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Research Article

Reinvestigate the Effect of *Punica granatum* Peel Ethanol Extract Against Paracetamol Nephrotoxicity in Rats

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ABSTRACT

Background: Paracetamol, also known as acetaminophen, is extensively used for pain relief and as an antipyretic drug. High dose of paracetamol is commonly known to induce hepatotoxicity and nephrotoxicity in humans and experimental animals. In purpose, to sustain kidney wellness, the impact of PGPE as a nephron-protecting plant was examined. Materials and methods: 35 rats (male and female), weighing 150-200 g, divided randomly into 5 groups, (A, B, C, D and E), received orally, normal saline (A and B), Silymarin 80mg/kg (C), 250 mg/kg PGPE (D), 500 mg/kg (E) for 7 days, on day 8 nephrotoxicity induced by single dose of Acetaminophen 750mg/kg. After 24 24-hour fasting period, rats were sacrificed, and blood samples were taken for screening the haematology and blood biochemical profile. Kidney tissue samples were taken in 10% formalin for histopathology testing. Results: PGPE impact on kidney function tests showed a dramatic elevation of serum creatinine. In groups, those dosed with PGPE, compared to the positive and negative control groups. Potassium levels were not influenced by administering the plant extract, while sodium levels were remarkably high in group (D), which received 250 mg/kg PGPE. Serum urea levels showed notable elevation in the positive control group; meanwhile, the levels were not significantly different among the PGPE groups and the negative control. The total WBCs count is reduced immensely by paracetamol administration. Moreover, it got even worse when a 500 mg/kg PGPE dose was used. RBCs, Hb, and PCV levels were dramatically lowered by paracetamol, and a greater reduction was observed by 500 mg/kg of PGPE.

INTRODUCTION

N-acetyl-para-aminophenol (APAP), also called Acetaminophen or paracetamol in various

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countries, is a non-opioid analgesic and antipyretic agent used to relieve pain and fever. Despite its well-established practical implementation, the exact mechanisms underlying its effect remain subtle (Smith et al, 2022).

Paracetamol is a non-steroidal anti-inflammatory, belonging to the aniline class. Hepatotoxicity and nephrotoxicity may occur from receiving a toxic dose of the drug. A toxic dose of paracetamol may lead to death in lab animals, in addition to humans. The appropriate dose of paracetamol for adults is 0.5 g every 4-6 hours, while the highest dose is 4 g. The determination of the dose in children depends on age and weight, which should exceed 4 mg/kg in 24 hours (Sara et al, 2022).

The mechanism of action of paracetamol is thought to be via the lowering of prostaglandin production in the brain and spinal cord (Nazish et al., 2017).

The heart, liver, and kidneys are the key organs accountable for drug detoxification, metabolism, and excretion. Due to their functions, these organs are constantly exposed to oxidative stress, due to lipid peroxidation, which triggers inflammatory signals. Dependently, raising pro-inflammatory cytokines, leading to remarkable diminution of some organs' ability to endure damage (Reham et al, 2018).

The metabolism of paracetamol occurs in the liver and is excreted in the urine mainly as inactive glucuronide and sulfate conjugates. The overdose of the drug leads to saturation of Glucouronyl transferases and Sulfotransferases, which prevents the action of cytochrome P450, generating (NAPQ1) in amounts that surpass the glutathione capacity for scavenging, initiating glutathione reduction (Mitchelli et al, 1973). The lack of glutathione leads to the accumulation of NAPQ1 molecules in hepatic cells. Then, oxidative stress

is triggered, leading to lipid peroxidation, pronounced in permanent cell membrane damage and eventually cell death (Sara et al, 2022).

Paracetamol's nephrotoxicity potential leads to excessive production and accumulation of reactive oxygen species in renal tubular cells. The reduction of antioxidant enzyme levels leads to morphological deterioration of the intracellular organelles. There is increasing evidence that paracetamol causes acute renal failure through activation of NF- κ B in the kidney (Bayan et al, 2023).

Alternative medicine has gained more consideration recently, mainly in developing countries. Numerous plants are believed to have curative properties used to control several diseases, after their potency was confirmed by intensive research.

One of those plants with therapeutic effects is Punica. Granatum peel, which represents a natural source of antioxidants. The analysis of plant peel revealed that it contains numerous phytochemical compounds, for example, it's documented as a rich source of phenol compounds, flavonoids, and phytosterols (Nadia et al, 2016). Also considered to be a source of poly saccharides and minerals, including potassium, nitrogen, magnesium, phosphorus, and sodium. Additionally, Punica. Granatum peel's content of tannin possesses free radical scavenging properties, which are highly susceptible to enzymatic and non-enzymatic hydrolysis mutually (Sara et al, 2022).

Objectives

- Investigate the nephroprotective properties of Punica. Granatum ethanol extract.
- Determination of the plant's peel phytochemical profile.



- Investigate the effect of ethanol extract on haematology profile.
- Evaluate effects of extract on serum biochemical parameters (kidney function tests).

2. MATERIALS AND METHODS

This experiment took place at the Central Veterinary Research Laboratory, Khartoum, Sudan. October 2020

2.1 Plant materials:

Punica. Granatum peel is obtained from the local market of traditional herbs. Extraction of the plant material was accomplished by using ethanol, following the method described by Sukhdev et al (2008). Concisely, 100 g of the plant peel was extracted using Soxhlet by adding 500 mL of ethanol (70%) at 68°C for 72 hours. The extract was cleaned using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary vacuum evaporator at 40-45 °C. The 100 g yielded 27.368g (27.37%).

2.1.1 Phytochemical profile tests: the phytochemical screening tests were done according to the standard procedures described by (Sukhdy et al, 2008; Srividya and Nehrota, 2003; and Chang et al, 2002).

2.2 Trial Protocol approval:

The regulatory laws regarding research ethics were followed, referring to the Sudanese Veterinary Council statutes.

2.3 Experimental Animals:

35 male and female rats were purchased from the Faculty of Veterinary Medicine, University of Khartoum. Rats' weight ranged between 150-200 Grams, grouped equally into 5 groups (n=7).

Located in stainless steel cages, fed a standard diet, and the admission to the water was ad libitum. The temperature throughout the trial ranged between 36-39 °C.

2.4 Drugs and chemicals:

Silymarin and paracetamol were obtained from the pharmacy at Khartoum Hospital Street. Xylazine and Ketamine were obtained from Sigma Aldrich. Ethanol and Formaldehyde were purchased from a laboratory solvents and reagents supply company in Khartoum.

2.5 Trial Design:

The five groups were sorted out as follows: Group A, assigned as a negative control (Normal saline); group B, allotted as a Positive control (Paracetamol); Group C assigned as a reference drug (Silymarin); group D received 250 mg/kg Punica. Granatum peel ethanol extract, and lastly, group E was dosed with 500 mg/kg Punica. Granatum peel ethanol extract, via stomach lavage. Animals were left for 2 weeks as an adaptation period, then the next described protocol was tracked:

Negative and Positive control groups (A and B, respectively), only distilled water was administered; meanwhile, the referenced drug group received 80 mg/kg for an uninterrupted 7 days. The treatment groups (D and E), dosed with 250 and 500 mg/kg of Punica. The ethanol extract was also applied for 7 consecutive days. Kidney damage was induced on day 8 by administering (750 mg/kg paracetamol, single dose) given orally to all groups, excluding the negative control group (A). Rats are contingent on fasting for 24 hours. On day 9, samples were taken. Previously to euthanasia, the blood samples were collected in plain and EDTA-coated tubes for the biochemistry lab for kidney function tests analysis, and to the



haematology lab for determination of the following parameters: blood cell count, Hb, PCV, MCV, MCH, MCHC, and platelets.

2.6 Blood Biochemistry Profile:

Blood samples were centrifuged at 3000 rpm for 10 min, serum was allocated in Eppendorf tubes and transferred to the lab for kidney function tests analysis, using BIOSYSTEM BTS-350 Apparatus.

2.7 Haematological Profile:

Complete blood picture determined by means of URIT 3010 Haematology analyser-China.

2.8 Histopathology Examination:

The lethargy of the rats in each group was achieved by Xylazine (5 mg/kg) and Ketamine (100 mg/kg). Animals were euthanised by cervical dislocation. Tissue samples were transferred to the pathology lab in 10% formaldehyde solution. After two days of preservation in 10% formaldehyde solution, kidney tissue samples were placed into an automatic tissue processor (Histos5, rapid microwave processor, Milastone-USA) and monitored for 12 hours. The samples were blocked with molten paraffin at 56-58 °C and those paraffin blocks were frozen at -10 °C in a refrigerator. After 4-5 µm-thick sections were cut, the paraffin blocks were stained with hematoxylin and eosin. The stained sections were examined under the light microscope.

2.9 Gas Chromatography-MS:

GC-MS Analysis of the ethanol extract of the experimental plant was carried out using QP2010-

Ultra Shimadzu, serial number 02052101565SA. Capillary column (RTX=5ms=30mmx0.25 µm). Samples were introduced using split mode. Helium gas (99.999%) was used as carrier gas at a sustained flow rate of 1.6 ml/min. The temperature program began at 50 °C with a rate of 10 °C /min to 300 °C, with a 10 °C hold time. The injection port temperature was 200 °C and the interface temperature was 250 °C. The samples were analysed by using scan mode in the range of 40-500m/z, charge-to-ratio, and the total run time was 14 min.

Identification of components:

Clarification on the mass spectrum of GC-MS was done by comparing their retention index and mass fragmentation patterns with those available in the library of the National Institute of Standards and Technology (NIST).

Statistical Analysis: Values are stated as mean (±SD). The statistical analysis was performed using one-way analysis of variance (T-TEST), using Statistix, version 10 Software, USA. *P* values (*P* < 0.05) were considered statistically significant when compared to the positive control group (B).

3. RESULTS

3.1 Phytochemical analysis:

Phytochemical profile analysis of Punica. Granatum peel ethanol extract showed high content of tannins and flavonoids, modest content of coumarins, and little content of saponins and alkaloids. Other compounds, such as triterpenes and anthraquinones, were not distinguished. (Table 1).



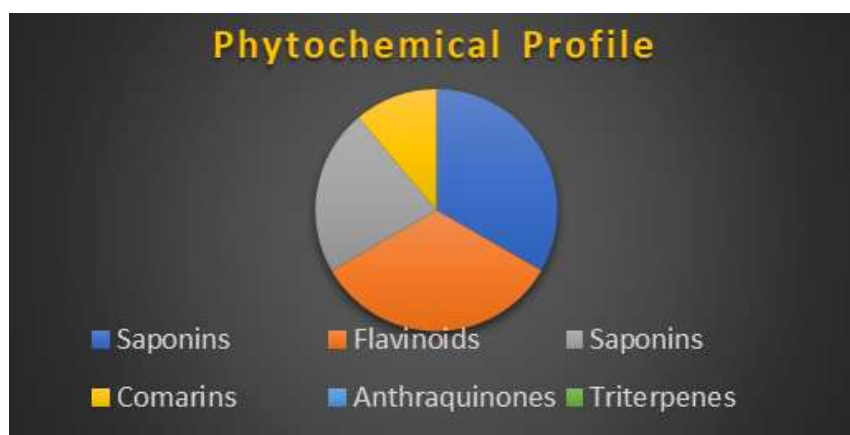


Figure (1): Phytochemical profile of *Punica granatum* peel.

3.2 GC analysis:

GC/MS analysis of *Punica granatum* peel ethanol extract revealed the existence of only two bioactive compounds: Sucrose and gamma sitosterol.

3.3 Haematology results:

Overall, the results showed no statistically significant difference observed concerning MCV, MCH, MCHC. Although the negative control group (A) reported significantly high WBCs count compared to the treatment groups. Group (C) showed numerically a high total count of WBCs. The negative control group revealed a statistically higher RBCs count ($p \leq 0.0006$), compared to

group E. For the time being, the RBCs count was only arithmetically higher compared to the other groups. Assessment of whole blood haemoglobin concentration disclosed notably high concentration in the negative control group samples compared to group B ($p \leq 0.04$) and E ($p < 0.01$), while the difference was not significant as compared to group C and D. PCV result values prevailed from group (D); were significantly low compared to those of the negative control group, which occupied the top with 47.7 %. The platelet count was significantly higher in the positive control group compared to group E, which was administered 500 mg/kg of PGPE. Group (D) came after group (B) without a significant difference compared to groups (A) and (C). (Table 2).

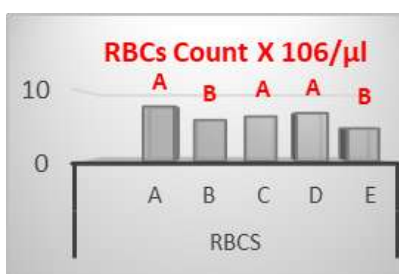


Figure (2)

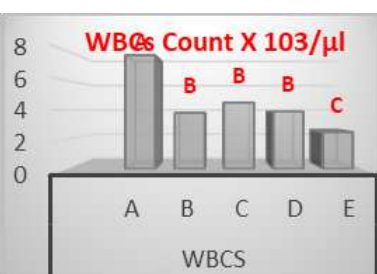


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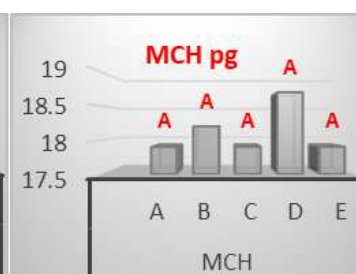


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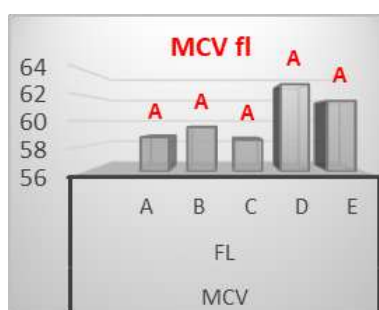


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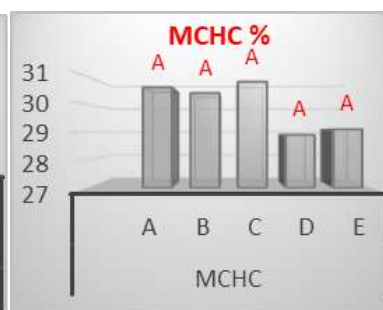


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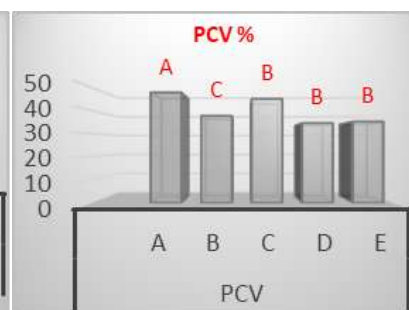


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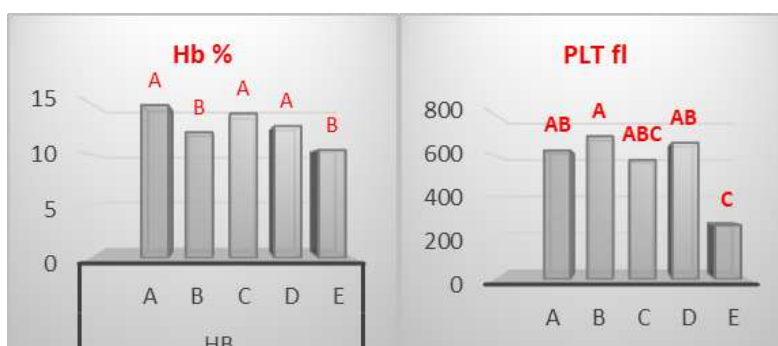


Figure (8):

Figure (9):

3.3 Kidney Function Tests:

The blood biochemical profile of kidney function tests showed, in terms of electrolytes, Potassium levels were not significantly different between groups. Numerically, the negative control group recorded the highest level, while the negative control group was at the bottom. Sodium levels results showed variation between different groups. The PGPE 250 mg/kg group reported the highest level, statistically significant compared to all groups except group (A); the reference drug group (C) came at the end of the list, recording the lowest sodium level.

The positive control group reported a significantly high urea level compared to all groups. Among the other groups, the levels of urea were not statistically different; however, arithmetically, the level was higher in group (C), compared to groups (A), (D), and (E). The creatinine analysis

unexpectedly showed high levels in groups administered with Punica. granatum peel ethanol extract, though the difference was not significant compared to the other groups. Group (E) occupied the top with 0.92 mg/dl, while the lowest level was reported in the negative control group.

The total protein result revealed arithmetically lower levels in the positive control group (B) compared to the other groups, except for the Silymarin group ($p < 0.05$). The highest levels of serum total protein were reported in the Silymarin-receiving group with 7.5 g/dl. Similarly, serum albumin results followed the same swing, showing a significantly high concentration in group (C), whereas the lowest level was reported in group (B). The difference was significant at ($p < 0.05$). Serum albumin concentration between groups (A), (D) and (E) showed no difference, neither statistically nor arithmetically.

Table 1: Effect of Punica.Granatum peel ethanol extract on kidney function tests

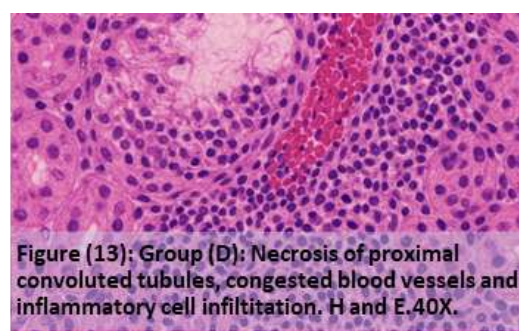
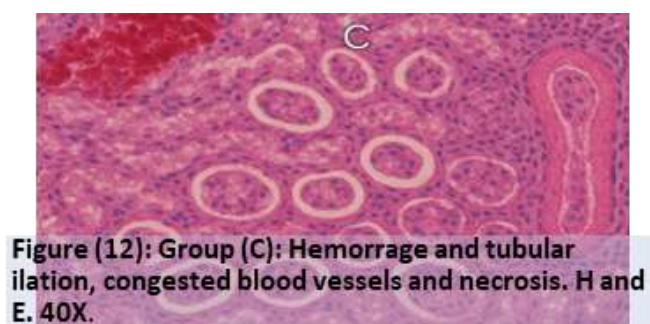
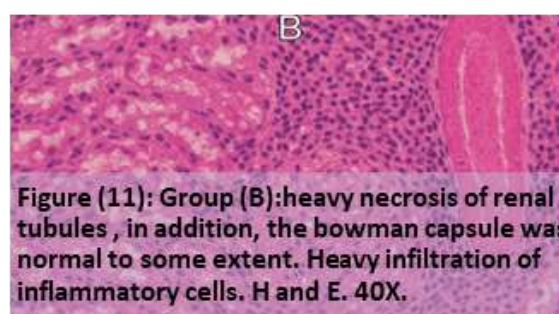
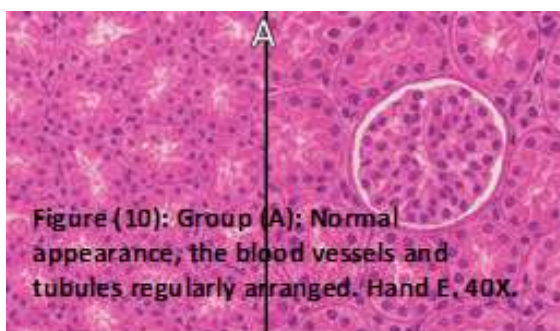
Parameter	Sodium (Na) mEq/L	Potassium (K) mEq/L	Urea mg/dl	TP g/dl	ALB g/dl	Creatinine mg/dl
Group						
A	147.33 ^{AB} ±2.	5.06 ^A ±0.52	42.6 ^B ±2.5	7.4 ^A ±0.4	2.8 ^{AB} ±0.19	0.63 ^{AB} ±0.09
B	140.2 ^{BC} ±2.1	3.9 ^A ±0.52	48.5 ^A ±2.5	6.3 ^B ±0.4	2.4 ^B ±0.19	0.67 ^B ±0.09
C	137.0 ^C ±2.1	4.78 ^A ±0.52	42.65 ^{AB} ±2.5	7.5 ^A ±0.4	3.0 ^A ±0.19	0.83 ^{AB} ±0.09
D	148.0 ^A ±2.1	4.3 ^A ±0.52	40.2 ^B ±2.5	7.1 ^A ±0.4	2.8 ^{AB} ±0.19	0.88 ^A ±0.09
E	139.5 ^C ±2.1	4.3 ^A ±0.52	40.3 ^B ±2.5	7.2 ^A ±0.4	2.8 ^{AB} ±0.19	0.92 ^A ±0.09

Data are means $\pm$ standard deviation. Means in the same column followed by the same letters are not significantly different at ($p < 0.05$).

3.4 Histopathology Results:

Histopathological inspection of the rat's kidney tissue section of group (A) showed a normal appearance. The tubules and the blood vessels were regularly arranged. The positive control group kidney sections revealed heavy necrosis of renal tubules (fragmentation of the nucleus); in addition, the Bowman's capsule was normal to some extent. Heavy infiltration of inflammatory cells and haemorrhage was observed. Moreover,

congestion was clear in the kidney blood vessels. Necrotic lesions were observed in group (C), accompanied by haemorrhage and tubular dilation. The blood vessels were congested and dilated, with remarkable necrosis in the convoluted tubules in group (D). Examination of the kidney sections revealed necrosis of the proximal convoluted tubules, thickening of the muscular layer of the blood vessels, and they were obviously congested. The inflammatory cells were infiltrated across the slide fields. Group (E), congestion and thickening of the muscular layer of vessels were noticed. Heavy infiltration of inflammatory cells was remarkable; tubular dilation and necrotic lesions were also observed.



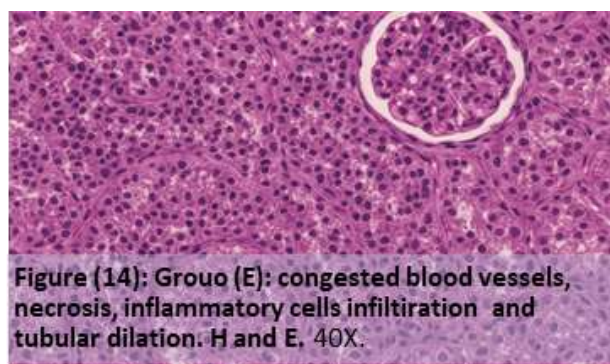


Figure (14): Group (E): congested blood vessels, necrosis, inflammatory cells infiltration and tubular dilation. H and E, 40X.

4. DISCUSSION:

Punica. Granatum's potency to act against toxic compounds could be attributed to the presence of large amounts of kaempferol and Gallic acid (Sara et al, 2022). These compounds can act as radical scavengers. An overdose of acetaminophen is known to initiate acute kidney toxicity. Phytochemical complexes disrupt PAPA metabolism by controlling the availability of paracetamol via various mechanisms. PAPA acts as an antagonist of enzymes, such as cytochrome P-450 enzymes, esterases, and uridine phosphate. Besides, governing paracetamol metabolism can be achieved by affecting transporters such as P-glycoprotein, organic anion transport polypeptides (Abdel-Daim et al, 2018).

The present experiment results, concerning RBCs, WBCs, Hb, PCV, and PLT, displayed a significant reduction in all stated parameters in the positive control group; furthermore, the low level was more noticeable in group (E), which was treated with 500mg/kg PGPE. These findings are in agreement with Igboh (2006) and his coworkers, who observed a reduction in WBCs count, Hb and PCV when the effect of alcohol and paracetamol on the features of the haematology of albino rats was investigated. The current experimental findings are also in line with another study by Pardeep et al (2021). Pardeep's results revealed a significant reduction in RBCs, WBCs, Hb, PCV, and PLT count in response to a high dose of paracetamol

compared to the negative control group. The notable decrease in the mentioned parameters levels in the paracetamol-treated group could be attributed to high exposure of the hemopoietic system to xenobiotics and their secondary metabolites, which have a negative influence on nutrient availability, such as Iron. The effect of xenobiotics and their metabolites may be developed to include the production of erythropoietic growth factor, responsible for cell proliferation and differentiation. Additionally, the influence may extend to deteriorate dynamic functions, which in turn lead to changes in blood parameters such as WBCs, RBCs, Hb, PCV and PLT (Uboh et al, 2010). The noteworthy drop in RBCs counts in group (E), which received the higher dose of PGPE, could be attributed to its significant content of tannin (phytochemical result). This postulation could be built on the annotations of (Majed et al, 2013), who studied and their coworkers, the outcome of different doses of Tannic acid on leukocytes and erythrocytes, their concluding findings stated that exposing erythrocytes to tannic acid resulted the appearance of eryptosis hallmarks, hence tannic acid generated erythrocytes shrinking and cell membrane ascent. Additional study by (Mehmet et al. 2016), who examined the effect of pomegranate on paracetamol-induced acute hepatic injury in mice. Their observations are in line with the present result, concerning RBCs, Hb and PCV; significantly declined values were detected. Since the reduction of RBCs count was notable

depending on dose, group (C), which received 250 mg/kg PGPE, showed no significant different count compared to group (A), we can assume that the drop of RBCs count in response to PGPE, is dose dependent, and primarily; suppose that; 250 mg/kg has likely protective effect against paracetamol-induced hepatotoxicity concerning RBCs count.

Leukocytopenia was the most remarkable change in the haematology profile. The immense decrease in leukocytes observed in group (E) could be attributed to the high content of PGPE of sucrose (GC analysis result). A study performed by (Obchi et al, 2009), to explore the effect of Aspartam and Sucrose on some biochemical and hematological parameters in albino Wister rats; they found out that administration of sucrose lead to significant decline in WBCs count, besides notable low Hb and PCV values, these results sustenance the present results, which could be justified by the effect of sucrose metabolites such as hydroxyl radicals on hematology profile, henceforward theses radicals irritate bone marrow depression noticeable as in-sufficient synthesis of RBCs and WBCs. Once more, the non-significant differences in WBCs count values among groups (A) and (C) support the postulation of the protective effect of 250mg/kg of PGPE against paracetamol toxicity.

The phytochemical analysis of PGPE shows that it contains numerous flavonoid compounds. The existence of these compounds may clarify the occurrence of low platelet levels in the 500 mg/kg dosed group. Hence, flavonoids are claimed to have antiplatelet activity, mainly through their effect on the arachidonic acid cascade (Jana et al, 2016). The anti-anaemic impact of the ethanol extract of punica granatum seeds was investigated by Shravan et al (2016). They observed an increase in Hb concentrations in a dose-dependent manner; in comparison with phenyl hydrazine-

induced anaemic rats, they attributed their observation to alkaloid compounds in Punica granatum seeds. The present result does not agree with their findings, where the Hb and PCV values were significantly ($p < 0.03$) and ($p < 0.01$), correspondingly, low in the 500 mg/kg dosed group; compared to the negative control group, while the variance was not significant between PGPE-treated groups regarding PCV values. The opposing results of the two studies may be due to the implementation of different parts of the Punica Granatum plant, in turn, the existence of alkaloids in distinct concentrations. A general view on haematology results throughout the comparison between the reference drug effect and the protection provided by PGPE at different doses, 250 mg/kg of PGPE, reversed the toxic effect of paracetamol and recorded a higher count of RBCs and PLTs. While Silymarin was better regarding the development of WBCs count, Hb and PCV values.

Sodium and potassium results revealed significantly higher blood Sodium values in the control group. The same result was observed in group D, compared to group C, which was dosed with 250 mg/kg PGPE. Generally, values between 137-148 m.equl/l are within the reference range. Potassium levels were not significantly different among groups. Although the control positive group reported the lowest level, the negative control group levels were the highest, whereas the treatment groups recorded almost the same level of potassium. Regarding potassium results, our current study finding is in line with what was reported by (Pakravan et al, 2007). When they studied the effect of high doses of paracetamol on blood potassium levels, they reported a dose-related incidence of hypokalemia.

Lipid peroxidation increases in response to paracetamol, leading to breakdown of the

antioxidant system, which in turn causes a reduction in serum total protein levels. That is exactly what triggered the remarkable decrease in serum total protein of the positive control group in our current study. That reduction was significant compared to other groups; meanwhile, the difference was not significant among the others.

Paracetamol, increases lipid peroxidation, in a manner may lead to collapse of antioxidants defense system and accordingly lead to decrease of serum total protein level, (Eesha et al, 2011), this statement, can clarify the scientific reason behind the significant decreased levels of serum total protein in positive control group compared to the rest groups, while there was no statistical disparity among other groups. Albumin represents 50% of plasma protein; it plays an anchor role to sustain osmotic pressure (Bern et al, 2015). A contrary result was obtained by (Mehmet et al, 2016), who reported no significant change in serum total protein levels between the paracetamol group and PGPE-treated groups. The difference in the obtained results may be attributed to different doses of PGPE used in the current trial. The current experimental result showed no significant difference in albumin levels between all groups except for the Silymarin-treated group, which recorded a high concentration of albumin in serum samples. That means paracetamol did not affect serum albumin level in this trial; this result agrees with Kanbur et al (2009), who reported that paracetamol does not affect serum albumin concentration. (Mehmet et al, 2016) also reported the same result. Albumin has a long half-life, and its concentration is reduced only when hepatopathy and portosystemic shunt are absent (Bern et al, 2015). Both doses of PGPE have the efficacy to reverse the alteration of protein levels.

The serum creatinine level was almost within the normal range (0.4-0.8 mg/dl), Brabra, (2014),

except for group E, which reported 0.92 mg/dl. The difference was significant when compared to group B (the control positive group). The recorded values between the other groups were not significantly different. Numerous previous studies documented the reducing effect of Punica granatum peel extract on blood creatinine levels. On the contrary, the result obtained from the current study could be attributed to synergistic toxicity with NAPQI, a reactive metabolite that depletes glutathione and causes oxidative stress (Jack et al, 2010). Despite the valuable content of antioxidants in Punica granatum, mixing PGPE with paracetamol, especially at a dose of 500 mg/kg, may cause renal tubular stress, impairing creatinine clearance. The elevation could be false and can be justified by the effect of tannin content in Punica granatum peel extract, which induces gastrointestinal irritation; reduces water absorption rates, leading to hemoconcentration and false elevation of serum creatinine. As we mentioned above, rats were subjected to 24 hours of fasting before sampling. Serum ketone body levels are elevated in response to fasting, especially under stressful conditions (paracetamol administration), which can lead to dehydration, acid-base imbalance, and renal stress, and may elevate creatinine. Another disagreement with previous studies could be attributed to dose differences or individual variations. The blood urea level was remarkably elevated in response to paracetamol administration; this result is in line with Abdalmonem and his co-workers, who reported that noticeably high blood urea values were observed after administering paracetamol at a dose of 2g/kg. The reason behind the elevation of urea in the control positive group is due to the oxidative stress resulting from glutathione diminution as a result of paracetamol reactive metabolite, after making covalent bonds with renal tissue, leading to cell death, which has been

correlated with electrolyte imbalance and blood urea instability (Alderman et al, 2002).

Rats dosed with *Punica granatum* at doses of 250 and 500 mg/kg showed a significant reduction in blood urea levels compared to the positive control group. The values were not significantly different from the reference drug group. Concluded results are in agreement with (Nabil et al, 2023), who reported a possible reversing effect of renal damage induced by phenylhydrazine; a remarkable decrease in blood urea level was observed in response to the plant extract dosing. Another study by Masoud et al (2024) reported similar results. Maoud and his co-worker concluded that *Punica granatum* peel extract has renoprotective properties against lithium-induced kidney damage.

CONCLUSION:

Numerous studies reported the beneficial influence of using *P. Granatum* peel on nephrotoxicity. their findings reported a positive shift in almost all kidney function parameters. Opposing results were obtained in this study: a high blood creatinine level was documented. In addition to histopathology results, which revealed necrosis, dilation of blood vessels, and infiltration of inflammatory cells, the mentioned findings represent crucial parameters and clues, along with other signs and symptoms, to the development of kidney failure.

RECOMMENDATIONS:

The observations excluded from the current study after reinvestigating the effect of *P. Granatum* peel ethanol extract on nephrotoxicity established by paracetamol raise awareness of the random use of medicinal herbs without governmental regulatory bodies. Despite the huge benefits we can obtain from using medicinal plants due to their rich

content of beneficial compounds, more research is needed to determine the toxicity of each compound and to explore the exact therapeutic dose for each condition, taking into consideration the variation of health status of every patient.

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