effect by preventing cellular injury and maintaining the GSH level.

The present study suggests that deleterious ROS or lipid peroxides induced by arsenic exposure can be mitigated by *T. aestivum* (Wheatgrass) extract which is reflected by a significant decline in LPO content and acid and APL, SGOT, SGPT activity, and a significant enhancement in b.wt., liver weight, LDH and GSH content in the liver as compared to the NaAsO₂-treated group in Swiss albino mice.

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