Trop J Nat Prod Res, April 2021; 5(4):749-752

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Curative Effect of Aqueous Seed Extract of *Citrus paradisi* against Carbon Tetrachloride-Induced Nephrotoxicity in Wistar Rats

Olagunju J. Abayomi[1], Akinola O. Oyewole[2]*, Joseph A. Olagunju[1], Olusegun O. Akinola[2]*, Omolola E. Omotosho[2]

[1]*Department of Medical Biochemistry, Lagos State University College of Medicine, Ikeja-Lagos, Nigeria*
[2]*Department of Biochemistry, Covenant University, P.M.B 1023, Km 10, Idiroko road, Canaan land,Ota, Ogun State, Nigeria*

ARTICLE INFO

Article history:
Received 03 November 2020
Revised 09 April 2021
Accepted 15 April 2021
Published online 03 May 2021

ABSTRACT

Carbon tetrachloride (CCl_4) is a possible nephrotoxin that leads to grievous and harmful chemical toxicity. *C.paradisi* contains phytochemicals,vitamins and flavonoids which possesses strong anti-inflammatory, and antioxidant properties. This study investigated the serum electrolytes levels and the efficacy of *C. paradisi* to ameliorate the effect of carbon tetrachloride-induced nephrotoxicity in Wistar rats. The aqueous seed extract of *C. paradisi* was administered at a dose of 125-500 mg/kg body weight. Thirty albino rats were assigned into 6 groups of 5 rats in each. Group I was the 1ml/kg normal saline while group II was intraperitoneally treated with 3 mL/kg of CCl_4 1 hour before oral treatment (*p.o.*) with 10 mL/kg of normal saline while groups III-VI rats were intraperitoneally treated with 3 mL/kg 30% CCl_4 1 hour before oral treatment with 10 mg/kg of ascorbic acid. Groups III-VI were administered with 125 mg/kg, 250 mg/kg, and 500 mg/kg of aqueous seed extract of *C.paradisi*. Results shows an increase in all the serum electrolytes except in serum phosphate as a result of contamination. The result insinuated that kidney damage was induced in the rats with ascorbic acid attenuating the effect of carbon tetrachloride at each dose. The treatment showed that aqueous seed extract of *C. paradisi* controls an increase in serum electrolytes.

Keywords: Nephrotoxicity, Carbon tetrachloride, Serum electrolytes, *Citrus paradisi*, Toxicology.

Introduction

C. paradisi, popularly known as grapefruit, is widely grown in most nations and its height is about 3-5 metres with bright yellow colorations[1]. Phytochemicals, flavonoids and vitamins are common features in grapefruit with powerful anti-inflammatory properties.[2-5] They are abundant in minerals and secondary metabolites which defends the body against diseases. The major bioflavonoid in grapefruit is naringen that gives grapefruit juice a bitter taste.[6-7] Naringen possesses anti-carcinogenic activity[6] and blockage of precise cytochrome P450 enzymes such as CYPIA2.[8]

Carbon tetrachloride (CCl_4) is commonly known as tetrachloromethane, Halon-104, and Refrigerant-10.[9] Carbon tetrachloride is particularly potent used in the generation of experimental models in oxidative stress in many pathophysiological occurrences.[9,10] Numerous researches have insinuated that carbon tetrachloride is the most formidable needed during the activation reactive oxygen species (ROS) in different tissues.[11] Due to the administration of carbon tetrachloride, superoxide anion, hydrogen peroxide and superoxide anion are produced because of oxidative stress leading to the generation of ROS.[12] It is disclosed that carbon tetra chloride-induced oxidative stress steers nephrotoxicity leading to several uncontrolled disorders causing general kidney failure.[13,14]

This situation that can enhance mortality and morbidity arising from disease conditions traceable to cellular oxidative stress calls for the search for a solution in substances that could attenuate oxidative stress. Such research had over the years been extended to natural products as a result of certain toxicities associated with synthetic ones. Cell proliferation, maturation, migration and adhesion helps cytokines to play a crucial role during cell immunity. During the upregulation of inflammation cytokines IL-1β, IL-2, IL-6, and TNFα are activated by the production of macrophages.[15] Several studies have be done to find out the potentials of herbal remedies against CCl_4 in order to improve antioxidant enzymes such as superoxide dismutase, catalase, enhancing the reduced glutathione content. Medicinal plants are playing important roles against different types of diseases, and have shown their immense curative exploits.[16] Plant extracts that contains natural antioxidants have shown beneficial ameliorative capabilities against damages generated by chemicals.[17] Several plants having profound curative usefulness.[18,19] The increasing propensity for the effectual treatment of oxidative stress-based diseases has inspired researchers to harness the antioxidant potential of therapeutic plants and plant-based active constituents.[20] The present study is aimed at evaluating the nephrotoxic property of aqueous seed extract of *C. paradisi* by investigating its effects on serum electrolytes at the oral dose of 125-500 mg/kg/day of the extract on the nephrotoxic features in Wistar rats.

Materials and Methods

All reagents and chemicals include diethyl ether, chloroform, and carbon tetra-chloride used during this work were of grade analytically. They were gotten from different sources such as BDH Chemicals, Poole, England, Sigma Chemical Company, Mo., USA.

Collection of plant material

Dried seeds of *C.paradisi* were collected from a cultivated farmland within Odorasanyi, Ijebu-Igbo,Ogun State, Nigeria in February 2015.

*Corresponding author. E mail: akinolasegun481@yahoo.com
Tel: +2348169157828

Citation: Abayomi OJ, Oyewole AO, Olagunju JA, Akinola OO, Omotosho OE. Curative Effect of Aqueous Seed Extract of *Citrus Paradisi* against Carbon Tetrachloride-Induced Nephrotoxicity in Wistar Rats. Trop J Nat Prod Res. 2021; 5(4):749-752. doi.org/10.26538/tjnpr/v5i4.25

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

For botanical identification and voucher specimen referencing was done at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria (Voucher specimen number: FHI 110359). Plant auhentication was done by a botanist in the Herbarium, Botany and Microbiology Department, the University of Lagos, Akoka, Lagos State.

Animals
Both sexes of Wistar rats were used weighing between 150-250g were procured from the Animal Care Section of the College of Medicine, University of Lagos. Rats were kept at an excellent state of 12hr light followed with 12 hours of darkness with a relative room temperature of about 25 and 26^{o}C and humidity of 65 ± 5%. Before experimentation, the animals were allowed to grow and acclimatized within a time frame of three weeks. They were housed in clean plastic cages with sawdust beddings and with a constant supply of food and water. The study was by following the ethical directives (2010/63/EU) for animal handling proposed by the European parliament and was approved by the Department of Biochemistry, Animal Ethical Committee, Lagos State University, Ojo, Lagos, Nigeria.

Preparation of aqueous seed extract of C. paradisi
Matured, ripe grapefruits (*C. paradisi*) were sliced into pieces and the seeds were mechanically removed. Seeds were sufficiently cleansed with distilled water several times at room temperature. The washed seeds were drained and water films adhered to the seeds were blotted out with the aid of a kitchen towel. The seeds were completely dried in an oven set at 45^{0}C and 300 g of the dried seeds were reduced into a powdery form. The ground seeds were soaked in distilled water at 4^{0}C for 48 hours while a Whatman No.1 was used to get the filtrate. A dark yellowish solid crude mass was gotten when the filtrate was evaporated conserved in a desiccator until it was ready for use.
Experimental Design and Grouping of Animals

Induction of carbon tetrachloride-induced nephrotoxicity
The method of drug-induced renal toxicity was used which was done using CCl_4 of about 30% diffused in olive oil according to Lu *et al*.[24] Animals were injected intraperitoneally (i.p.) by carbon tetrachloride about 30% in olive oil at the range of 3 ml/kg body weight to induce nephrotoxicity followed by appropriate treatments.

Grouping of animals and oral treatment with aqueous seed extract C. paradisi
Thirty Wistar rats were assigned into 6 groups of 5 rats each (I-VI). The differences in their weight did not exceed 20%. The group I was the control (1 mL/kg of 0.9% normal saline) while the group II rats were intraperitoneally treated with 3 mL/kg of 30% CCl_4 1 hour before oral treatment with 10 mL/kg of normal saline, while groups III-VI rats were treated with 3 mL/kg 30% CCl_4 1 hour before oral treatment (p.o.) with 10 mg/kg of ascorbic acid. The groups III-IV were administered with 125 mg/kg, 250 mg/kg and 500 mg/kg of aqueous seed extract of *C. paradisi*. The treatments lasted for a period of seven days. The rats were sacrificed under the influence of diethyl ether anesthesia. The standard reference drug used was ascorbic acid which is a well-known potent antioxidant.

Animal sacrifice and collection of blood
Animals were fasted for 24hours before sacrifice and were anesthesized by positioning them in a jar closed containing cotton wool immersed with diethyl ether anesthetic. After anesthesia, blood samples were obtained through cardiac puncture into plain sample bottles (for serum preparation) and heparinized tubes (for plasma preparation).

Preparation of serum
A Uniscope Laboratory Centrifuge (Model SM 902V, Surgifriend Medicals, England) was used to centrifuge the blood samples after being allowed to clot at room temperature for a period of 6 hours at 3000 rpm for 20 minutes to allow the sera to separate. The portions of the sera that were cleared were aspirated off and the residues discarded. The separated sera were stored wet-frozen until ready for use.

Removal of organs
Following bleeding of the animals by cardiac puncture, the entire bodies of the animals were swabbed with cotton wool soaked with ethanol. After this, the abdominal cavities of the animals were excised through a midline abdominal incision to show the abdomen and to excise the kidney. The excised kidney samples were washed thoroughly in ice-cold normal saline several times to remove gross fat and blood. The thoroughly washed kidney samples in ice-cold normal saline were then stored wet-frozen at -80^{0}C in a Revco freezer until ready for use.

Estimation of serum electrolytes
An ISE 6000 BYY SFRI Spectrophotometer was used to determine the serum electrolyte levels of sodium, chloride, phosphate, bicarbonate, potassium, and calcium in an automated manner. The machine calibrates automatically for the whole parameters to show. When this is done, the samples are allotted into a probe in which the tune button is pressed on the screen of the aforementioned machine. The result for the electrolyte levels of bicarbonate, calcium, phosphate, chloride, potassium and sodium are shown on the machine when it notifies with a screen display to remove the sample.

Statistical analysis
Statistical data were presented as mean ± (SEM). SPSS 25 was used to analyze the dissimilarities between the means, while P values < 0.05 were taken to be statistically significant. Multiple comparisons were done using Turkey's test.

Results and Discussion

Findings for serum electrolytes levels across the different treatment groups are presented in Figures 1-6. Serum bicarbonate levels were highest amongst test subjects in group II and lowest amongst test subjects in group VI. The difference in serum bicarbonate levels between the test groups was however not statistically significant.
There was a significant increase in serum chloride levels amongst the test subjects as seen in figure 2. Serum chloride levels were reduced with increasing extract concentration and varied significantly across all test groups as compared to control group II. Test subjects that received 125mg/Kg of plant extracts in addition to CCl_4 and ascorbic acid (group IV) showed significantly higher serum chloride levels as compared to those in group 1(normal saline). Similar patterns were observed between test subjects in groups IV/V compared to group III. Significant differences in serum sodium levels were only found between test groups V/VI and group II. There were no statistically significant differences observed between groups I/III and any of the test groups (Figure 3). Serum potassium levels were reduced with increasing extract concentration. Significant differences in serum potassium were observed between control group II and test groups V and VI. There was also a significant increase in serum potassium levels in groups IV and V as compared to control group I (Figure 4).
No changes were observed in serum calcium and phosphate levels between test and control groups (Figures 5 and 6). The kidney is a vital excretory organ in mammals in charge of the excretion of nitrogen, and nucleic acid metabolic waste products (urea, creatinine, and uric acid). Impaired renal function is responsible for the accumulation of toxic materials in the blood. Treatment of these major disorders and renal replacement theory is the major management for them. High levels of urea and other substances that contain nitrogen in the bloodstream are the major causes of symptoms like headache, vomiting, and loss of [21] pain as a result of some disorders such as thrombosis in the blood vessels leads to stretching of the fibrous tissue capsule surrounding the kidney.[22] Thirst or dehydration occurs if the kidney is injured as a result of fluid reduction before physical examinations.[22] Physical examination are very important because they show evidence to show the effect of the kidney problem, such as a rash in interstitial nephritis.[22] When the body cannot excrete sufficient fluid from the body can lead to peripheral edema and pulmonary

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

edema.[23] Carbon tetrachloride intoxication caused a rise in the serum Na^+, K^+, Cl^-, Ca^{2+}, and HCO_3^- (Group II) when compared to the untreated normal rats (Group I). It also caused significant decline in the serum PO_4^{2-} levels when compared to normal rats. This is indicative of the fact that carbon tetrachloride appears to compromise kidney function. However, vitamin C reversed the changes caused by carbon tetrachloride intoxication (Group III). Oral dosing with graded dose of aqueous seed extract of *C. paradisi* (125mg/kg-500mg/kg body weight) also attenuated the effect of carbon tetrachloride. While treatment attenuated the effects of carbon tetrachloride in a dose-dependent manner in all parameters, serum phosphate did not follow this trend. The observation concerning phosphate may be due to contamination in the course of analyses. The mechanism of action is as a result of antioxidant/free radical scavenging activity occasioned by the active principles in the extract. The fluid levels in the body are being controlled by serum electrolytes which helps in the maintenance of normal pH, fluid levels. The free radicals have been discovered in the pathogenesis of membrane damage in carbon tetrachloride intoxication. Hence, this membrane damage plays a crucial role in generating the cascade reaction resulting in the cellular death of the tissues.[24]

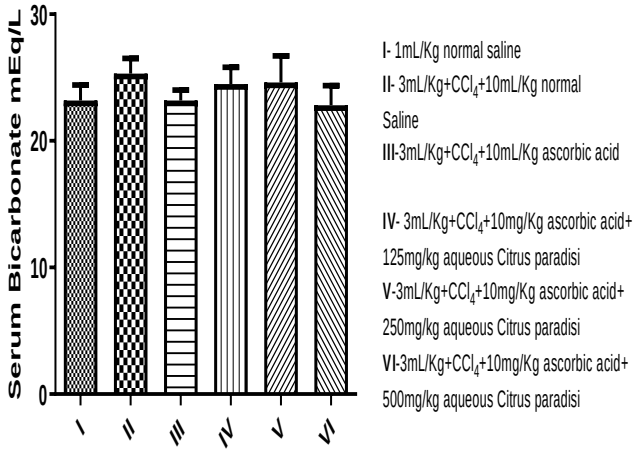

Figure 1: Serum bicarbonate of Normal, Treated and Untreated nephrotoxic rats. Each value represents Mean ± SEM of five.

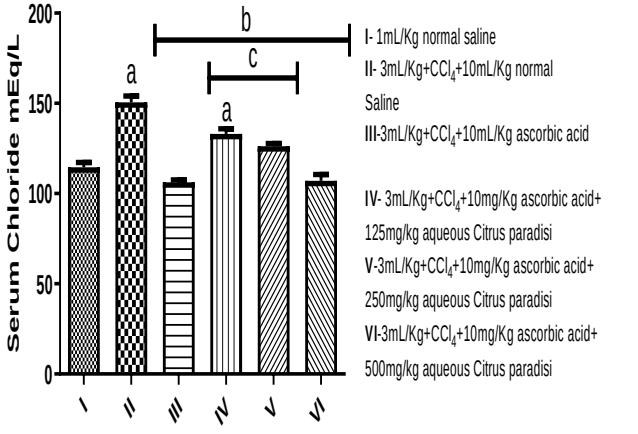

Figure 2: Serum chloride of Normal, Treated, and Untreated nephrotoxic rats. Each value represents Mean ± SEM of five

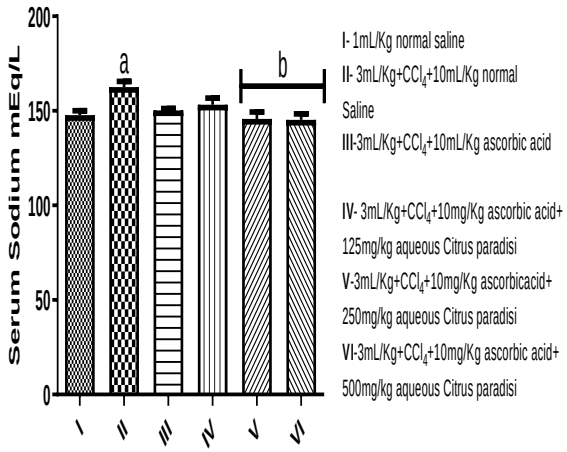

Figure 3: Serum sodium of Normal, Treated, and Untreated nephrotoxic rats. Each value represents Mean ± SEM of five.

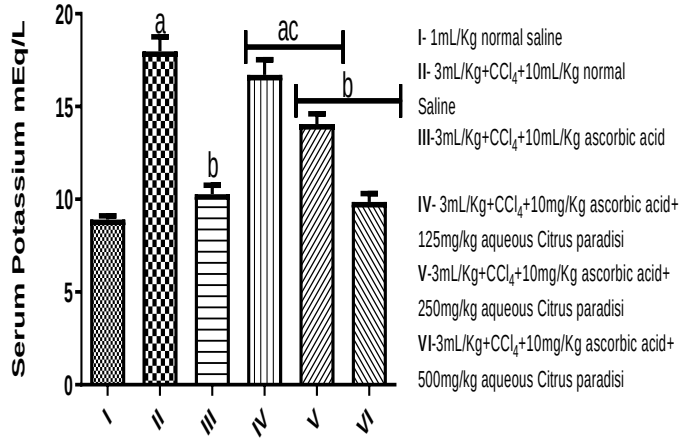

Figure 4: Serum potassium of Normal, Treated, and Untreated nephrotoxic rats. Each value represents Mean ± SEM of five.

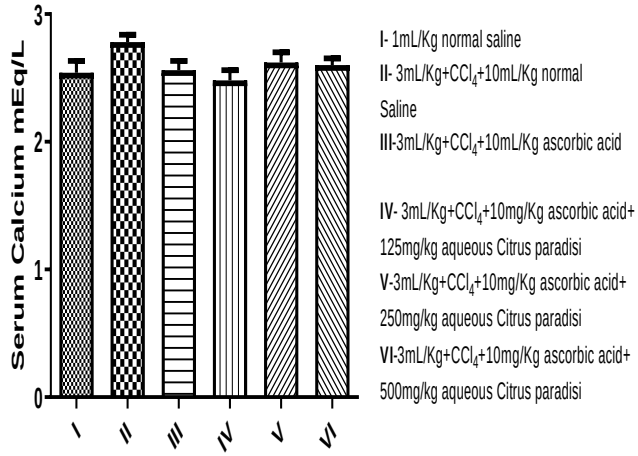

Figure 5: Serum calcium of Normal, Treated, and Untreated nephrotoxic rats. Each value represents Mean ± SEM of five.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

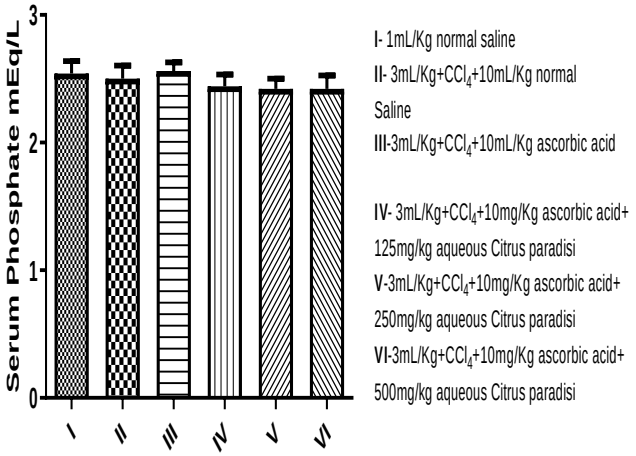

Figure 6: Serum phosphate of Normal, Treated, and Untreated nephrotoxic rats. Each value represents Mean ± SEM of five

Conclusion

The aqueous seed extract of *C.paradisi* possesses active agents that are nephroprotective even in the face of severe carbon tetrachloride intoxication. The mechanism of ascertained as a result of the antioxidant/free radical scavenging activity occasioned by the active principles in the extract.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1) Adeneye AA. Methanol seed extract of *Citrus paradisi* Macfad lowers blood glucose, lipids and cardiovascular risk indices in normal Wistar rats. Nig Q J Hosp Med. 2008; 18(1):16-20.
2) Chiba H, Uehara M, Wu J, Wang X, Masuyama R, Suzuki K, Ishimi Y. Hesperidin, a citrus flavonoid,inhibits bone loss and decreases serum and hepatic lipids in ovariectomized mice. J Nutr. 2003; 133:1892-1897.
3) Gao K, Henning SM, Niu Y, Youssefian AA, Seeram NP, Xu A, Heber D. The citrus flavonoid naringenin stimulates DNA repair in prostate cancer cells. J Nutr Biochem. 2006; 17:89-95.
4) Peluso MR. Flavonoids attenuate cardiovascular disease, inhibit phosphodiesterase and modulate lipid homeostasis in adipose tissue and liver. Exp Biol Med. 2006; 231:1287-1299.
5) Liu Y, Heying E, Tanumihardjo SA. History,global distribution and nutritional importance of citrus fruits. Comp Rev Food Sci Food Saf. 2012; 11:530-545.
6) Armando C, Maythe S, Beatriz NP. Antioxidant activity of grapefruit seed extract on vegetable oils. J Sci Food Agric. 1998; 77:463-467.
7) Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC. A Prospective study of tomato products, Lycopene and prostate cancer risk. J Natl Cancer Inst. 2002; **94**:391-398.
8) Gao K, Henning SM, Niu Y, Youssefian AA, Seeram NP, Xu, Heber D. The citrus flavonoid naringenin stimulates DNA repair in prostate cancer cells. J Nutr Biochem. 2006; **17**:89-95.
9) Yoshioka H, Usuda H, Fukuishi N, Nonogaki T, Onosaka S. Carbon tetrachloride-induced nephrotoxicity in mice is prevented by pretreatment with zinc sulfate. Biol Pharm Bull. 2016; **39**:1042-1046.
10) Samad A, Umar Ijaz M, Ashraf A, Sajid M, Imran M, Abbas K, Zafar S, AlGhanim KA, Al-Misned F, Al-Mulahim N, Mahboob S. Methanolic extract of *Nepeta paulsenii* as an ameliorative agent against CCl_4 induced testicular damage in male albino rats. J King Saud Univ. – *Sci*. 2020; 32(1):1168-1174.
11) Kamisan FH, Yahya F, Mamat SS. "Effect of methanol extract of Dicranopteris linearis against carbon tetrachloride induced acute liver injury in rats. BMC Compl Altern Med. 2014; 14:123.
12) Ritter C, Reinke A, Andrades M. "Protective effect of N-acetylcysteine and deferoxamine on carbon tetrachlorideinduced acute hepatic failure in rats. Crit Care Med. 2004; **32**(10):2079-2083.
13) Khan MR and Zehra H. Amelioration of CCl_4-induced nephrotoxicity by *Oxalis corniculata* in rat. Exp Toxicol Pathol. 2013; 65:327-334.
14) Naz K, Khan MR, Shah NA, Sattar S, Noureen F, Awan ML. Pistacia chinensis: a potent ameliorator of CCl_4 induced lung and thyroid toxicity in rat model. Biomed Res Int. 2014; 192906-192908
15) Hamid H, Liu D, Abdulrahim Y. "Amelioration of CCl_4-induced liver injury in rats by selenizing Astralagus polysaccharides: role of pro inflammatory cytokines, oxidative stress and hepatic stellate cell. Res Vet Sci. 2014; 114:202-221.
16) Zhao R, Jia G, Yu Y, Song B, Hu C, Wu L. Regulating activity of polysaccharides from *Portulaca oleracea L*. on dendritic cells of mice immunized against foot-and-mouth disease. Pak Vet J. 2020; 40(1):7-12
17) Bakr AF. Assessment of ginger extract and ginger nanoparticles protective activity against acetaminophen-induced hepatotoxicity and nephrotoxicity in Rats English. Pak Vet J. 2019; 39(4):479-486.
18) Bora KS and Sharma .The genus Artemisia: a comprehensive review. Pharm Biol. 2020; 49:101-109.
19) Bari MN, Saleem U, Faisal MN, Mehmood MH, Asif M, Majeed W,Anwar H, Muzaffar H, Mahmood A, Abdelsadik A. Anti-hyperglycemic efficacy of *Derris ovalifolia* in alloxan-induced diabetic wister rats. Pak Vet J. 2020; 40(1):108-112.
20) Wang J, Zhu H, Zhang C, Wang H, Yang Z. Protective effects of baicalein against cadmium-induced oxidative stress in rat testes. Pak Vet J. 2019; **39** (2):216-220.
21) Skorecki K, Green J, Brenner, B. Chronic renal failure. Harrison's Principles of Internal Medicine. New York: McGraw-Hill; 2005. 1653-1663 p.
22) Brady H and Brenner B. Chronic renal failure. Principles of Internal Medicine (16th edition) New York, NY: McGraw-Hill; 2004. 1644-1653 p.
23) Haliwell B and Gutteridge J. Free radicals in Biology and Medicine. Clarendon Press Oxford; 2006; 112-116p.
24) Lu KL, Tsai CC, Ho LK, Lin CC, Chang YS. Preventive effect of the Taiwan folk medicine *Ixeris laevigata* var. oldhami on a-naphthyl-isothiocyanate and carbon tetrachloride-induced acute liver injury in rats. Phytother Res. 2002; 16:S45e50.