PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF *ZIZYPHUSSPINA CHRISTI* FRUIT AGONIST PARACETAMOL INDUCE HEPATOAND HAEMATOTOXICITY IN ADULT MALE RABBITS

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ABSTRACT

Zizyphusspina-christi has a common name in Iraq "Nabka",it has been used in traditional medicine.The present study was designed to elucidate the protective effect of aqueous extract of ziziphusspina Christi on hepato and haematotxicity induced by paracetamol in adult male rabbits. Eighteen adult male rabbits were divided randomly into 3 groups of 6 rabbits each and were treated for four weeks as follows:control group, second (T1) groupreceived orally (300 mg/kg B.W.) of paracetmoldailyand third (T2) Groupreceived orally (200 mg/kg B.W.) of crude extraction of zizyphyusspinadaily with (300 mg /kg B.W.) of paracetmoldaily. The results indicated that paracetamol administration induced hepato and haematotoxcity manifested by significant($P<0.05$) elevation in liver enzymes (AST and ALT) and reduction in total protein and albumin concentration with significant ($P<0.05$)decrease in RBC, Hb, PCV, MCH, MCHC and Neutrophiles whereas it increase in WBC, MCV, Lymphocyte and monocytes as a compared with control group.On the other hand, animals treatment with aqueous extract of zizyphusspinachristiin group T2 showed a significant ameliorated in biochemical andhaematologicalparameters change induce by paracetamol.It could be concluded that oral administration of Zizyphusextract have hepatoprotectiveand antianemic role agonist hepato and haematotoxicity induce by paracetamol.

INTRODUCTION

Paracetamol (Acetaminophen) is a common chemotherapeutic analgesic and antipyretic drug available as an over the counter prescription. It has only weak anti-inflammatory activity and belonging to the para-aminophenol class of the non-steroidal anti-inflammatory drugs (NSAIDs) (1). It is the active substance of phenacetin and is derived from coal tar (2). The word ofparacetamol is derive from the chemical names for the compound:

para-acetylaminophenol and N-acetyl-para-aminophenol (3).It is widely used for the headache, relief of mild to moderate pain and fever, it is a major component in numerous cold and flu remedies (4). Although, it is consider safe for human use at therapeutic doses, but when taken acute higher dose more than 1000 mg per single dose and more than 4000 mg per day for adults is related with significant hepatotoxicity(5). It's metabolized in the liver where its major metabolites include inactive glucuronidation and sulfation of paracetamol, which are excreted by the kidneys. Only a small amount of paracetamol is metabolized by the hepatic cytochrome P450 enzyme system (its CYP2E1 and CYP1A2 isoenzymes) which is formation toxic highly reactive metabolite N-acetyl p-benzoquinoneimine (NAPQI) (6). The NAPQI is reactive with glutathione and formation the glutathione conjugates. About 90% from the glutathione is exhausted following acetaminophen toxicity(7). Acetaminophen high dose leads to the accumulation of N-acetyl-p-benzoquinoneimine, which causes oxidative stress and glutathione depletion leads to cell injury and death (8). Excessive use of acetaminophen causes hepatotoxicity and analgesic nephropathy in the liver and kidney (9), and induces hepatotoxicity as an animal's models,(10). In addition to hepatotoxcity, however, it cause activation for coagulation system and haemolyticanaemia in patient after ingestion paracetmol(11).

Zizyphusspinachristi known as Christ's Thorn Jujube and it has a common name ‘‘Nabka”, and locally known as sidr, it is one of the plants commonly used in Iraqi folk medicine for the treatment of various diseases .it's a native plant that grows in warm temperature and subtropical regions especially in Middle East. Zizyphusspina-christi belongs to the family Rhamnaceae (12). The mature fruit is red to purplish-black and wrinkled, looking like a small date. The fruit has a single hard seed, similar to an olive stone. It is used as both a delicious fruit and an effective herbal remedy with therapeutic effects in various diseases (13). Zizyphus species are commonly used in alternative medicine for the treatment of various diseases such as digestive disorders, liver complaints, urinary troubles, weakness, loss of appetite, obesity, diabetes, bronchitis, fever, insomnia, pharyngitis, anemia, diarrhea, and skin infections (14).From previous studies showed the Zizyphusspina-christi have medicinal properties as antibacterial, antidiabetes, analgesic, antifungal, anti-hyperglycemic and antinociceptive activities (15). Chemical analysis of its fruit has shown the present flavonoids, alkaloids, triterpenoids, saponins, lipids, proteins, free sugar and mucilage (16). Another phytochemical studies on the different species of the genus Zizyphusrevealed its containcyclopeptide, alkaloids, flavonoids, sterols, tannins, and triterpenoidsaponins (17),(18). Many researcher documented the fruit extract of Z. spina Christi have

antitumor(19), CNS depressant(20), antioxidant and antifertility activities (21).Thus, the aims of this study were designed to determine whether the protective effect of aqueous extract of Ziziphusspinachristifruit on the liver and hematopoietic organs agonist paracetamol which induce hepato- and haematotoxicity in male Rabbits

MATERIAL AND METHODS

Plant: Zizyphusspina Christi fruits were purchased from Shatra market in April, 2015 andidentified and authenticated by Plant department of technical institute shatra. The fruit were washed, then their seed were separated from fruits and removed, the samples were shade dried at room temperature and finely powdered using mortar and pestle.

Preparation of Aqueous Fruit Extract

Zizyphusspinachristi fruits were dried and ground to powder,100 gms of the fruits powder was dissolved in 250 ml of water for 2 hours. The suspension was then heated at 60-65ºC for thirty min. The extract was collected separately and repeated the processes three times with the residual powder and collected the extract in each time. The extracts were collective and passed through fine cotton cloth. The filtrates were evaporated by rotavapor. A dark semisolid material was obtained and stored at 0-4ºC until use (22). A known amount of the residual extracts were dissolved in distilled water and administered orally to the rabbits via Gavage needle.

Experimental animal

Eighteenmale local rabbits (weighing between 1,5-2kg) were used this study. Animals were housed in iron cages in a conditioned room (22-25°C) with light/dark cycle of 12:12 hrs per day in animal house of the animal resources department in a technical institute in shatra. Animals had free access to water and standard pellet diet along the experimental.

Experimental design:

The animals (male rabbits) were divided randomly into (3 groups) 6 animals per group & were treated daily for 30 days as follows:

A- Control group: rabbits of this group received (5 ml/kg B.W.) of tap water by oral dosage using gavages needle.

B- T1 Group: rabbits of this group were received (300 mg/kg B.W.) of paracetamol solution once daily (5).

C- T2 Group: rabbits of this group were received (200 mg/kg B.W.) of crude extraction of Zizyphusspina (21) and after 30 min. received (300 mg /kg B.W.) of paracetamol solution daily (5).

Collection of Samples

After four weeks of treatment, the animals were anesthetized by intramuscular injection of (ketamine 30mg/Kg B.W &Xylazine 5mg/Kg B.W). Blood samples were collected by cardiac puncture, 5 ml of blood samples were collected from the heart andsome of the blood collected in the EDTA tubes used to study the hematological parameters and the remaining blood collected in test tubes without any anticoagulant stand for 30 min at 37˚C to allow coagulation (23). Serum was separated from coagulated blood sample by centrifugation at 3000 RPM for 15 min, and kept by freezing at -20˚C until used for measuring of the biochemistry assay.

Determination of hematological parameter

Red blood cells (RBC), packed cell volume (PCV), Hemoglobin (Hb), white blood cell count (WBC), differential WBC count were determined by standard methods. Packed cell volume (PCV) was determined by a microhematocrit method using a microhematocrit centrifuge. Hemoglobin concentration was determined by the Cyanomethemoglobin photometric method. The red cells (RBC) andwhite blood cells (WBC)were counted by using double improved Neubauer counting chamber, whereas Differential white blood cell count calculate by blood smear prepare and stained by Leishman's stain to derive the percentage of each type of the white blood cells.Erythrocyte indices including Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC) were calculated from values of RBC, PCV and Hb as follows equations 1, 2, 3 (24):

$$MCH = \frac{Hb \times 10}{RBCs} \quad (1)$$

$$MCV = \frac{PCV \times 10}{RBCs} \quad (2)$$

$$MCHC = \frac{Hb \times 100}{PCV} \quad (3)$$

Biochemical Assays:

Serum aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity were enzymatically determined by using standard assay (SYRBIO chemical-kitbased on the method of Reitman and Frankel in 1957 (25).Determination of Serum total proteins carried out by using Biuret method, Proteins form a violet color complex in present copper ions in alkaline solution(26).Albumin was measured in serum base on method performed by Doumasin 1971 (27). Albumin reacts with bromocresol green to yield green color.

Statistical analysis:

Result were expressed as Mean ± standard error. Statistical analysis of data was performed on the basis one- way analysis of variance (ANOVA) statistical SPSS software, version 21) followed by Dunnett‟s post hoc test was performed for inter-group comparison using the LSD.Values of $p<0.05$ were considered statistically significant(28).

RESULT

The result in table (1) shown that treatment of GroupT1by paracetamol daily for 30 days led to significant ($P<0.05$) increase in ALT, AST concentrations as a compared with control groups and decrease significantly ($P<0.05$) in total protein and albumin concentration as a compared with control group. Results also clarified that co-administration ofaqueous extract of Zizyphusspina-christi fruits and paracetoml significantly $p<0.05$decrease the change recorded in liver enzyme concentrations and enhanced total protein and albumin concentration near to a normal level.

Table (1) Effect of aqueous extract of Zizyphusspina-christi fruits on liver enzymes, total protein and albumin in male rabbits treated with paracetamol.

Animal group / Parameter	Control group	Group T1	Group T2
ALT (U/I)	14.12 ± 0.88^{c}	36.66 ± 1.01^{a}	19.24 ± 0.73^{b}
AST (U/I)	17.76 ± 0.52^{c}	40.50 ± 1.60^{a}	24.96 ± 0.81^{b}
TP (g/dl)	6.50 ± 0.10^{a}	3.92 ± 0.06^{c}	5.38 ± 0.20^{b}
Albumin (g/dl)	3.48 ± 0.11^{a}	2.62 ± 0.10^{b}	3.22 ± 0.07^{a}

-Values are expressed as mean ± SE, n = 6 each group
- Different lettersin the same raw refer to significant differences ($p<0.05$) vs. control.

The obtained resultsin Table (2) revealed a significant decrease ($p<0.05$)the erythrocyte values in group T1 which received paracetamol orally this observation is reflected by decline RBC,haemoglobin concentration, haematocrit, MCH, MCHC and elevated in MCV level when a compared with control group. On the other hand rabbit that treated with paracetamol plus aqueous extract of ZizyphusSpina-Christi L. fruits showed improve blood component (RBC, Hb, PCV and MCHC) as a compared with group T1 which received paracetamol.

Table (2) Effect of aqueous extract of ZizyphusSpina-Christi fruits on erythrocyte values in male rabbits treated with paracetamol.

Animal group / Parameter	Control group	Group T1	Group T2
RBC (10^6/mm^3)	5.93 ± 0.14^{a}	4.72 ± 0.16^{c}	5.40 ± 0.17^{b}
Hb (g/dl)	11.03 ± 0.27^{a}	8.24 ± 0.27^{c}	9.84 ± 0.19^{b}
PCV (%)	35.82 ± 0.18^{a}	31.08 ± 0.25179^{c}	33.84 ± 0.40^{b}
MCV (fl)	60.40 ± 1.20^{bc}	65.84 ± 1.28^{a}	62.66 ± 0.90^{ab}
MCH (Pg)	18.60 ± 0.24^{a}	17.45 ± 0.39^{bc}	18.22 ± 0.31^{ab}
MCHC (%)	30.79 ± 0.75^{a}	26.51 ± 0.57^{b}	29.07 ± 0.36^{a}

- Values are expressed as mean ± SE, n = 6 each group
- Different letters in the same raw refer to significant differences ($p<0.05$) vs. control.

Our results about white blood cell (WBC) and differential count in table (3) indicted the group-T1 which treated with paracetamol rabbits exhibited significant ($P<0.05$) increase in white blood cell (WBC),increase in lymphocyte and monocytes and decrease neutrophils when compared to control group.Results also clarified that co-administration ofaqueous extract of ZizyphusSpina-Christi fruits and paracetamol significantly$p<0.05$ elevation of leucocyte count as a compared with group T1,while no significant difference of differential count when compared with control group.

Table (3) Effect of aqueous extract of Zizyphusspina-christifruits on leucocyte and differential count male rabbits treated with paracetamol.

Parameter \ Animal Group	Control group	Group T1	Group T2
WBC $10^3/mm^3$	9.68 ± 0.23^c	$16.04 \pm 0..37^a$	10.98 ± 0.34^b
Lymphocytes	71.96 ± 0.72^b	79.66 ± 0.73^a	73.74 ± 0.96^b
Neutrophils	22.40 ± 0.72^a	12.90 ± 0.68^b	20.80 ± 0.68^a
Monocytes	4.14 ± 0.20^b	5.08 ± 0.29^a	4.36 ± 0.19^b

- Values are expressed as mean ± SE, n = 6 each group
- Different letters in the same raw refer to significant differences ($p<0.05$) vs. control.

DISCUSSION

Paracetamol is commonly used as an analgesic and antipyretic drug at therapeutic doses. However, the overdose of paracetamol cause hepatatoxicity and oxidative stress, the obtain result indicated chronic paracetamol consumption induces severe liver injury and liver necrosisas monitored by the elevation liver enzyme (AST and ALT) and reduction in total protein and albumin concentration in group T1 which received paracetamol may be paracetamol overdose caused formation reactive oxygen speciesand induce oxidative stress which led to hepatotoxcicity, also elevation may be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. Serum AST and ALT are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage (29). This result in agreement withRadosavljevićin 2010 who reported overdose of paracetamol produce oxidative stress caused severe liver injury with liver cell necrosis(30). Liver enzymes elevation may be a reflection of radical-mediated lipid peroxidation of liver cell membrane, this result similar with Reid in 2005 who observed paracetamol metabolic in liverby cytochrome p450 which produce a toxic metabolite, N-acetyl-p-benzoquinone imine(NAPQI) which caused depletes liver glutathione thereby inducing oxidative stress and binds to hepatic cell and mitochondrial proteins leading to hepatocellular necrosis and increased lipid peroxidation in hepatic tissues(31). Also NAPQI were binds to cysteine residues on proteins

to form acetaminophen adducts which excellent correlate of acetaminophen toxicity (32).It can be propose that this may be this binding led to protein miss folding –unfolding causing its reduction. therefore, free radicals produced after paracetamol treatment may mediated oxidation, proteolysis and degradation of albumin leading to its reduction (33). Also prolonged paracetamol consumption caused destruction of the hepatic cells and hepatic dysfunction led to impairs protein synthesis and decrease in the serum total protein, albumin and globulin concentration (34).Our result agree with previous study that animal exposure to paracetamol caused elevation in activity of ALT and AST and depression in total protein. (35).

The current study revealed that oral administration of aqueous extract of Zizyphusspina-christifruits in group T2have ameliorated effect agonist hepatotoxicity which induce by paracetamol, our result shown reduction liver enzymes AST and ALT in addition elevation total protein and albumin in group T2 when compared with group T1 which expose to paracetamol may be due to phytochemical compound in extract of Zizyphusspina-christiwhich act as antioxidant substance serve a inhibition free radicals-induced lipid peroxidation and suppression paracetamol toxicity. phytochemical analysis of Zizyphusspina-christi revealed present cyclopeptide alkaloids, flavonoids, sterols, tannins, and triterpenoidsaponins (18), therefor, this compound act antioxidant activity for scavengers free radical (14), This result agree with Yossef in 2011 who reportedZizyphusspina reduction the serum levels of liver enzymes and restored normal levels of endogenous antioxidants and it have protective effect and antioxidant activity agonist oxidative stress and hepatic toxicity(12).

Oral administration of paracetamol induced anemia indicated from result obtain shown decrease in the erythrocytic count, haemoglobin content and haematocrit value, MCH and MCHC in groupT1 which treated with paracetamol as a compared with control group. The reduction in erythrocyte value may be due to increase free radicals, reactive oxygen species, and peroxide radicals after paracetamol administration which lead to hemolysis anemia(36), also paracetamol caused hepatotixcity and impairs protein synthesis and decrease in the serum total protein, albumin and globulin concentration, therefore, insufficiency of protein synthesis that mainly induces decrease of essential amino acids and shortage of energy source of protein synthesis incorporated in hemoglobin production and anemia (36). Our result in accordance with previous study that paracetamol caused destruction RBC, and induce thrombocytopenia and haemolyticanaemia(37).

From result Zizyphusspina-christi have protective effect agonist haematotoxicity induce by parcetamol in group T2 which received Zizyphusspina-christi and paracetamol when compared with group T1 which treated with paracetamol may be due Zizyphusspina-christicontain phytochemical compound include peptide,cyclopeptide, alkaloids, balsams, carbohydrates, saponins, steroids, terpenes. polyphenol, flavonoids, sterols, tannins, betulinic acid, triterpenoidal and glycosides (38),(39).These compound are well known haemopoietic factors that have direct effect on the production of blood and antioxidant substance sever on inhibition free radical, prevent hemolytic anemia and improve blood compenent (40),(41).This result agree withPitchaiahin 2015who reported Zizyphus fruit have anti-anaemic activity by enhanced the red blood cell count and hemoglobin concentration(42).

In the current work, chronic administration of paracetamol inducedsignificant increase in total WBC, lymphocytes and monocytes may be due to oxidative stress which induce by paracetamol, (43), this oxidative stress caused elevation WBC count(44), Our result agree with Samuel in 2015 who reported paracetamol consumption reduced RBC count and PCV, and elevated WBC count (45). Co-adminstration of ZizyphusSpina-Christi L fruit and parcetamol shown decrease WBC as a compared with group T1 and restore differential count near to control group. It has been suggested Zizyphusspina-christi fruit have antioxidant compound act to inhibition oxidative stress and have antiflammatery activity led to return WBC count and differential count near to normal value(46).

It may conclude that paracetamol induce hepato and haematotoxicity and Zizyphus extract have hepatoprotective, antioxidant and antianemicactivity agonist hepato-and haematotoxicity induce by paracetamol.

الدور الوقائي للمستخلص المائي لفاكهة نبات السدر zizyphusspinachristi ضد البراستيمول المستحدث التسمم الكبدي والدموي في ذكور الارانب البالغة

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الخلاصة

نبات السدر Zizyphusspina-christi يمتلك اسم شائع في العراق "النبق " والذي استخدم في الطب الشعبي. صممت هذه الدراسة لتوضيح الدور الوقائي للمستخلص المائي لفاكهة نبات السدر ضد التسمم الكبدي والدموي الذي يحدثهالبراستيول في ذكور الارانب البالغة . استخدم ثمانية عشر من ذكور الارانب البالغة قسمت عشوائيا الى ثلاثة مجاميع ست ارانب لكل مجموعة وعولجت لمدة اربع اسابيع وكالاتي :الاولى مجموعة السيطرة ، الثانية مجموعة T1 تتناول فمويا 300 ملغرام /كيلو غرام من وزن الجسم من البراستيمول يوميا والثالثة مجموعة T2 تتناول فمويا 200 ملغرام/ كيلو غرام من وزن

الجسم من المستخلص المائي لفاكهة نبات السدر يوميا مع 300 ملغرام / كيلو غرام من وزن الجسم من البراستيمول يوميا. اكدت النتائج ان اعطاء البراستيمول استحدث التسمم الكبدي والدموي الذي تصف بالارتفاع المعنوي (P<0.05)في انزيمات الكبد (ASTو ALT) وانخفاض في تركيز البروتين الكلي والالبومين مع نقصان معنوي (P<0.05)في عدد كريات الدم الحمر ،خضاب الدم ، معدل حجم كرات الدم المرصوص ، متوسط هيموغلوبين الكرية ومتوسط تركيز هيموغلوبين الكرية والعدلات بينما ارتفاع في متوسط حجم الكرية ،عدد كريات الدم البيض ،الخلايا اللمفاوية وخلايا وحيدة النواة . من الناحية الاخرى ، الحيوانات التي عولجت بالمستخلص المائي لثمار نبات السدرZiziphusspina Christi في مجموعة T2 لوحظ تحسن معنوي في تغيرات القيم الكيموحيوية والدموية التياستحدثة بواسطة البراستيول . نستنتج من ذلك ان اعطاء مستخلص نبات السدر يمتلك دور وقاية الكبد ومضاد فقر الدم ضدالتسمم الكبدي والدموي الذي استحدث بواسطة البراستيمول.

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