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The Preventive Effect of Pomegranate Peel Powder (Punica Granatum) Against Ethanol-Induced Oxidative Stress on Various Biochemical Parameters in Albino Rats

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A B S T R A C T

The point of this study was to find out if pomegranate (Punica granatum) peel powder (PPP), could protect against liver oxidative stress caused by ethanol (E) in rats. We randomly divided 24 Wistar rats, weighing 190–250 g, into four groups 6 rats for each. The negative control group fed a basic diet (pellet), the positive control (ethanolic) group was given 70% ethanol at a dose of 0.005 ml/g to cause oxidative stress. Group III (E&PPP 50%) was orally treated with PPP 50% in addition to a basal diet after one hour of dosing with 70% ethanol for 0.005 ml/g, and group IV (E&PPP 80%) was orally treated with PPP 75% in addition to a basal diet after one hour of dosing with 70% ethanol for 0.005 ml/g. After 30 days, the rats were euthanized, and prior to this, blood samples were collected in specialized tubes to isolate the serum for the intended tests. In the ethanolic group of rats, serum total protein, globulin, albumin, AST, ALP, ALT, and MDA levels went up significantly, while glutathione GSH levels went down significantly. PPP diminished ethanol-induced amounts of total protein, globulin, albumin, AST, ALP, ALT, and MDA in treatment group E&PPP 80%, decreased less than the ethanolic group in E&PPP 50%, compared to the control group. Consequently, the study shows that PPP protects liver from ethanol's toxicity by scavenging free radicals and reducing ROS levels, thereby mitigating hepatocyte damage.

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1. INTRODUCTION

The pomegranate, *Punica granatum* (family: Punicaceae), is a delectable fruit recognized for its powerful antioxidant attributes. *P.granatum* is extensively farmed for the manufacturing of pomegranate juice. However, the pomegranate peel powder (PPP), a byproduct of the pomegranate industry, constitutes roughly 77% of the entire fruit(Hamed et al.,2021) . PP possesses elevated antioxidant levels compared to pomegranate juice, indicating its potential utility as a nutritional supplement in animal feed(Badawi M. E.& Gomaa A. M, 2016). PPP has numerous natural antioxidant components, including polyphenols and polysaccharides(Ibrahium M.,2010)(Bachoual R.et al.,2011) that yield antioxidative, anti-infective, antibacterial, hepatoprotective, antiatherogenic, antidiarrheal, and antimutagenic effects(El-Houseiny W. et al.,2021). PPP demonstrated anti-inflammatory and anti-atherogenic properties relative to HFD controls(Salama A. A. et al.,2019).It has an anti-ulcer activity (Abdulzahra A. & Al-Salih, H. ,2022). Pomegranate flowers contain polyphenols that can protect liver enzymes and prevent NAFLD. Punicalagin, an ellagitannin, can reduce hyperlipidemia and hepatic lipid accumulation in rats. Pomegranate extracts have antioxidant, anti-inflammatory, hypoglycemic, and hepatoprotective properties, potentially aiding in the prevention and management of NAFLD (Zamanian M. et al.,2023). Consumption of alcohol, non-steroidal anti-inflammatory medications, tobacco, an inadequate diet, and both physical and psychological stress typically result in gastric ulcers (Ajibo D. et al. ,2023). These factors may induce oxidative stress (Suzuki H. et al.,2021) . Ethanol induces ulceration of the stomach mucosa by generating highly deleterious free radicals that act as necrotic agents(Ofusori A. et al., 2019) . Ethanol disrupts stomach mucus secretion, modifies the permeability of endothelial cells (mucosa), and diminishes mucus production, rendering gastric mucosal cells more susceptible to free radicals (Hossen M. et al. ,2021). Oxidative stress (OS) refers to the imbalance between pro-oxidant processes induced by reactive

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oxygen species (ROS) and an organism's capacity to mitigate their overproduction or manage the resultant damage. Multiple studies have shown that increased reactive oxygen species (ROS) production in gastrointestinal illnesses leads to inflammation and further enhances ROS synthesis (Ghazizadeh H. et al.,2020) (Caliri A. et al., 2021). The present work may provide a rapid and secure method to mitigate oxidative stress induced by high doses of ethanol in rats through the application of three distinct quantities of pomegranate (Punica granatum) peel powder (PPP).

2. MATERIALS AND METHODS

2.1. Peel Collection and Preparation

Pomegranate fruit was brought from local suq, which lies in Karbala city. The pomegranate's external part (husk) was collected, dried, grounded with an electrical grinder, and then saved within 25 C ± 2 for later use.

2.2. Research Design and Methods

Twenty-four male growing albino rats (*Rattus norvegicus*) aged between 8 - 10 weeks, weighing 190–250 g was utilized for the study. The present study sourced animals from the animal house at the College of Pharmacy, University of Karbala. The procedure adheres to the requirements set forth by the National Institutes of Health (NIH), and the research design received approval from the local committee. The animals are contained in groups within enclosures, with unimpeded access to sustenance and hydration. Following two-weeks acclimatization period, the animals were partitioned into three equal groups:

- Group I (control) Animals of this group fed on basal diet (pellet).
- Group II (Ethanolic) 70% ethanol for 0.005 ml/g to induce oxidative stress (Beiranvand M. et al., 2021)(Abdel-Kawi S. et al.,2023).
- Group III (E&PPP 50%) was orally treated with PPP 30% in addition to basal diet (Abd Elsabor R. et al., 2018)(Hanani Z. et al.,2018) after one hour of dosing with 70% ethanol for 0.005 ml/g.
- Group IV (E&PPP 80%) was orally treated with PPP 75% in addition to basal diet, after one hour of dosing with 70% ethanol for 0.005 ml/g.

Daily food consumption was documented, while body weight was measured weekly. Upon the conclusion of the experiment (30 days).

2.3. Data Analysis

All results are shown as the mean ± standard deviation. ANOVA was employed to assess the statistical significance of the experimental data, with a significance level of $P < .05$. We may utilize the Excel 2013 software as a quantitative tool to conduct this statistical analysis.

3. RESULTS AND DISCUSSION

3.1. The Effects of Ethanol on the Biochemical Parameter Levels

Table 1 showed significant differences in all biochemical parameter levels, i.e., there was a significant increase ($P < 0.05$) in all of (Total protein, Globulin, Albumin, AST, ALP, ALT, MDA) in Ethanol group compared to control and treatment E&PPP 30%, E&PPP 75% groups. While there was a significant decrease in and GSH in Ethanol group compared to control and treatment E&PPP 30%, E&PPP 75% groups, may be altered by ethanol action in this group (Preedy V. et al.,1988) This may be elucidated by the elevated levels of ethanol-induced inflammation in the ethanol group (Xu H. et al.,2024) , Ethanol induces oxidative stress through the generation of reactive oxygen species (ROS)(Gugliandolo E. et al.,2021). It has a pivotal role in various illnesses, including stomach ulcers (Yoo J. et al., 2018) . Because of ethanol-induced hepatotoxicity (Yoo J. et al., 2018), the AST level was increased in the ethanol group compared to control, and increase level of ALP, ALT in this group. It goes back to the same reason (Liu Q. et al., 2024) (Arumugam M. K. et al., 2022).

ALT is predominantly located in non-mitochondrial regions of liver cells, whereas roughly 80% of AST is in the mitochondria of liver cells. Damage to liver cells releases ALT and AST into the serum, leading to an increase in serum AST and ALT levels. The invasion of cancer cells damages normal liver cells in liver cancer patients, leading to a rise in ALT and AST levels (Yang J., 2010). Ethanol induced oxidative stress (Jedidi S. et al., 2022), and destroyed mitochondria (Zhao H. et al., 2021).Therefore, it decreases the hepatic GSH and SOD (Schlorff E. et al., 1999), in addition to increasing hepatic MDA leve (Alsaif M. A., 2007) there for the levels of GSH in the second group reduced to 14.10 ± 0.30 compared to normal level in the control group 16.78 ± 0.40.

Table 1. The values are evidently average ± SD value, n = 6 in each group, [a] show the difference in statistics. With a control group, [b] statistical disparity according to CRG group, ($P < 0.05$).

Parameters	Control	Ethanol	E&PPP 50%	E&PPP 75%
Total protein (mg/dL)	05.85 ± 0.20	08.11 ± 0.14 [a]	07.50 ± 0.10 [a]	06.28 ± 0.12 [b]
Globulin (mg/dL)	01.83 ± 0.10	03.05 ± 0.10 [a]	02.49 ± 0.30 [a]	02.02 ± 0.15 [b]
Albumin (mg/dL)	04.02 ± 0.50	06.56 ± 0.20 [a]	05.02 ± 0.40 [a]	04.27 ± 0.33 [b]
AST (µ/L)	45.30 ± 0.40	65.00 ± 0.80 [a]	56.00 ± 0.60 [a b]	46.50 ± 0.80 [b]
ALP (µ/L)	20.60 ± 0.50	32.00 ± 0.40[a]	28.00 ± 0.45 [a b]	21.90 ± 0.70 [b]
ALT (µ/L)	21.00 ± 0.40	24.20 ± 0.30 [a]	22.50 ± 0.40 [a b]	20.50 ± 0.50 [b]
GSH (µ/L)	16.78 ± 0.40	14.10 ± 0.30 [a]	16.90 ± 0.10 [a b]	16.10 ± 0.40 [b]
MDA (µ/L)	04.30 ± 1.33	09.50 ± 1.60 [a]	06.50 ± 1.00 [b]	05.20 ± 1.30 [b]

3.2. The Effects of E&PPP on the Total Protein, Globulin, and Albumin Levels

Table 1 showed significant differences decrease ($P > 0.05$) in all of Total protein, Globulin and Albumin in E&PPP 75% group compare to E&PPP 30% group and ethanol groups, and no significant differences in E&PPP 75% group compare to the control group. Perhaps the reason is due to the effect of the high concentration of food containing pomegranate peels, which in turn contain many effective antioxidants and anti-ethanol substances such as gallic acid, ellagic acid, punicalagin, luteolin, catechin, rutin, hydrobenzoic acid, quercetin, punicalins) and caffeic acid (Mahmood A. M. & Jabar H. L., 2023).

Continuous exposure of the liver to a specific quantity of ethanol prompts hepatocytes to progressively release proteins (Vildhede A. et al., 2015) to mitigate the effects of ethanol or other oxidizing agents encountered, because of hepatocytes play a pivotal role in liver inflammation (Gong J. et al., 2023) Consequently, the concentrations of total serum proteins, globulin, and albumin elevate, mirroring the increases observed in the ethanol group (Niemelä O., 2001) . In the in E&PPP 30% and in E&PPP 75% groups, pomegranate peel powder effectively restored the usual amounts of these proteins, aiming to rehabilitate liver functions (Faddladdeen K. A. & Ojaimi A. A., 2019) . Consequently, we observed that the PPP 30% group, administered pomegranate powder at a concentration of 30%, could not restore normal liver functions, in contrast to the PPP 80%group, which received a greater concentration of 75%. The data unequivocally demonstrated a reduction in the concentration of total proteins, albumin, and globulin.

3.3. The Effects Of E&PPP on the AST, ALT, ALP Levels

Table 1 showed significant differences decrease ($P > 0.05$) in all of AST, ALT and ALP in E&PPP 75% group compare to E&PPP 30% group and ethanol groups, and no significant differences in E&PPP 75% group compare to the control group. AST, ALP and ALT, are important biomarkers of liver function (Akanya O. H. et al., 2015). The levels of these enzymes rise significantly when the liver is exposed to inflammatory substances such as sebastin (Palipoch S. & Punsawad C., 2013), CCL4 (Kostic T. et al., 2022), and ethanol (Høiseth G. et al., 2022) After giving ethanol to the ethanol group, we saw that the levels of these enzymes went up, while they went down in the PPP 30% and 80% treatment groups. Still, the higher amount of pomegranate peels in the fourth group (PPP 80%) had a big effect on getting protein levels back to normal. ALT is an enzyme typically found in hepatic and cardiac cells. Elevated ALT levels in the blood indicate liver or cardiac impairment when either organ is compromised. In hepatocellular damage, enzymes typically found in the cytosol are liberated into the bloodstream. Their presence in plasma serves as valuable indicators for assessing the degree and nature of hepatocellular injury (Pari L. & Murugan P., 2004).

We use ALT and AST to assess the hepatocellular integrity of liver tissue. ALT is mostly located in the liver, whereas AST is typically present in comparable concentrations in the liver, heart, muscle, kidney, and brain. Consequently, ALT exhibits more specificity for the liver compared to AST. The normal range for both ALT and AST in humans is 25 U/L to 50 U/L (Dollah M. A. et al., 2013). ALP increase by ethanol according to the table , Ethanol exposure results in alcoholic fatty liver, inflammation, necrosis, fibrosis and cirrhosis (Rostami H. et al., 2020) , Alkaline phosphatase (ALP) is a principal liver enzyme and a significant indicator of hepatic physiological health (Xue M. et al., 2017). In 1920, it was discovered that this enzyme elevated in hepatic disorders (Kaneko J. J. et al., 2018) . Alkaline phosphatase (EC3.1.3.1) facilitates the hydrolysis of organic phosphates, including proteins, nucleotides, and alkaloids, in an alkaline environment. The enzyme exists in several forms inside the bloodstream. It is also prevalent in the liver and bones (Fischbach F. & Zawta B., 1992) . It is important to know that pharmaceutical drugs, like vitamin E and corticosteroids, which are often anti-inflammatory and antioxidant, are often used to treat liver damage caused by drinking too much alcohol. However, using them for too long or in the wrong Excessive quantities may lead to complications. A high intake of vitamin E elevates the hazard of prostate cancer in men (Klein E. et al., 2012).

3.4. The Effects of E&PPP on the GSH and MDA Levels

The alterations in GSH content across various groups are presented in Table 1, demonstrating that rats treated Ethanol alone demonstrated a substantial decrease in plasma glutathione (GSH) levels. PPP supplementation elevated plasma glutathione levels. This outcome corresponds with that of (Ashoush I. S. et al., 2013) and (Oh S. I. et al., 1998) (Xue M. et al., 2022) . Glutathione (GSH) is essential for the detoxification of xenobiotics and the maintenance of cellular redox balance (Georgiou-Siafis S. K. & Tsiftsoglou A. S., 2023) . A decrease at the cellular level is often viewed as a sign of oxidative stress. It is clear that this lower level of this natural antioxidant is linked to oxidative stress caused by ethanol, which is shown by the production of harmful acetaldehyde and other reactive molecules inside the cell. The observed increase in GST activity seems to be a planned response aimed at blocking the harmful byproducts produced during the metabolism of ethanol (Macdonald I. O. et al., 2010) . PPP, an agricultural by-product with bioactive polyphenols, to prevent oxidative stress-related pathogenesis (Al-Gubory K. H. et al., 2010). The effect of the PPP in the two treatment groups (E&PPP 80% and E&PPP 30%) was apparent, with an increase in GSH levels in the lower concentration group E&PPP 30%. However, the significant differences removed in the higher concentration group E&PPP 30% compared to the control group.

The significant increase (09.50 ± 1.60) in MDI in the ethanol group compared to the control group was due to high concentration and continuous ethanol dosing (Aggul A. G. et al., 2022). This elevation was due to a rise in oxidants, which then increased free radicals caused by ethanol-damaged liver cells [15]. Research demonstrates that ethanol can provoke lipid peroxidation in cellular membranes via the generation of reactive oxygen species (ROS), resulting in cellular damage (Arthur I., 2001) . The treatment group with the lower concentration E&PPP 30% endeavored to diminish these oxidants utilizing PPP 06.50 ± 1.00. However, it appears that the brief treatment duration and the low PPP concentration precluded the restoration of normal levels of this substance in comparison to the treatment group with the higher concentration E&PPP 80%, which successfully reinstated normal levels 05.20 ± 1.30, exhibiting no significant differences from the control group 04.30 ± 1.33. Without a doubt, the results reveal that PPP therapy improves the antioxidant defense system when ethanol causes oxidative damage (Kumar D. et al., 2013)

4. CONCLUSION

The findings indicate that the mechanisms contributing to heightened oxidative stress following ethanol ingestion encompass the excessive generation of free radicals, a decrease in endogenous antioxidants, and an elevation in MDA release. This work demonstrates that the protective effect of PPP against ethanol-induced liver damage is dependent on the antioxidant activities of phenolic components, including gallic acid, ellagic acid, punicalagin, luteolin, catechin, rutin, hydrobenzoic acid, and potentially glycosides and coumarin derivatives found in pomegranate peel. As the concentration of PPP rises, the dietary levels of these chemicals increase, hence enhancing the protective effect of PPP. The principal mechanism via which PPP alleviates hepatocyte damage is by scavenging free radicals generated by ethanol and its synergistic effects, resulting in elevated levels of GSH and reduced levels of MDA, total protein, globulin, albumin, AST, ALT, ALP proteins, alongside diminished levels of ROS.

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