Punica. Granatum peel ethanol extract may exhibit liver protection activity against hepatotoxicity induced by paracetamol in rats

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Abstract

Background: Paracetamol is extensively used analgesic and antipyretic drug. High dose of paracetamol is well known as hepatotoxicity motivator. In order to maintain the hepatic heath, the effect of PGPE as hepatoprotective plant evaluated. ***Materials and methods***: 35 male rats weighing 150-200 g, sorted 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 hepatotoxicity induced by single dose of paracetamol 750mg/kg. after 24 hours of fasting, rats were sacrificed, blood samples collected for determination of hematology and biochemistry profile. Liver tissue samples were taken in 10% formalin for histopathology examination. ***Results:*** the high elevation of liver enzymes ***(ALT, AST and ALP***, occurred by the single high dose of paracetamol was remarkably reversed by PGPE administration, while serum level of albumin was not significantly altered. Total WBCs count massively lowered by paracetamol administration, and got worse by dosing 500mg/kg PGPE. RBCs, Hb and PCV values expressively reduced by paracetamol and more reduction induced by 500 mg/kg of PGPE.

Key Words: Punica. Granatum peel, Paracetamol, Liver injury, phytochemical, Silymarin.

Abbreviations: RBCs: *Red blood cells.* ***WBCs:*** *White blood cells.* ***PCV.*** *Packed cell volume.* ***Hb:*** *Hemoglobin.* ***MCV:*** *Mean cell volume.* ***MCH:*** *Mean corpuscular hemoglobin.* ***MCHC:*** *Mean corpuscular hemoglobin concentration.* ***PLT.*** *Platelets.* ***ALT:*** *Alanine aminotransferase.* ***AST.*** *Aspartate aminotransferase.* ***ALP.*** *Alkaline phosphatase.* ***T.P:*** *Total protein.* ***ALB:*** *Albumin.* ***D.B:*** *Direct bilirubin.* ***IND.B:*** *Indirect bilirubin.* ***PGPE.*** *Punica granatum peel extract.* ***APAP.*** *N-acetyl-para-aminophenol.* ***NAPQI.*** *N-acetyl-p-benzoquinone imine.* ***NAC:*** *N-acetyl cysteine.*

1. Introduction

Paracetamol is the common name of acetaminophen; it represents one of the further most extensively used over-counter drug. Acetaminophen is used as analgesic and antipyretic, for both adult and children **(Laura *et al,* 2003).** It's classified as a member of aniline Non steroid anti-inflammatory drug. The toxic dose of paracetamol may originate liver necrosis, nephrotoxicity and may cause death in lab animals as well as in human. The conservative dose of paracetamol for adults is 0.5 gm every 4-6 hours, the supreme allowed dose is 4 grams. Regarding the children; the dose is age and weight dependent, which it should be less than 4 mg/kg in 24 hours. **(Nadia *et al,*2016).** Paracetamol over dose; saturates Glucouronyl transferases and Sulfotrans transferases averting the metabolism of the drug by cytochrome P450; which produces NAPQ1 in quantities exceeds the ability of glutathione to scavenge, causing glutathione diminish (**Mitchelli *et al*, 1973)**. The consequences of un replace up of glutathione; is an accumulation of **NAPQ1**in hepatic cells, oxidative stress leads to lipid peroxidation triggering a permanent cell membrane damage,

hence forward cell death **(Doyon., 2009).** Liver is a vigorous organ, so issues related to it represents one of the major health problems around the world. The vital functions from which liver health importance raise is including, detoxification of endogenous and exogenous damaging materials, glycogen storage, plasma protein synthesis. Therefore, several metabolic alterations are connected to liver cells injuries (**Erfidan *et al,* 2016)**. Reactive oxygen and reactive nitrogen species, are playing a critical role in instigation and advancement of liver allied illnesses, such as, Non-alcoholic steatosis, alcoholic and viral hepatitis and hepatocellular carcinoma **(Nagata *et al,* 2007).** Liver fibrosis development may lead to cirrhosis, which is linked to liver cancer. The percentage of patients with liver cirrhosis, who further progressed to liver cancer is about 10-20% **(Emerit *et al,* 2006).**

There contrary side effects of steroids, anti-viral and vaccines, which are used to cure liver diseases if administrated for long term. According to extensive studies, the prevention against the oxidative stress related to liver pathologies, can be achieved by natural products possess high potent anti-oxidant activity and defensive role against paracetamol liver damage. The principal anti-dote in paracetamol toxicity occasions is N-acetyl cysteine **(NAC); (Mitchelli *et al.,* 1973).** Yet, hepatocyte toxicity may continue developing when NAC is **administrated (Doyon and Schwartz 2009).**

Therapeutic preparations had been generated from nutritional components. The usage of traditional medicine, lately, had surge attention.in developing countries. Numerous plants, have therapeutic competence, this potency had been confirmed and applied to control several diseases.

Punica.Granatum peel, symbolizes a native source of anti-oxidants. The evaluation of ***Punica. Granatum*** peel, revealed occurrence of various phytochemical compounds, for example, it's recognized as rich source of phenol compounds, flavonoids and phytosterols **(Nadia *et al,*2016).** In addition to poly saccharides and minerals counting, potassium, nitrogen, magnesium, phosphorous and sodium **(Al-Rawahi *et al,* 2014).** Furthermore, ***Punica. Granatum*** peel's content of tannin, has free radical scavenging activities, which is extremely vulnerable to enzymatic and non-enzymatic hydrolysis mutually **(Seeram *et al,* 2005).**

Table 1: **phytochemical screening of *Punica. Granatum* peel ethanol extract.**

CONSTITUENT	ETHANOL EXTRACT
SAPONINS	+
COMARINS	++
ALKALOIDS	+
FALVINOIDS	+++
TANINS	+++
TRITERPENES	-
ANTHRAQUINONES	-

Objectives

- The investigate hepatoprotective activity of ***Punica Granatum*** ethanol extract.
- Screening the phytochemical profile.
- Figure out the influence of ethanol extract on hematology parameters.
- Exploring effects of extract on serum biochemical profile.
- Defining the bioactive constituents of ***Punica Granatum*** ethanol extract.

2. Materials and Methods

The trial conducted on October **2020**, at Central Veterinary Research Laboratory-Khartoum-Sudan.

2.1 Plant materials:

Punica. Granatum peel purchased from local market for traditional herbs, Plant extraction: Extraction of plant material was achieved using ethanol according to the method described by **(Sukhdev *et al,* 2008).** Briefly 100 g of plant extracted by soxhlet by adding 500 ml of ethanol (**70%**) at 68°C for **72** hours. The extract was filtered using whatman No 1filter paper, then concentrated using rotary vacuum evaporator **at 40-45° C.** The **100 g** yielded **27.368g (27.37%).**

2.1.1 Phytochemical screening: the phytochemical screening was carried out according to the standard procedure described by **(Sukhdy *et al*, 2008; Srividya and Nehrota., 2003 and Chang *et al,* 2002).**

2.2 *Experimental protocol approval*:

This work is ethically approver by the Sudanese veterinary council, the regulatory regulations and laws are concerning research ethics are followed.

2.3 Animals:

35 male and female rats brought from veterinary medicine College-University of Khartoum. rats weighed between **(150-200 Grams),** divided evenly into 5 groups **(n=7).** Placed in stainless steel cages, fed standard diet and the access to the water was *ad labium*. The temperature during the experiment was between **36-39º C.**

2.4 Drugs and chemicals:

Silymarin and paracetamol were purchased from pharmacy at Khartoum hospital street. Xylazine and Ketamine obtained from Sigma Aldrich. Ethanol and Formaldehyde brought from laboratory solvent and reagents supplying company in Khartoum.

2.5 Experimental Model:

the five groups sorted as follows; **group A** served as negative control (Normal saline); **group B** assigned as Positive control (Paracetamol); **group C** is a reference drug (Silymarin); **group D** dosed by **250** mg/kg ***Punica. Granatum*** ethanol extract; and finally, **group E** received **500** mg/kg ***Punica. Granatum*** ethanol extract; using stomach lavage. Animals allowed adapt for 2 weeks, followed by tracking the following protocol:

Negative and Positive control groups, (**A** and **B** respectively), received only distilled water, while referenced drug group dosed by 80 mg/kg for consecutive 7 days. The treatment groups (**D** and **E)**, received **250 and 500 mg/kg** of ***Punica. Granatum*** peel ethanol extract for also for sequential 7days. Liver injury was induced on day 8 by administrating (**750 mg/kg paracetamol**, ***single dose***) given orally to all groups except negative control group (**A**). Rats subjected for fasting for 24 hours. On day 9 samples were taken. Prior to euthanasia blood samples collected in plain and EDTA coated tubes for biochemistry lab to perform liver function tests and hematology lab for blood cells count, **Hb**, **PCV, MCV, MCH, MCHC** and platelets determination.

2.6 Blood biochemistry profile analysis: samples were centrifuged at 3000 rpm for 10 min, serum collected in Eppendorf tubes and sent to the lab for liver function tests determination using ***BIOSYSTEM BTS-350*** Apparatus.

2.7 Hematology:

complete blood picture determined using, ***URIT 3010 Hematology analyzer-China.***

2.8 Histopathology analysis: The drowsiness of the rats in each group was accomplished by Xylazine (5 mg/kg) and Ketamine (100 mg/kg). Animals euthanized by cervical dislocation. Tissue samples taken in 10% formaldehyde solution were forwarded to pathology lab. The liver tissue samples perceived in 10% formaldehyde for two days then placed into automatic tissue processor (Histos5, rapid microwave processor, Milastone-**USA)** and monitored for 12 hours. The samples were blocked with molten paraffin at 56-58 0C and those paraffin blocks froze at -10º C in a refrigerator. After 4-5µ thick sections were sliced the paraffin blocks were stained with hematoxylin eosin. The stained sections were inspected under a light microscope.

2.9 Gas Chromatography-MS:

GC-MS Analysis of the ethanol extract in the selected plant was performed using **QP2010-Ultra Shimadzu**, with serial number **02052101565SA**. capillary column (RTX=5ms=30mmx0.25 µm). Samples were injected using split mode. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1.6 ml/min. temperature program started at 50ºC with rate 10 c/min to 300ºC as final temperature degree, with 10 hold time. the injection port temperature was ^{200}ºC and the interface temperature was 250 ºC. The samples were analyzed by using scan mode in the range of 40-500m/z, charges to ratio and the total run time was 14 min.

Identification of components:

Interpretation on 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 expressed as mean (±SD). The statistical analysis was performed using one-way analysis of variance (T-TEST) using **Statisix version 10 Software**, USA. *P* values **($P < 0.05$)** was considered statistically significant when compared to (acetaminophen alone) control.

3- Results

3.1 Phytochemical analysis:

Phytochemical screening of ***Punica. Granatum*** peel ethanol extract revealed, high content of tannins and flavonoids, moderate content of cumarins and low content of saponins and alkaloids. While other compounds such as triteoentens and anthraquinones were not detected**. (Table 1).**

3.2 GC analysis:

GC/MS analysis of ***punica, granatum*** peel ethanol extract, showed the presence of only two bioactive compounds; Sucrose and gamma sitosterol. **(Figure 1).**

3.3 Hematology parameters:

Generally, there was no statistically significant difference reported regarding **MCV. MCH, MCHC**. However, the negative control group recorded significant high **WBCs** count compared to treatment groups, among which there was no statistical difference between group (B); (C) and (D). However, group (C), showed numerically higher total count of **WBCs.** Statistically the negative control group revealed high total **RBCs** count ($p < 0.0006$), compared to group E, meanwhile the **RBCs** count was only numerically higher in comparison with the rest of groups. Estimation of whole blood hemoglobin concentration revealed, remarkably high concentration in 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 obtained from group (D) was significantly low compared to that of the negative control group, which came on the top with 47.7 %. Platelets count was significantly high in positive control group compared to group E, which received 500mg/kg of **PGPE.** Group (D), came secondly after group (B) without significant difference compared to group (A) and (C). (**Table 2).**

3.4 Liver function tests:

Regarding biochemical profile, the positive control group (B) showed highly significant level of serum **AST,** compared to group A ($p \leq 0.02$), D ($p < 0.02$) and group E ($p < 0.03$). group (C) which received Silymarin as reference drug recorded the second-high level of AST with 156 IU/L. The concentration of serum **AST** was not remarkable between **PGPE** groups, however, numerically; 500 mg/kg dose group (E), showed higher level. Group (E), recorded 59.6 IU/L of serum ALT, this level was not significant compared to group (B), and Silymarin received group **(RD).** While group (D), which dosed by 250 mg/kg **PGPE,** showed 47.6 IU/L; this result was not statistically different compared to the negative control group (A). alkaline phosphatase was significantly high ($p < 0.002$), in group (B) compared to group (A), (C) and group (D). **PGPE** groups level of **ALP**, was only numerically different, with higher level 118 IU/L, in upper dose group.

Total protein result obtained from this trial revealed, no significant lower concentration in positive control group (B), compared to the other groups except for the Silymarin dosed group ($p < 0.05$). The highest level of serum total protein observed in Silymarin received group with 7.5 g/dl. Meanwhile serum albumin result, followed the same trend of total protein result and showed significant high concentration in group (C), while the minimum level recoded in group (B) the difference was significant at ($p < 0.05$). Serum albumin concentration between group (A), (D) and (E), was not different neither statistically, nor numerically. Indirect bilirubin level was significantly high in positive control group, followed by Silymarin group (C), with 35 mg/dl. The difference between group (A) and (D) was not significant. Direct bilirubin result showed, statistically no significant difference between all groups, but on the level of numbers the highest concentration observed in group (B), while the lowest one noticed in group (D); **(Table 3).**

3.5 Histopathology Results:

Histopathological examination of rat liver section of group (A), revealed; normal hepatic cells, sinusoidal and central vein dilatation, in addition to congestion of the central vein. Acetamiphen group liver sections, showed; severe hepatocellular necrosis (fragmentation of nucleus), also sinusoidal dilatation and hemorrhage was clearly noticed. Reference drug group liver tissue sections showed, dilatation and congestion of the central vein, necrosis appeared clearly in portal area and the bile duct lining epithelium. Heavy hepatocellular necrosis was also observed (nuclear fragmentation). Among the observations of this group sections, sinusoidal dilatation and congestion, furthermore hemorrhage was noted too. Group (D), examination of liver section under lens 40, showed, sinusoidal and central vein dilatation, also the central vein was obviously congested. Group (E), sinusoidal and central vein dilatation, clear central vein congestion, heavy necrosis of hepatic cells, bile duct and portal area. Furthermore, hemorrhage of liver parenchyma was noted, in addition to infiltration of inflammatory cells.

4- Discussion

Punica. Granatum; constituents, have the capability to reverse and to protect against the poisonous effect in the liver by decreasing the oxidative stress and inflammatory response **(Jeyadevi *et al,* 2019).** The hepatoprotective effect of **PGPE** could be attributed to the existence of great quantity of Kaempferol and Gallic acid **(Van Elswijk *et al,* 2004).** which are work as radical scavenging compounds (**Kim and Lee., 2009).** Overdose of paracetamol is known to origin acute liver toxicity **(Tiittareli *et al,* 2017),** phytochemical complexes can disturb the **PAPAP** metabolism by controlling the availability of paracetamol in various mechanisms, such as working as antagonist to many enzymes like, cytochrome **p-450** enzymes, esterase uridine phosphate. Also governing paracetamol metabolism could be through affecting transporters such as, p-glycoprotein, organic anion transport polypeptides **(Abdel-Daim *et al,* 2018).**

The findings of the current experiment; regarding, **RBCs, WBCs, Hb, PCV** and **PLT;** showed significant decrease in all mentioned parameters in paracetamol administrated group, moreover, the reduction more pronounced in group (E), which

treated with 500mg/kg **PGPE**. These results are in an accordance with what reported by **(Igboh., 2006);** and his coworkers, who noticed reduction in **WBCs** count, **Hb** and **PCV**; when they studied the effect of alcohol and paracetamol on aspect of the hematology of albino rats. Conformation of the present experiment results from another study held by **(Pardeep *et al,* 2021),** their observations revealed significant decrease in **RBCs, WBCs, Hb, PCV** and **PLT** count, in response to high dose of paracetamol compared to the negative control group. The remarkable decrease in mentioned parameters values, in paracetamol treated group could be attributed to high susceptibility of hemopoietic system to xenobiotic and their secondary metabolites, which have negative impact on nutrients availability such as Iron. The effect of xenobiotic and its metabolites may expand to involve the production erythropoietic growth factor responsible for cell proliferation and differentiation, moreover the influence may expand to deteriorate vital functions, which in return lead to alterations in different blood parameters such as **WBCs, RBCs, Hb, PCV** and **PLT, (Uboh *et al,* 2010).** The remarkable reduction of **RBCs** counts in group (E), which received the higher dose of **PGPE**, could be attributed its substantial content of tannin (phytochemical result). This assumption could be built on the observations of **(Majed *et al,* 2013),** who studied-and his coworkers, the effect of different doses of Tannic acid on leukocytes depleted erythrocytes, their final results revealed that, exposing erythrocytes to tannic acids resulted appearance of eryptosis hall marks, hence tannic acid triggered erythrocytes shrinking and cell membrane scramble. Another study by **(Mehmet *et al.* 2016),** investigated the pomegranate effect on paracetamol induced acute hepatic damage in mice; their observations came in line with the current study result, regarding **RBCs, Hb** and **PCV,** significant decreased values were observed. Since the reduction of **RBCs** count was remarkable dose only, whereas group (C), which received 250 mg/kg **PGPE,** showed no significant different count compared to group (A), we can assume the reduction of **RBCs** count in response to **PGPE**, is dose dependent, and primarily suppose the 250 mg/kg has potential protective effect against paracetamol-induced hepatotoxicity in regard to **RBCs** count.

Leukocytopenia observed as one of the most remarkable hematology profile alterations. The massive reduction leukocytes noticed in group (E), could be referred to the high content of **PGPE** of sucrose (GC analysis result). A study carried out 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 findings support the current trial results, which could be justified by the influence of sucrose metabolites such as hydroxyl radicals on hematology profile, henceforward theses radicals provoke bone marrow depression pronounced in in sufficient synthesis of **RBCs** and **WBCs**. Once more the non-significant different **WBCs** count values among group (A) and (C), synergize the possibility of the protective effect of 250mg/kg of **PGPE** against paracetamol toxicity.

The phytochemical analysis of **PGPE**, revealed it contains high number of flavonoids compounds. The presence of these compounds may explain the notable low count of platelets in 500 mg/kg dosed group, hence the flavonoids claimed to have anti platelets effect, principally through their effect on arachidonic acid cascade **(Jana *et al,* 2016).** The ant anemic activity of ethanol extract of ***punica granatum*** seeds; examined by **(Shravon *et al,* 2016),** they observed an incensement in **Hb** concentrations in dose dependent manner; in comparison with phenyl hydrazine-induced anemic rats, they attributed their observation to alkaloids compounds in punica granatum seeds. The present trial result does not agree with their findings, where the **Hb** and **PCV** values were significantly ($p < 0.03$) and ($p < 0.01$) respectively, low in 500 mg/kg dosed group; compared to the negative control group, while the difference was not significant among **PGPE** treated groups in regard to **PCV** values. The contrary findings of the two trials might be a result using different parts of Punica Granatum in addition the difference in concentration of alkaloids in different plant's parts. General view on hematology result; taking the comparison between the reference drug protection effect, and the defense given by the **PGPE** different doses, the observations revealed 250 mg/kg of **PGPE,** the poisonous effect of paracetamol and showed higher count of **RBCs** and **PLTs**. Reversely. Silymarin; was better in developing the values of, **WBCs** count, **Hb** and **PCV**.

AST and **ALT**, are excellent markers of hepatic damage, hence both of them represent a part of gluconeogenesis process. Since **AST** is found in various tissues other than liver; it considered low sensitive evident of liver injury. In contrast, the highest level of **ALT** is mainly found in liver, therefore **ALT** is more specific to evaluate liver health condition.

Blood biochemistry analysis revealed that, paracetamol induced hepatic damage. The current trial result showed, significant elevation of serum **ALT, AST** and **ALP** levels in response to paracetamol administration, compared to the negative control group (A). Treatment with 250mg/kg of **PGPE**, reduced the mentioned enzymes significantly, where the administration of 500mg/kg, resulted in significant reduction ($p < 0.04$), compared paracetamol group only in regard to AST, while, the ALT and ALP serum values were only numerically lower in **PGPE** treated group. The present result is in an accordance with the result obtained by (**Nadia *et al,* 2016**), when investigated the effect of methanol extract of pomegranate peels in acetaminophen induced hepatotoxicity, their observations confirmed the hepatoprotective

effect of **PGPE** extract, therefore the serum levels of **ALT** and AST, were significantly decreased in treated groups. Similarly**, (Mehmet *et al,* 2016),** reported significant increase of liver enzymes (**ALT, AST** and **ALP)**, in paracetamol treated group, and observed significant decrease in levels of those enzymes in groups received different doses of ***Punica Garanatum***. Also, aqueous extract of **PGPE** leaves, was tested against hepatotoxicity induced by carbon tetrachloride in rats, administrating ***Punica Granatum*** leaves extract by stomach lavage in dose 250 and 500 mg/kg, lowered down the serum levels of **ALT**, **AST** and **ALP, (Manoj *et al,* 2018).** The explanation of liver injury by mean of paracetamol and consequently the increase of serum liver enzymes concentration is that, paracetamol trigger lipid peroxidation, which in turn lead to disturbing cell membrane integrity and release liver enzymes into the blood stream. Efficient management of liver enzymes, bilirubin and total protein values, represents mile stone step towards upgrading the hepatic function, and the effectiveness of any drug is depending on its ability to reduce the harmful influence of hepatotoxins, moreover, restore the hepatic function. Though different doses of **PGPE**, that used in this trial had a positive effect in restoring liver function concerning liver enzymes; but the observation in this trial elucidated that 250mg/kg dose more effective the 500 mg/kg one.

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 decrease 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, and play an anchor role to sustain the osmatic pressure, **(Bern *et al,* 2015).** Contrary result was obtained by **(Mehmet *et al,* 2016**), who reported no significant change of serum total protein levels between paracetamol group and **PGPE** treated group, the difference in obtained result may be raised from different doses of PGPE used in the current trial. The current experimental result showed no significant difference in albumin levels between all group except for **Silymarin** treated group, which recorded high concentration of albumin in serum samples. That means paracetamol had no effect on serum albumin level in this trial, this result agrees with, **(Kanbur *et al,* 2009),** who reported that paracetamol has no effect on serum albumin concentration. **(Mehmet *et al,* 2016),** also reported the same result. Albumin has long half-life, and its concentration is reduced only when hepatopathy and Porto-systemic shunt are dispersed**, (Bern *et al,* 2015).** The both doses of **PGPE,** had the efficacy to converse the alteration of reduced protein due to administration of paracetamol.

Liver modify, bilirubin into form that the body can get rid of, it termed as conjugated or direct bilirubin. This bilirubin moves from the liver into the small intestine and very small amount is passed through the kidney and expelled in urine. Elevation of total bilirubin companied with elevation in direct bilirubin, indicates liver disease. The observation of bilirubin levels in this trial showed numerical high concentration of direct bilirubin in positive control group, in comparison with all groups. This may indicate that, liver injury induced by paracetamol affected the ability of liver process the bilirubin in the bile duct, or the amount of produced bilirubin represent overload on declined liver function. In direct bilirubin concentration was significantly high in positive control group compared to group (A), (D) and (E), ($p < 0.02$), ($p < 0.005$) and ($p < 0.03$), restively. This result along with law hemoglobin concentration are signs of anemia occurred in group (B), in response to high dose of paracetamol. The ***Punica Granatum*** peel ethanolic extract had the capacity reverse the harmful effect of paracetamol and started to restore the liver function which reflected in reduction of direct bilirubin.

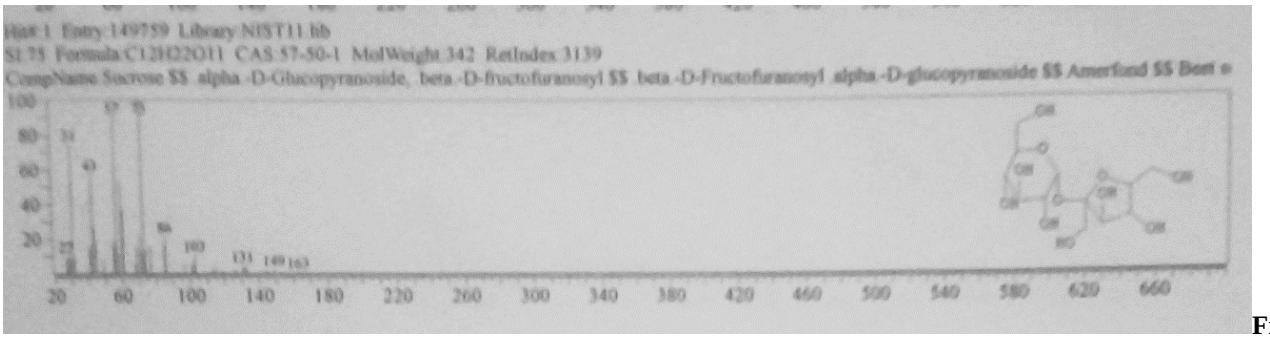

Figure (1): *GC analysis result of Punica Granatum phytochemical constituents, Sucrose chromatograph.*

Table 2: *Effect of* ***Punica.Granatum*** *peel ethanol extract on hematological parameters*

PARAMETER	*RBCS* $10^6/\mu L$	*WBCS* $10^3/\mu L$	*MCV* *FL*	*MCH* *PG*	*MCHC* *%*	*PCV* *%*	*HB* *G/DL*	*PLT* *FL*
GROUPS								
A	$7.84^{A}\pm1.2$	$7.7^{A}\pm1.9$	$58.7^{A}\pm1.7$	$17.9^{A}\pm0.31$	$30.6^{A}\pm0.79$	$47.7^{A}\pm5.9$	$14.72^{A}\pm1.7$	$622^{AB}\pm196$
B	$5.9^{B}\pm1.2$	$3.8^{B}\pm1.9$	$59.5^{A}\pm1.7$	$18.2^{A}\pm0.31$	$30.4^{A}\pm0.79$	$37.6^{C}\pm5.9$	$12.1^{B}\pm1.7$	$690.8^{A}\pm196$
C	$6.4^{A}\pm1.2$	$4.5^{B}\pm1.9$	$58.5^{A}\pm1.7$	$17.9^{A}\pm0.31$	$30.8^{A}\pm0.79$	$44.9^{AB}\pm5.9$	$13.9^{A}\pm1.7$	$576^{ABC}\pm196$
D	$6.89^{A}\pm1.2$	$3.9^{B}\pm1.9$	$62.9^{A}\pm1.7$	$18.7^{A}\pm0.31$	$28.9^{A}\pm0.79$	$34.5.5^{BC}\pm5.9$	$12.7^{A}\pm1.7$	$659.3^{AB}\pm196$
E	$4.7^{B}\pm1.2$	$2.5^{C}\pm1.9$	$61.6^{A}\pm1.7$	$17.9^{A}\pm0.31$	$29.1^{A}\pm0.79$	$35.1^{BC}\pm5.9$	$10.4^{B}\pm1.7$	$261^{C}\pm196$

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

Table 3: *Effect of* ***Punica.Granatum*** *peel ethanol extract on biochemical profile*

PARAMETER	*AST* *IU/L*	*ALT* *IU/L*	*ALP* *IU/L*	*TP* *G/DL*	*ALB* *G/DL*	*DB* *MG/DL*	*INDB* *MG/DL*
GROUP							
A	$107.6^{C}\pm21.2$	$44.3^{B}\pm6.4$	$96.7^{C}\pm14$	$7.4^{A}\pm0.4$	$2.8^{AB}\pm0.19$	$0.16^{A}\pm0.01$	$0.26^{B}\pm0.04$
B	$167.3^{A}\pm21.2$	$60^{A}\pm6.4$	$130.3^{A}\pm14$	$6.3^{B}\pm0.4$	$2.4^{B}\pm0.19$	$0.19^{A}\pm0.01$	$0.39^{A}\pm0.04$
C	$156.3^{A}\pm21.2$	$55.1^{A}\pm6.4$	$92.3^{C}\pm14$	$7.5^{A}\pm0.4$	$3.0^{A}\pm0.19$	$0.16^{A}\pm0.01$	$0.35^{AB}\pm0.04$
D	$127^{B}\pm21.2$	$47.6^{B}\pm6.4$	$110.6^{BC}\pm14$	$7.1^{A}\pm0.4$	$2.8^{AB}\pm0.19$	$0.15^{A}\pm0.01$	$0.29^{B}\pm0.04$
E	$139.7^{B}\pm21.2$	$59.6^{A}\pm6.4$	$118^{AB}\pm14$	$7.2^{A}\pm0.4$	$2.8^{AB}\pm0.19$	$0.17^{A}\pm0.01$	$0.28^{B}\pm0.04$

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

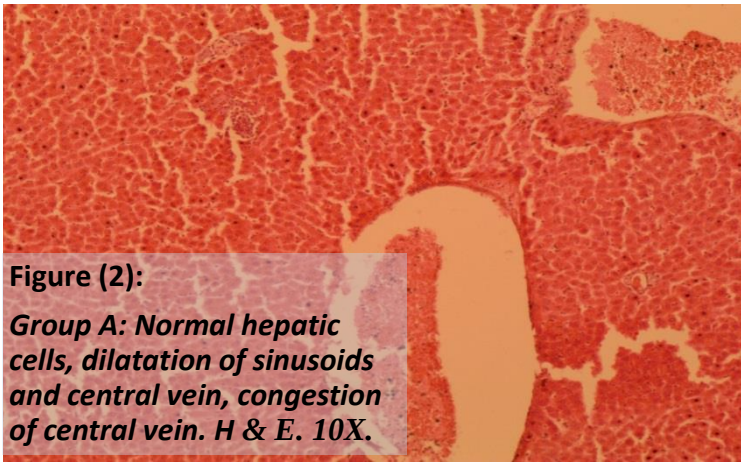

Figure (2):

Group A: Normal hepatic cells, dilatation of sinusoids and central vein, congestion of central vein. H & E. 10X.

Figure (3):

Group (B): severe hepatocellular necrosis, portal area necrosis and sinusoidal hemorrhage. H & E, 40X.

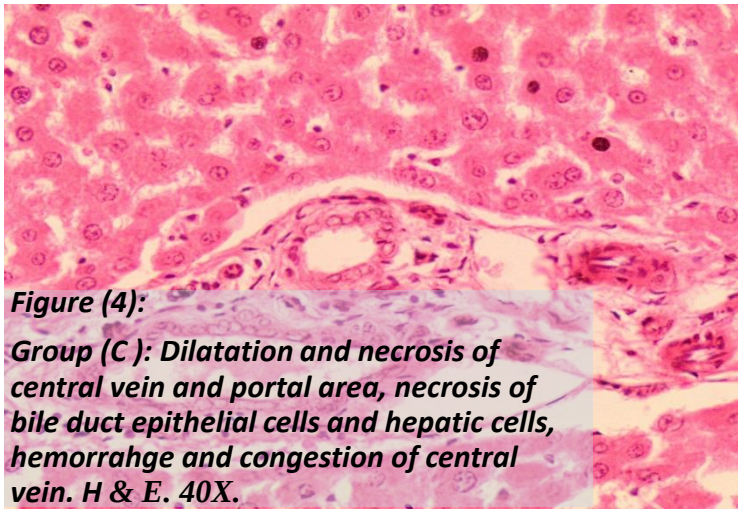

Figure (4):

Group (C): Dilatation and necrosis of central vein and portal area, necrosis of bile duct epithelial cells and hepatic cells, hemorrahge and congestion of central vein. H & E. 40X.

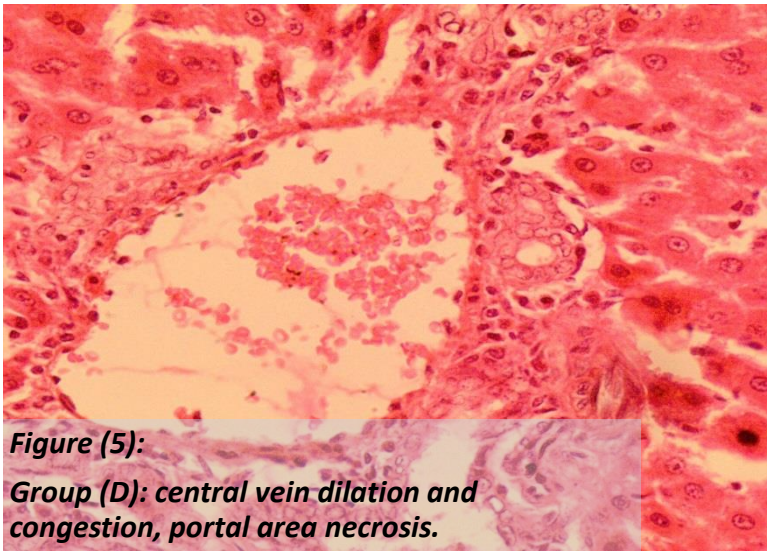

Figure (5):

Group (D): central vein dilation and congestion, portal area necrosis.

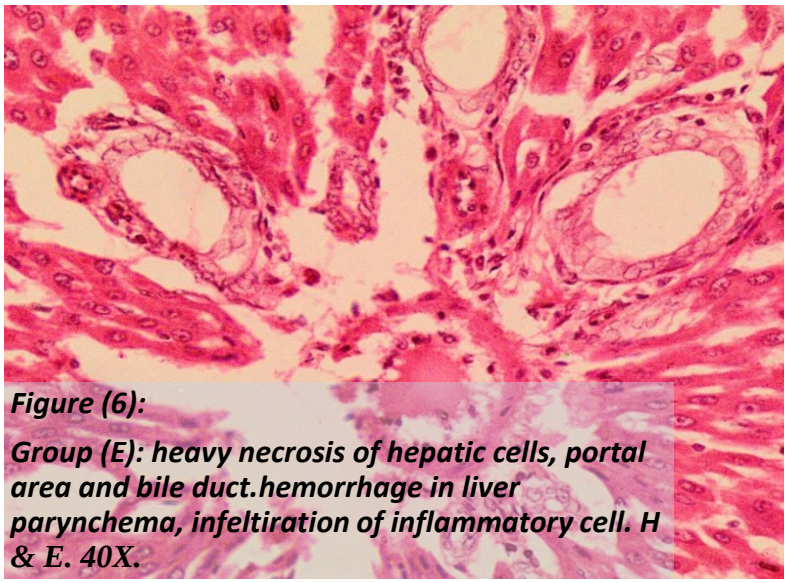

Figure (6):

Group (E): heavy necrosis of hepatic cells, portal area and bile duct.hemorrhage in liver parynchema, infeltiration of inflammatory cell. H & E. 40X.

Histopathology findings of group **(B),** are in agreement with Aiyalu and Mustafa, 2012, who reported severe necrosis in rate administrated paracetamol (2g/Kg body weight); orally. Group (D); which administrated (250 mg/Kg body weight); PGPE orally, showed necrosis in portal area, dilation of central vein and sinusoids. the mentioned results are partially in line with **(Nadia *et al.*, 2011),** report, that drenching rats methanolic extract of ***Punnica. Granatum*** showed necrosis and inflammation. The limited necrotic lesion in portal area could be attributed to the protective effect of this dose, and the presence of the lesion in this might be due exposure to blood streams which carry high concentration of paracetamol, as this area represents the gate to the liver tissue. The current observation of necrosis was confined in portal area, no evidence of lesion dissemination among the whole hepatic tissue. Nevertheless, the part of inflammation occurrence is totally agreed with findings in group (E); which dosed 500mg/Kg body weight **PGPE.** Oxidative stress, represents one of responsible factors of hepatotoxicity. Paracetamol suggested to induce hepatotoxicity via initiation of oxidative stress status and generation of free radicals. Since **PG**, contains compounds classified as antioxidants, such as polyphenols and flavonoids, it may exhibit protection effect against the oxidative stress, free radicals' production, subsequently, minimize the incidence of liver injury.

Conclusion

The current study results revealed potent capacity of ***Punica Granatum*** peel ethanolic extract to prevent and diminish the hepatic toxicity originated from using high dose of paracetamol. 250 mg/kg dose is preferable over 500 mg/kg. the lower dose achieved protection and reversed the poisonous effect of paracetamol and had a quick response to liver injury revealed clearly in significant reduction of liver enzymes levels.

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