Impact of *Rute chalepensis* Ethanol Extract in Ochratoxin Treated Male Rats: A Biochemical and Histopathological Study

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ABSTRACT--- ***The objective of this study was to explore the ameliorating effects of Ruta chalepensis ethanol extract on some serum biochemical markers such as, alanine aminotransferase; ALT, aspartate aminotransferase; AST, urea and creatinine concentrations, and histopathological changes in liver and kidney tissues of Ochratoxin treated male rats. Fifty adult male rats were randomly allocated in five equal groups. Male rats were daily administered, for 42 days, with drinking water (negative control; G1), whereas other groups were orally administered with single dose of Ochratoxin (1 mg/kg, bw). G2 was untreated and served as positive control, while G3, G4 and G5 groups were daily treated, for 42 days, with 200, 300 and 450 mg/kg bw, of Ruta chalepensis ethanol extract, respectively. Fasting blood samples were collected by cardiac puncture at the end of the experiment, for assessment of serum ALT, AST, urea, total protein, and creatinine concentrations. Samples from livers and kidneys were taken for histopathological examination. In spite of the deviations of serum ALT, AST, urea, creatinine and total protein concentrations due to deleterious effect of Ochratoxin (G2 group) compared with control (G1), treated of male rats with Ruta chalepensis ethanol extract (G3, G4, and G5) returned them to nearly normal range. These results were in compatible with the regenerating effect of Ruta chalepensis ethanol extract which have been seen in liver and kidney tissues, where they showed necrotic and degenerative changes due to Ochratoxin toxicity. In conclusion, Ruta chalepensis ethanol extract has an efficient role as antitoxin and/or antioxidant agent.***

Keywords-- Ochratoxin, Ruta chalepensis, ALT, AST, urea, creatinine, liver, kidney

1. INTRODUCTION

Medicinal plants have been used as alternative medicine due to its content of various substances that can be used as treatment of chronic as well as infectious diseases. One of traditiona medicine is *Ruta chalepensis* L. which belongs to the family *Rutaceae*. Its flowers are cymes with 4-5 sepals, 4-5 petals, 8-10 stames and a superior ovary. *Ruta chalepensis* is a perennial herb that widely distributed in the Mediterranean area[1] and also was introduced in America after the Spanish conquest[2]. It is one of the most frequently used plants for medicinal purposes[3,4]. Oil glands, that are principally present in leaves, give its strong deterrent odors[5,6]. *Ruta chalepensis* pharmacological properties, attributed to the high content of alkaloids[7-9], such as furocoumarins[7], coumarins[9] and furoquinolone alkaloids[10], flavonoids, phenols, amino acids and saponins found in the leaves and stems of the plant[11]. *Ruta Chalepensis* is used, in the traditional medicine for the treatment of various disorders, as analgesic and antipyretic and for the treatment of rheumatism and mental disorders[1]. Also it have emmenagogue, abortificient, antihelmintic and spasmolytic effects[12] as well as its potency as anti-infamatory[13], anti-helminthic[14], antifungal[15], antifertility, anticonvulsant and sedative[16,17]. In children, infused *Ruta chalepensis* leave extract has been used for treatment of convulsion and other nervous disorders. In Africa, the aqueous decoction of the leaves is used for the treatment of fever[4]. More than fifty chemical composition of *Ruta chalepensis* essential oil were studied by many research teams in Iran[18], Greece[19], Turkey[20] and India[21,22].

Ochratoxin-A (OTA) is a mycotoxin produced mainly by *Aspergillus* molds (such as *Aspergillus ochraceus*) and *Penicillium* molds (such as *Penicillium verrucosum*). It is toxic, mainly to the kidney and the hepatic tissues of humans and livestock, and is suggested to be related to Balkan human nephropathy, which is an endemic disease in Balkan countries. Its carcinogenicity has been demonstrated in animals but there have not been enough evidences in humans[23,24]. The absorbed Ochratoxin into the body is distributed at a high concentration in the kidneys. Its side effects attributed to the inhibition of the synthesis of proteins, DNA and RNA in the cells. It shows renal toxicity by inhibiting various enzyme activities in the kidneys[25]. Ruminants are resist the toxic effects of Ochratoxin because the rumen is highly capable of degrading and converting ochratoxin-A into phenylalanine and ochratoxin-α, which has low toxic effects, therefore this type of poisoning is not very much likely to occur; however, calves with immature rumen sensitive to Ochratoxin.

Therefore, the present study was designed to investigate the biochemical deviations and hepatotoxic and nephrotoxic effects of Ochratoxin as well as the ameliorating role of *Ruta chalepensis* ethanolic extract in males rats, as a model of mammals.

2. MATERIALS AND METHODS

2.1.Experimental animals:

Fifty adult male rats (aged 10 weeks and weighed 155±5.6 g) were randomly allocated in five equal groups and treated as follow: G1 group (negative control) was orally administered with drinking water daily for 42 days, G2 group (positive control) was orally administered with single dose of Ochratoxin (1 mg/kg, bw), while G3, G4 and G5 groups were administered with single dose of Ochratoxin (1 mg/kg, bw) and treated, for 42 days, with 200, 300 and 450 mg/kg bw, of *Ruta chalepensis* ethanol extract, respectively. Fasting blood samples were collected by cardiac puncture at the end of the experiment, for assessment of serum ALT, AST, urea, total protein, and creatinine concentrations. Samples from livers and kidneys were taken and fixed in 10% formalin neutral buffer solution for histopathological examination.

2.2.Ochratoxins:

Ochratoxins: was provided by Sigma Aldrich Company, UK.

2.3.Preparation of ethanolic extracts of *Ruta chalepensis*:

The *Rute chalepensis* plant was classified by the biologist Dr. Yass Khudhair Abbas, College of Sciences, Di-Qar University. *Rute chalepensis* leaves were powdered using electrical grinder. Twenty grams were taken and extracted with 70% ethanol in soxhlet apparatus within 24 hours. Then, the extract was placed in Petri dishes and put in the oven for dryness at 40 °C within 48 hours. The resulted dry extract was stored at -20 °C until use[26].

2.4.Serum preparation:

Blood was collected in test tubes with cap and allowed for 20 minutes to clot, and then serum was separated after centrifugation of collected blood at 4000 rpm for 10 minutes[27]. Each serum sample was divided nearly into 6 divisions and put in eppendroff tubes (0.5 ml) and kept at -20 °C until assessment of the biochemical parameters.

2.5.Biochemical assay:

Urea and Creatinine concentrations were assessed using kits of spectrophotometer provided by US bio, USA, whereas ALT and AST concentrations were assessed using ELISA kits provided by US bio, USA.

2.6.Microscopic examination:

Liver and kidney tissue sections were processed and stained with Haematoxylin and Eosin stain according to Luna[28] and examined under light microscope.

2.7.Statistical analysis:

Results were expressed as mean ± standard deviation. Comparisons between groups were performed using one way analysis of variance (ANOVA1) and newman- keuls. Differences were considered to be significant at the level of $P<0.05$. Statistical analysis was carried out using the GraphPad Prism (SAS Institute, Inc., USA).

3. RESULTS

3.1.Serum biochemical markers:

The results illustrated in figure (1) showed significant elevation ($p<0.05$) of serum urea (A), creatinine (B), AST (C) and ALT (D) concentrations in Ochratoxin supplemented group (G2) compared with control (G1), whereas those treated with *Ruta chalepensis* ethanol extract (G3, G4 and G5) revealed gradual decrease of biochemical parameters, mentioned above, in a pattern of dose dependent, where G5 showed the more significant decrease ($p<0.05$) among treated groups, so that the means of G5 group reached the normal levels that have been recorded by control group.

3.2.Histopathological changes:

Histological section, obtained from control male rat kidneys, revealed the presence of normal architecture of glomeruli and renal convoluted tubules, whereas those obtained from Ochratoxin supplemented male rats revealed degenerative and necrotic changes in the epithelial cells which lining convoluted tubules, with mild distraction and atrophy of glomeruli,

dilation in renal tubules and presence of sever hemorrhage in renal tissue. Treatment with different doses of *Ruta chalepensis* ethanol extract showed different gradual degrees of proliferative improvement (figure 2), where G3 group male rats revealed presence of several glomeruli appeared normal in structure after proliferation and few others appeared atrophied with the presence of tubular bisophylium (regeneration), moreover, there is normal proliferative events in the epithelial cells which lining the tubules, and the glomeruli showed high cellularity, enlarge size, and sever hemorrhage in the renal tissue. G4 male rats revealed the presence of proliferation, large and circled glomeruli, with the presence of tubular basophiles (regeneration) in the renal convoluted tubules, and there are mild hemorrhage in renal tissue. G5 male rats revealed the presence of proliferation, large and circled glomeruli, with the presence of tubular basophiles (regeneration) in the renal convoluted tubules, and there is no hemorrhage in renal tissue.

Histological sections obtained from livers of male rats of control group shows normal radial arrange around central vein and hepatocyte showed with hexagonal shape with acidophilic cytoplasmic and central permanent nuclei in liver tissue, whereas in ochratoxins supplemented male rats (G2) explained extensive necrosis in the hepatic tissue, loss of radially arrangement of hepatic cords around the central vein, congestion and hyperaplasia of bile ducts. G3 group revealed some of cell showed binucleated, degeneration of hepatocytes, normal central vein, present of radial arrangement of hepatocyte and mild proliferation in liver tissue. In G4 and G5 groups, histological sections showed normal radial arrangement of hepatocyte, clear regeneration of hepatocyte which showed vacuolated and binucleated, mild dilation of sinusoids in liver tissues.

4. DISCUSSION

Except G2 group, which has been supplemented with Ochratoxin, all other male rats showed normal activity and body health throughout the experimental period. This finding indicated that treatment of male rats for 42 days with the three given doses of *Ruta chalepensis* ethanol extract (200, 300 and 450 mg/kg, bw) in combination with single dose supplementation of Ochratoxin (1 mg/kg, bw) has ameliorating effect on general body health of male rats. Ochratoxin supplemented group showed dullness of male rats. This change could attributed to the toxic effect of Ochratoxin, since it is a type of mycotoxins produced by some *Aspergillus* species, where its harmful toxic effects have been proved by many researchers[29]. Ochratoxin A is the most prevalent and relevant fungal toxin of this group, while Ochratoxins B and C are of lesser importance. Ochratoxin A is possibly a human carcinogen and is of special interest as it can be accumulated in the meat of animals, therefore exposure to Ochratoxins through diet can cause acute toxicity in mammalian kidneys[29]. On the other hand it has been postulated that *Ruta chalepensis* has impact to relieve the pain associated with the physical symptoms of complaints such as gout, rheumatism, and sciatica. Along with alleviating the uncomfortable effects of gas and colic, rue was thought to expel worms from the body. Throughout the years of its use, rue has been used to promote menstruation. It is also used as a digestive tonic and to stimulate the appetite[32]. The ameliorating role of *Ruta chalepensis* ethanol extract, reported in the present study, could attributed to its beneficial compounds found in its oil alkaloids (acridone-, quinolone-, furoquinolone- and quaternary furoquinolines), as well as coumarins (furano-) and essential oils[30]. It has been found that a beneficial tea or infusion can be sipped to calm the nerves, increase the appetite or to ease croupy symptoms. Also its oil made with rue can be applied to areas suffering from sciatica or to ease chest congestion. Homeopathic preparations are available to treat arthritis and joint pain[33]. The essential oils from aerial parts of *Ruta chalepensis* plants harvested at different stages of growth in northern India contained 19 components, representing 85.4–93.3% of the oil, where the major components of oil are 2-undecanone, 2-nonanone, 2-nonyl-acetate and 2-dodecanone, whereas those isolated from the roots are the furoquinolin, kokusaginin, skimmianin and graveolin, acridone and chaloridon[31], whereas from dried aerial parts, the major compounds isolated include the furoquinolin alkaloids kokusaginin, skimmianin, graveolin, γ-fagarin and dictamnin, the acridone alkaloid arborinin, and the (furano-)coumarins bergapten (or 5-methoxypsoralen) and chalepensin[34,35].

In the present study, we tried to find out how *Ruta chalepensis* ethanol extract could improves the pharmacological intervention in healthy adult male rats against Ochratoxins. The presenting clinical, biochemical and histological findings revealed that *Ruta chalepensis* ethanol extract administration had benefit improvement of antioxidants activity in the toxic animal model, by reducing the complications of normal metabolic outcomes.

5. REFERENCES

[1] Iauk L., Mangano K., Rapisarda A., Ragusa S., Maiolino L., Musumeci R., Costanzo R., Serra A., 2004: Berberis aetnensis C. persl. Extracts: antimicrobial properties and interaction with ciprofloxacin. *J. Ethno pharmacology.*, , *90*, 267-272.

[2] Zeichen R., Rey A., Arganara E., Bindstein E. 2000.: Perinatal toxicology of Ruta chalepensis (Rutaceae) in mice. *J. Ethno pharmacology, 69*(2), 93-8.

[3] Arenas P., G.P. Savitry. 1994 La ruda: *Ruta chalepensis (L.)* Rutaceae. Dominguezia. 11:7–25.

[4] AlSaid M.S., Tariq M., AlYahya M.A., Rafatullah S., Ginnawi O.T., Ageel A.M. 1990.: The role of ethno pharmacology in drug development. *J. Ethno pharmacology, 28*,305-312.

[5] Cabrera A., and Zardini E. 1978. *Manual de la flora de los Alrededores de Beunos Aires (Ed) Acme Buenos Aires*, Argentina, , p. 234.
[6] Trease G.E. and Evans W.Ch. 1980. Pharmacognosy, Baillière Tindal Press, London, , p. 488.
[7] Günaydin K. and Savci S. 2005. Phytochemical studies on *Ruta chalepensİs* (LAM.) lamarck *Nat. Prod. Res.*, , 19, 203-210.
[8] Ulubelen, A., B. Terem, E. Tuzlaci, K. F. Cheng, and Y. C. Kong. 1986. Alkaloids and coumarins from *Ruta chalepensis*. Phytochemistry 25: 2692 2693.
[9] Ulubelen, A. and H. Guner. 1988. Isolation of dehydromoskachan C from Ruta chalepensis var. Latifolia. J. Nat. Prod. 51: 1012-1013.
[10] Mohr N., Budzikiewicz H., EI-Tawil B. A. H. and EI-Beih F. K. A., 1982. Further furoquinolone alkaloids from *Ruta chalepensis*. Phytochemistry, ,21 (7), 1838-1839.
[11] Hnatyszyn O,. Arenas P., Moreno A.R., Rondina R., Coussio J.D. 1974. *Plantas Revista de la Sociedad Cientifica*, , p. 23.
[12] Di-Stasi L.C., Hiruma C.A., Guimaraes C.M. 1994. Medical plants popularly used in Brazilian Amazon. *Fitoterapia*, , *65*, 529–540.
[13] Atta A. H., Alkofahi A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. Journal of Ethnopharmacology. 1998;60(2):117–124.
[14] Calzada F., YépezMulia L., Aguilar A. 2006.: In vitro susceptibility of Entamoeba histolytica and Giardia lamblia to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders.. *J. Ethno pharmacology*, , *108*, 367-370.
[15] Ali-Shtayeh M.S., Abu-Ghdeib S.I. 1999.: Antifungal activity of plant extracts against dermatophytes. *Mycoses*, , *42*, 665-672.
[16] Ulubenlen A., Ertugrul L., Birman H., Yigit R., Erseven G., Olgac V. 1993.: Antifungal activity of plant extracts against dermatophytes. *Phytotherapy Res., 8*, 233-236.
[17] Aguilar-Santamaria, L. and Tortoriello, J. Anticonvulsant and sedative effects of crude extracts of Ternstroemia pringlei and *Ruta chalepensis*. Phytotherapy Research 1996; 10: 531–533.
[18] Rustaiyan A, Khossravi M, Sultani-Lotfabadi F, Yari M, Masoudi S, Monfared A (2002). Constituents of the essential oil of *Ruta chalepensis L*. from Iran. J. Essent. Oil Res., 14: 378-379.
[19] Tzakou O.*, Couladis M. 2001. The essential oil of Micromeria graeca (L.) Bentham et Reichenb. growing in Greece. Flav. Fragr. J., 16, 107-109.
[20] Baser, K.H.C., T. Ozek and S.H. Beis, 1996. Constituents of the essential oil of *Ruta chalepensis L*. from Turkey. J. Essent. Oil Res.,8: 413-414.
[21] Bagchi G.D. Dwivedi P.D., Mandal S., Naqvi A.A., Kumar S. 2003.: Essential oil constituents of Ruta chalepensis plants grown in India . *Indian Perfumer, 47*, 39-41.
[22] Bagchi, G.D., P.D. Dwivedi, A. Singh, F. Haider and A.A. Naqvi, 2003. Variations in essential oil constituents at different growth stages of *Ruta chalepensis* on cultivation at north Indian plains. J. Essent. Oil Res., 15: 263-264.
[23] Food and Agricultural Materials Inspection Center, Association of Feed Analysis Methods.2009. Methods of Analysis in Feeds and Feed Additives.
[24] Japanese Society for Food Hygiene and Safety. 2010. Encyclopedia of Food Safety.
[25] Braunberg R.C. *et al*. 1994: Nat. Toxins, 2, 124.
[26] Harborne 1999. Phytochemical dictionary: Handbook of bioactive compounds from plants 2nd (Ed).
[27] Laessig R.H. Westgard, J.O. and Carey R.N.1976. Assessment of a serum separator device for obtaining serum specimens suitable for clinical analyses. Clin Chem. 22:235–239.
[28] Luna L.G. 1968. Manual of histologic staining methods for the armed force institute of Toronto, London, pathology 3rd . Mc Graw Hill book Company , NY, 12-31.
[29] Keeper-Goodman T. and Scott P.M. 1989 :Risk assessment of the mycotoxin Ochratoxin. Biomed. Environ. Sci. ;2:179–248.
[30] Baerts M. and Lehmann J. 2010. *Ruta chalepensis*. [Internet] Prelude Medicinal Plants Database. Metafro-Infosys, Royal Museum for Central Africa, Tervuren, Belgium http://www.metafro.be/prelude. Accessed August 2010.
[31] Gedif T. and Hahn H.J. 2003. The use of medicinal plants in self-care in rural central Ethiopia. Journal of Ethno pharmacology 87: 155–161.
[32] Kloos H. Tekle A. Yohannes L. Yosef, A. & Lemma A. 1978. Preliminary studies of traditional medicinal plants in nineteen markets in Ethiopia: use patterns and public health aspects. Ethiopian Medical Journal 16: 33–43.
[33] Van Wyk B.E. Van Oudtshoorn B. Gericke N. 1997. Medicinal plants of South Africa. Briza Publications, Pretoria, South Africa. 304 pp.

[34] Yineger H. Kelbessa E. Bekele T. Lulekal E. 2007. Ethnoveterinary medicinal plants at Bale Mountains national park, Ethiopia. Journal of Ethnopharmacology 112: 55–70.

[35] Enis Ben Bnina [a], Saoussen Hammami [a], Majda Daamii-remadi [b], Hichem Ben Jannet, Zine Mighri [a] (2010):Chemical Composition And Antimicrobial Effects Of Tunisian *Ruta Chalepensis* L. Essential Oils, Journal de la Société Chimique de Tunisie,1-9.

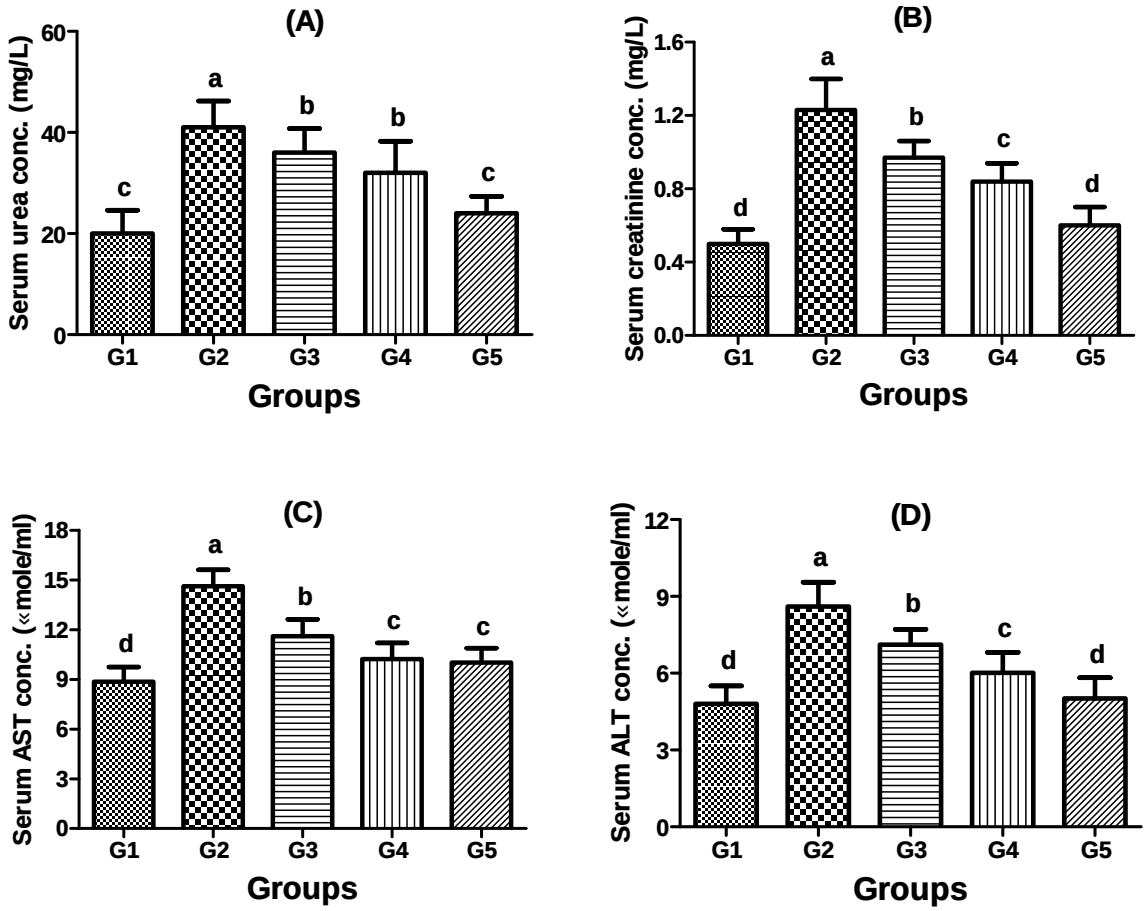

Figure 1: Serum urea (A), creatinine (B), AST (C) and ALT (D) concentrations in Ochatoxin toxic male rats treated with *Ruta chalepensis* ethanolic extract.

Values presented as M±SD.

Different letters denote significant difference ($p<0.05$) among exoerimental groups.

G1 (negative control): 10 male rats were orally administered with drinking water daily for 42 days.

G2 (positive control): 10 male rats were orally administered with single dose of Ochratoxin (1 mg/kg, bw).

G3: 10 male rats were orally administered with single dose of Ochratoxin (1 mg/kg, bw) and treated, for 42 days, with 200 mg/kg bw, of *Ruta chalepensis* ethanol extract.

G4: 10 male rats were orally administered with single dose of Ochratoxin (1 mg/kg, bw) and treated, for 42 days, with 300 mg/kg bw, of *Ruta chalepensis* ethanol extract.

G5: 10 male rats were orally administered with single dose of Ochratoxin (1 mg/kg, bw) and treated, for 42 days, with 450 mg/kg bw, of *Ruta chalepensis* ethanol extract.

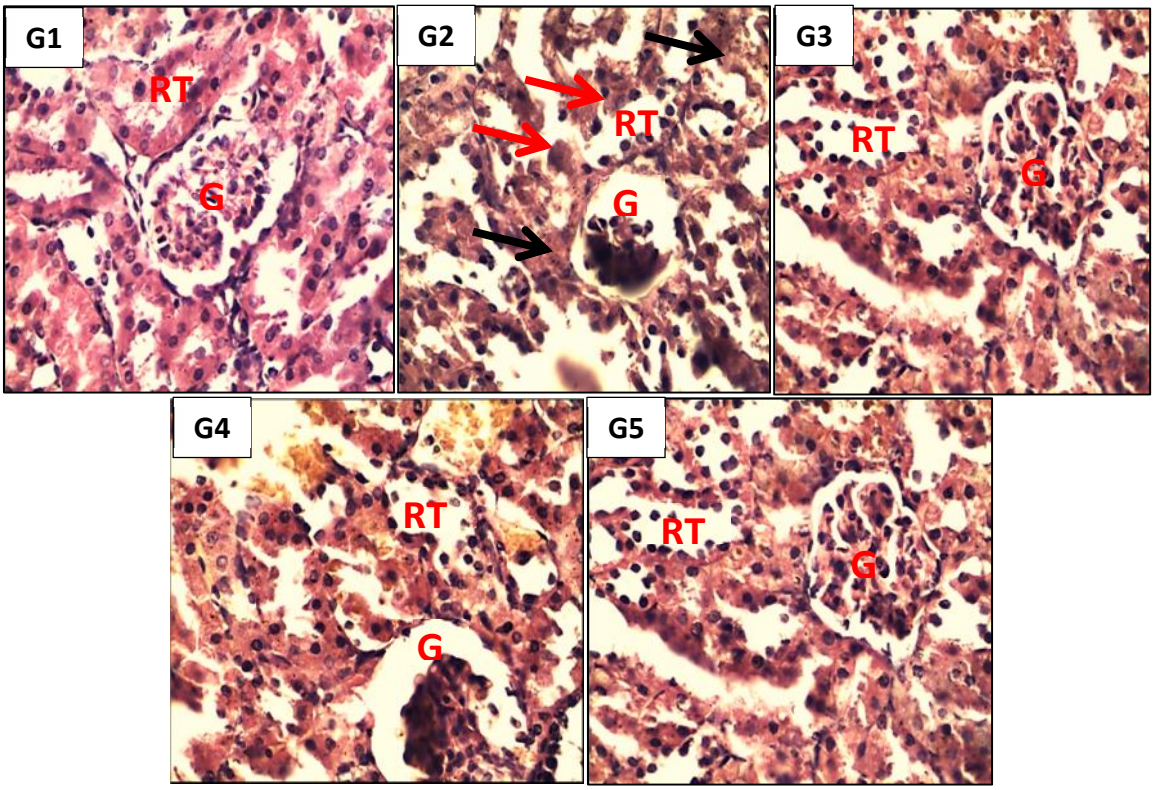

Figure 2: Histological sections of kidneys obtained from male rats showed normal architecture of glomeruli (G) and renal tubules (RT) in control group (G1), whereas males treated with Ochratoxin (G2 group) showed degenerative (red arrows) and necrotic (black arrows) changes in the epithelial cells which lining the convoluted tubules (RT), with mild distraction and atrophy of glomeruli (G) and dilation in renal tubules with the presence of sever hemorrhage in renal tissue. Ochratoxin toxic male rats treated with 200 (G3), 300 (G4), and 450 (G5) mg of *Ruta chalepensis* ethanol extract/ kg of body weight revealed gradual degrees of proliferative and regenerative event in both glomeruli and renal tubules. H&E 400x.

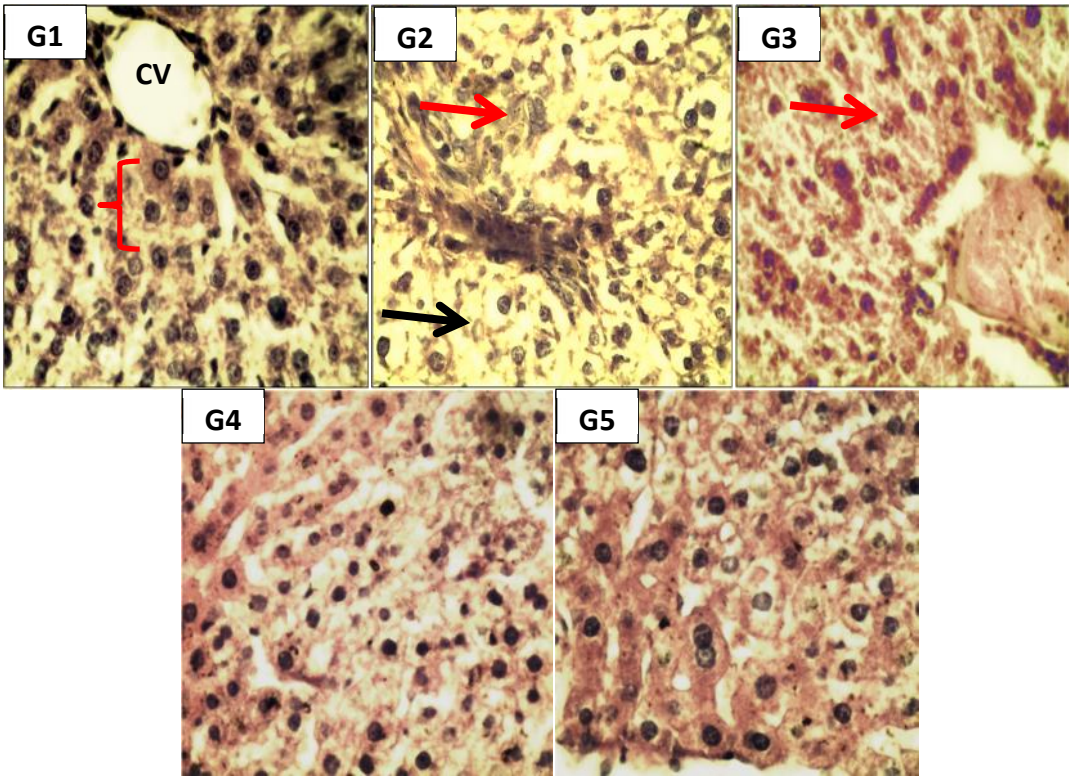

Figure 3: Histological sections of livers obtained from male rats showed normal architecture of central vein (CV) and hepatic cords (red curve) in control group (G1), whereas males treated with Ochratoxin (G2 group) showed degenerative (red arrows) and necrotic (black arrows) changes in the hepatocytes with disarrangement of hepatic cords. Ochratoxin toxic male rats treated with 200 (G3), 300 (G4), and 450 (G5) mg of *Ruta chalepensis* ethanol extract/ kg of body weight revealed gradual proliferative and regenerative changes with reference to that G5 group revealed higher degree of regeneration. H&E 400x.