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Management of Bisphenol-A Induced Hepato-Renal Disorder in Wistar Rat by Wheatgrass Aqueous Extract Co-Administration

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Abstract

Bisphenol-A (or BPA) is a renowned endocrine disruptor in current use. This study concentrated on the restorative effects of wheatgrass methanolic extract (WG-ME) on the liver and kidney in rats treated with BPA. For this purpose, 18 Wistar rats were divided into 3 groups: Control; BPA (100mg/kg); BPA+ WG-ME (100 mg/kg BPA + 200mg/kg WG-ME) by oral gavage for 28 days study period. Dissected liver and kidney were procured and stored at -20°C for the evaluation of cellular antioxidant status. For the analysis of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine levels, which have a role in measuring liver and kidney toxicity, blood samples were collected. In parallel, BPA decreases cellular antioxidant levels super-di-oxide (SOD), catalase and GPx in liver and kidney. The hepato-renal functional tests and antioxidant enzyme levels were significantly improved with the addition of WG-ME oral consumption. These findings provided that protective effect of WG-ME improving BPA-induced metabolic alterations.

Keywords: BPA, wheatgrass, liver function enzymes, renal toxicity markers, antioxidant enzymes

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Introduction

Xenoestrogens, is a known endocrine-disrupting chemicals (EDCs), have extensively adverse effects on human and animal health (Dimogerontas, G., & Liapi, C. 2013). Bisphenol-A (BPA), one of the most significant xenoestrogens, is produced at approximately 7.2 million tons globally and increases 5% yearly (Tarafdar A. et al., 2022). BPA is often used in the production of poly-carbonates such as toys; epoxy resins as in water pipes, medical, electrical and paper goods, and thermal paper (Hoekstra & Simoneau, 2013). It is also used in producing materials that remain constantly in contact with food and water, such as in packaging, bottles, and cans etc (Michalowicz, 2014; Makris et al., 2013). Previous studies reported BPA toxicity increases reactive oxygen species generation (ROS) in hepatocytes notably as a result decreased activity of antioxidant enzymes and genes and increased free radical production in cells. This results in increased reactive oxygen species (Linillos-Pradillo, 2023). The liver is the primary metabolic organ for many xenobiotics, including BPA, making it a target organ than other organs even at lower doses (Inoue, 2005, Völkel, 2002). A hypothesized hepatocyte dysfunction may lead to declension of cellular homoeostasis in other organs such as kidney with various complications increased absorption of toxins in the body, impotency in drug metabolism in the liver, inadequate digestion, and made infection prone (Bindhumol, V. 2003, Hadem, 2019).

Wheatgrass (*Triticum aestivum* L.), a member of Poaceae family, is cultivated worldwide. Presence of essential components such as polyphenolic and flavonoid compounds might be accountable for the remedial benefits wheatgrass showed (Chauhan, 2014). Many research reported curative and restorative effects of WG-ME, such as anti-mutagenic, anti-tumor properties (Sundaresan et al., 2015; Wasonga, C.,). However, there is no study done on the hepato-renal protective effects of WG-ME against BPA induced harmful effects in animal model. This study examined the beneficial role of WG-ME against the BPA triggered ailments on the liver and kidney using toxicity markers that are essential in evaluating hepatocyte-renal functions in serum.

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Methods

Chemicals and Other Reagents

BPA was obtained from Hi-Media (India), ALT (Cat. No: DF143), AST (Cat. No: DF41A), ALP (Cat. No: DC150), urea (Cat. No: DC150) and creatinine (Cat. No: DC150) kits were purchased (India).

Animals, Ethics Statement and Research Design

Animal experiments were performed at the Vidyasagar University (Approval No:). Eighteen adult Wistar albino rats 6-8 weeks old were used in this study, housed in standard rat cages in an environment with 12 hours of day and night, RT 22±2^{o}C, and humidity 50±10%. First 8 days were for acclimatization and 28days treatment period. The rats were divided into 3 group (n=6) with close mean body weights. The treatment was as following: 1. Control: 1ml of olive oil, 2. BPA: 100 mg/kg body weight of BPA dissolved in 1ml of olive oil (Abbas et al., 2021), (Laws et al., 2000). 3. BPA+WG-ME (n=6): 100 mg/kg body weight of BPA + 200 mg/kg of WG-ME, by oral gavage. During the treatment period, the BPA was prepared daily for fresh treatment. The time interval was 45 minutes applied between the BPA gavage and WG-ME in co-administered group.

Measurement of liver-kidney Function Test (LFT)

In this study to analyze extent of damage on liver and kidney by BPA-induction and if WG-ME has protective effects on that, certain necessary liver and kidney functional test enzymes were evaluated in the serum samples of different groups of rat. Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and creatinine were measured following the ready to use biochemical kits. The units are given in U/MG of serum.

Spectrophotometric assay of super oxide dismutase (SOD), catalase and GPx activities:

For SOD, organ slices of liver and kidney were homogenized in 100 mmol /L chilled Tris-HCl buffer. Then the homogenized tissue was centrifuged at 10000 x g for 20 min under chilled condition and supernatant was collected. Supernatant was mixed with equal volume of 10 mM pyrogallol and 50 mM Tris-HCl and reading was taken at 420 nm using Tris-HCL as blank

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(Kakkar et al. 1984).

Same concentration of supernatant was used for analysis of catalase activity. The supernatant was then mixed with H_2O_2 (30%) and the reading was taken at 240 nm (Sinha 1972).

For measuring peroxidase activity supernatant was prepared from liver and kidney tissues in same manner. A chemical mixture of sodium azide (1.0 mM), glutathione reductase, reduced glutathione (200 mM) and NADPH was prepared. Upon addition of this mixture with supernatant, H_2O_2 was also added for the final reading that was taken at 340 nm (Wendel 1980).

Results

Hepato-renal functional test enzymes and cellular anti-oxidant status:

In order to confirm the hepato-cellular degeneration, activities of ALT, AST, and ALP were estimated. A noticeably decrease in these enzymatic activities in BPA-induced model group when compared to the normal control group ($P<0.001$; Table.1). The toxic effects on kidney was confirmed by significantly high levels of urea and creatinine in BPA-fed rats comparison. The results showed prominent decrease in hepatic and renal SOD, catalase and GPx activities in BPA-induced group of rats when compared to the normal control group ($P<0.001$; Fig.1 & Higher values of SOD, catalase and GPx activities level were observed towards normalcy in the supplemented group when comparing the BPA treated group with control group of this study ($P<0.001$; Fig. A & B).

Table.1 revealed a significant statistical increase in the mean values of lipid and kidney toxicity biomarkers.

	AST	**ALT**	**ALP**	**Urea**	**Creatinine**
Control	83.37±3.37	45.69±3.00	76.9±3.73	47.26±2.45	0.80±0.06
BPA	120.47±4.49***	76.29±3.61***	117.15±4.42***	69.07±2.59***	1.13±0.04***
BPA+WG-ME	97.07±2.53$^{\#\#\#}$	56.72±3.54$^{\#}$	97.45±2.05$^{\#\#}$	58.31±1.86$^{\#\#}$	0.92±0.07$^{\#\#}$

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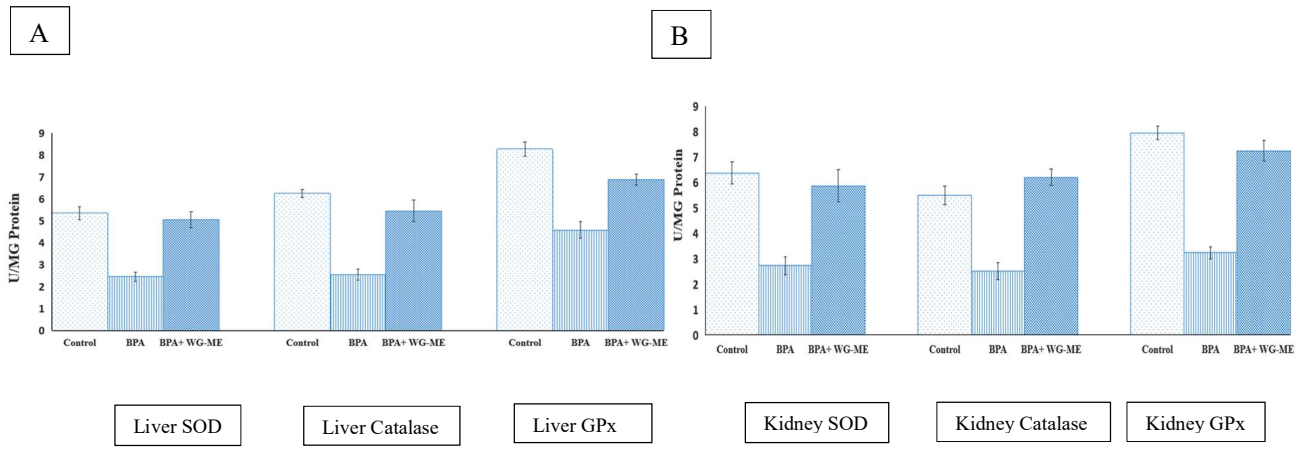

Figure 1. Effect of BPA and WG-ME on Serum Liver Function Test (LFT) Enzymes. Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) values are expressed as IU/mL of rat serum samples. *; significant differences compared to the control group ($p<0.05$), #; significant differences according to BPA group ($p<0.05$), WG-ME; shows that the difference between groups is insignificant ($p>0.05$). with the control group ($P<0.01$;) .

Discussion

SOD converts toxic radical super-oxide anion into H_2O_2, whereas the catalase and glutathione peroxidase produce water from hydrogen peroxide. In this study, the status of oxidative stress could confirmed by the level of enzymatic antioxidants. To study the toxic effects of BPA, an endocrine disrupting chemical that spreads, absorption through the skin causing damage to the kidneys and liver (Uzunhisarcikli & Aslanturk, 2019). Although studies investigating the combined effects of BPA and WG-ME on the liver are very limited, it was thought that this effect of WG-ME might be due to its strong antioxidant effects. Liver enzymes ALT, AST, ALP and LDH are released into the bloodstream following inflammation and necrosis and are reliable biomarkers for indicating liver injury (Giannini et al., 2005) Our findings showed that BPA exposure caused liver damage including, periportal inflammation and augmented serum

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levels of LDH, ALP and AST. Evaluation of the effects of 30-day BPA application on LFT enzymes is presented in Figure 1. BPA caused a statistically significant increase in AST, ALT, ALP activities in the BPA group compared to the control group ($p<0.05$). Data presented in our study demonstrate that high dose of BPA 100 mg/kg significantly increased the serum levels of liver functional biomarkers ALT, AST, ALP in BPA-treated rats (Figure 1), accepted as commonly used key tool (Tian et al., 2019) in the detection of liver damage (Center, 2007). ALP, a hypercritical marker enzyme for the examination of hepatobiliary disorders (Nangia & Yadav, 2021). The possible primary reason for the cytotoxic effect induced by BPA in the liver and kidney is attributed to increased oxidative damage (Abbas et al., 2021). BPA not only trigger the accumulation of reactive oxygen species in hepatic cells, but also decrease the serum levels of enzymatic antioxidants i.e SOD, catalase and GPx (Hassan et al., 2012) related to our results in this study. Similarly, oral BPA treatment cause degeneration, necrosis and kidney damage (Bordbar et al., 2021; Mourad & Khadrawy et al., 2012). The current study's most crucial starting point was determining WG-ME's protective actions against BPA induced hepato and renal toxicity. Al-Seeni et al. (2016) reported that has hepato-protective effect at 500mg/kg of b.w (Al-Seeni et al., 2016). Based on the evidence found, the improvement of abnormally increased ALT, AST, ALP when WG-ME administered simultaneously with BPA administration was evaluated as a remarkable finding that was interpreted as WG-ME reduced BPA-induced liver damage. WG-ME applied simultaneously with BPA showed significant decreases in renal toxicity bio-marker levels compared to the BPA group. The difference between the control and WG-ME groups for all enzymes was significant ($p>0.000$) (Figure.1). In the current study, in parallel with other studies, it was demonstrated WG-ME provide exciting and significant improvements in renal degeneration and necrosis. Therefore, the present study aimed to evaluate the potential of WG-ME to prevent BPA-induced liver injury in rats.

Conclusion

BPA administration causes complications characterized by cellular degeneration in the liver and kidney result in an increased serum liver kidney toxicity biomarkers and decreased

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enzymatic antioxidants. Significant findings have been obtained that co-administration of WG-ME provided recovery to a great extent by reducing the aforementioned BPA-induced adversities and maintaining physiological homoeostasis.

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

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