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Induction of Acute Renal Failure to Study of Some Physiological and Histological Criteria in Rats Before and After Treatment with *Eruca Sative* Oil Extract of Leaves

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Abstract

Objective : the objective of the study was to test oil extract of *Eruca sativa* leaves by trying to treat acute renal failure that induced by injected Cisplatin intraperitoneally with 7.5 mg/kg of body weight (single dose) as animal model and measuring some predicate biomarkers and histopathological sections.

Methods: the oil extract of *Eruca sativa* leaves was prepared by using petroleum ether according to the method of Stahl ,different concentration prepared by using dry gum method (4:2;1) 1000mg/kg,750kg/kg and 500mg/kg , acute renal failure induced in male of rats by injected Cisplatin intraperitoneally with 7.5 mg/kg of body weight (single dose) functional common kidney biomarker was done blood urea nitrogen, serum creatinine and novel biomarker measured copeptin(CPP) , kidney injury molecule-1(KIM-1) and Neutrophils associated gelatinase lipocalin(NAGL) also histopathological sections done foe hematoxylin & eosin and trichome stain (modified masson's).

Result : there is significant increase of Serum creatinine(Scr.), and blood urea nitrogen(BUN) in cisplatin group when compared with control group, also indicate there is significant increase in serum biomarkers for kidney injury CPP , KIM-1, and NAGL when compared with control group. After the treatment with oil extract of *Eruca sative* leaves by different concentrations 1000 ,750 and 500 mg/kg all these Scr. , , BUN, CPP , KIM-1and NAGL were significantly decrease when compared with cisplatin group. Histopathological changes in kidneys were observed as show interstitial inflammation, tubular cellular swelling diffuse cellular swelling, tubular necrosis , mild inflammation and fibrosis in cisplatin group when compared with control group and after the treatment the damages were not found or minimize .

Conclusion : The biomarkers CPP, KIM-1 and NAGL may be good predictive indicator of acute renal failure, plant extract may be has a highly effect for treating the renal failure and this was shown through the sections of the tissue in addition to biomarkers tests which reveal the induced acute renal failure groups showed highly response to treatment.

Keywords : *Eruca sativa* ,adenine , induced acute renal failure , copeptin , kidney injury molecule-1 , Neutrophils associated gelatinase lipocalin

INTRODUCTION

Kidneys are dynamic organs that perform the main control system for maintain the body homeostasis they are influenced by different chemicals and drugs that may impact there functions[1].Kidney The kidneys of human are mainly involved in filtering and concentrating different materials and chemical agents that may arrive a high concentration and become poisonous[2]. Renal failure (RF) a state of kidney in which fails for removing and concentrating metabolic end products from the blood, regulating the fluid, electrolytes, and regulation the pH in the extracellular fluids. There are different causes may lead to kidney disease, systemic disease, and/or urologic defects not related with kidney [3]The kidney have important and the main role in excretion of many chemicals and drugs . so the renal failure may lead to reservation, of these compounds, which may accumulate gradually to toxic levels [4-7]Whereas some models of renal failure animal are used to evaluate the damage of organs pathogenesis[8]. So the Pilling up of solutes in the blood and tissues is at most because inability of the injury kidneys to filter the blood plasma and eject the wastes of metabolism and the undesirable materials, therefore the remaining solutes are called uremic toxins when they participate to the impairment of the physiological and biochemical functions of the kidneys as the renal failure[9].Acute renal failure (ARF) is unexpected obstruction in kidney function from interruption, decrease of the circulation, or disease in the renal tissue. with the treatment can usually be reversible. else, it can progress to form end-stage renal disease (ESRD) or chronic renal failure CRF [10,11]. The mortality rate from ARF has not altered substantially from the 1960s [12,13]. due to ARF is seen in high ratio usually in older persons than before, and it is frequently associated on other life-threatening cases, like trauma, shock, and sepsis .In ARF, the glomerular filtration rate (GFR) is decline , that lead to the excretion of nitrogenous wastes is decrease also, fluid and electrolyte balance cannot be stable and constant [14]. Some factors, including drugs such as antibiotics, are involved as reasons of renal failure in patients[15].Nephrotoxic drugs are responsible for acute renal failure and chronic renal failure in hospital and outpatient settings. Among elderly indi¬viduals [16-18]. Cisplatin is an anti-neoplastic meidaction which can be choice as therapy for treatment of many cancers, like neck, head, ovary, testis, lung and breast cancer, While it side effects include gastro toxicity , ototoxicity, and reaction of allergic, cisplatin major side effect is nephrotoxicity [19-21]. *Eruca sativa* Is one of the medicinal plant known as an aphrodisiac. It has several antioxidant constituents including glucosinolates (and their degradation products; thiocyanates and isothiocyanates), flavonoids and carotenoids [22]. Glucosinolates have several biological activities including anticarcinogenic, antifungal,

antibacterial in addition to their antioxidant effects[23]. Different studies persist to discover new biomarkers that are identification the kidney disease, and understanding the intensity and development of renal failure when examination noninvasively in urine and blood. The biomarker includes copeptin CPP, kidney injury molecule 1(KIM-1), Neutrophil gelatinase-associated lipocalin (NGAL) [24.] CPP is a glycosylated polypeptide consist of 39 amino acids and harbor a leucine-rich core segment (C-terminal part of the vasopressin prohormone), [25,26].as a response to physiological stress the Concentrations of copepein in plasma is increase[27]. KIM-1 is a type I transmembrane glycoprotein which is not found in normal kidneys. The elevation in releasing of this protein was found at highly levels on the proximal tubule cells specially on the apical membrane after nephrotoxic or ischemic injury [28]. And in rats it is widely induced in the tubules after ischaemic or toxic injury [29,30]. KIM-1 is released into the circulation where kidney injury in rodents and humans with ARF and CRF[31]. Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein(25kDa) that related to the family of lipocalin protein, is produced in epithelial cells and neutrophils in most tissues, it is a marker of tubular injury of kidney [32].also NGAL, called lipocalin-2 osteopontin (bone phosphoprotein), located in activated neutrophils, a coordinate with its function as an innate antibacterial factor by interfering with bacterial iron uptake [33]. NGAL may eventually have important role in predicting both acute and chronic renal failure [34].

MATERIALS AND METHODS

COLLECTION AND EXTRACTION OF PLANT

The fresh leaves of *Eruca sativa* were purchased from the local market, then cleaned and dried. Then this leaves were cut to little pieces and by blender were crushed to produce powder which The extraction of oil from leaves done according to the method of [35] by addition 150 ml petroleum ether with 20 g of leaves powder and by using Soxholate for 24 hour, 40-60 C , the solvent evaporated by using the Rotary evaporator at 60 C to complete the evaporation process of solvent , and the remaining oil were used for concentrations preparation. The emulsion was prepared in the faculty of pharmacy / kufa university in the pharmaceutics and industrial pharmacy Department. by using dry gum method, This method is referred as (4:2:1) because for every 4 portions by volume of oil, 2 portions of water and 1 portion of Acatia are added in preparing the primary emulsion[36]. At first Acacia was weighted by weighing balance and taken into dried cleaned blender Then measured amount of oil added to acacia and mixed homogenously until cracking sound produced. When the mixture became sticky , distilled water was added and mixed well to form emulsion which is creamy white,- in a ratio of(4:2:1)then the emulsion was diluted with distil water to obtain the concentrations 500 mg/ml. also The Ethanol extraction done by using the powder of dried leaves 20 g added to 200 ml of ethanol 95% in the extraction thimble of the Soxholate for 24 hour for 60 C [37]. the solvent evaporated by using the Rotary evaporator at 40 C to complete the evaporation process of solvent , and the remaining extract were stored at 5 C. [38].

Phytochemical detection of the active components in plant leaves extracts:

Eruac sativa ethanolic extract was subjected to qualitative phytochemical analysis for Alkaloids, Phenolic compounds ,Glycosides, Flavonoid , Saponins , Tannins and coumarins by standard procedures using [39].

Experimental animals

Using Healthy adult 30 male rats (*Rattus norvegicus*) weighting 150-220 gm. The animals were placed in the animal house of Faculty of Science, University of Kufa , with standard environment situations temperature (25-28 C°) and 12 hour light-dark cycle , The study protocol was done with agreement by the ethical committee of the Department of Biology- Faculty of Science -University of Kufa . rats kept in animal house for acclimation to the laboratory condition for one weeks before using them. study performed during the period from September, 2016 to February, 2017.

Study protocol

Thirty adult male rats were used, each group was formed 6 rats as the following :

Group (1) rats were administrated of acatia by intra gastric intubation at dose 100 mg/kg of body weight (as control) for two weeks .

Group (2) rats were administrated of acatia by intra gastric intubation at dose 100 mg/kg. for one week, then injected Cisplatin (Cipla/India) intraperitoneally with 7.5 mg/kg of body weight (single dose) [40,41].and complete the administration of acatia by intra gastric intubation at dose 100 mg/kg B.W. for one week (as cisplatin group).

Group (3) rats were administrated of emulsion by intra gastric intubation at dose 500 mg/kg. B.W. for one week, then injected intraperitoneally with Cisplatin 7.5 mg/kg of B.W. (single dose) and complete the administration of emulsion by intra gastric intubation at dose 500 mg/kg for the second week.

Group (4) rats were administrated of emulsion by intra gastric intubation at dose 750 mg/kg B.W. for one week, then injected intraperitoneally with 7.5 mg/kg of B.W. (single dose) and complete the administration of emulsion by intra gastric intubation at dose 750 mg/kg B.W. for the second week.

Group (5) rats were administrated of emulsion by intra gastric intubation at dose 1000 mg/kg OF B.W. for one week ,then injected intraperitoneally with 7.5 mg/kg of body weight (single dose) then complete administration of emulsion by intra gastric intubation at dose 1000 mg/kg for the second week. After the second week of experiment all the rats were sacrificed for :

- Measurement of Creatinine , Blood Urea Nitrogen (BUN) ,CPP,KIM-1, and NAGL .
- Study the Histopathological changes in the Kidney .

The present study done and complete in laboratory of advanced researches of biology department in faculty of science and also in medical genetic laboratory/ Kufa

University. During the period from September 2016 and to February 2017

Animals sacrificing

The rats were sacrificed by using an anesthetizing mixture of xylazine 0.1 ml and ketamine 0.5 ml [42].after heart puncture and blood collection the blood placed in test tube containing gel which leave for 30 minutes in room temperature and then used for getting serum by centrifugation at 3000 rpm for 15 minutes to separate serum and put in epindroff tubes which kept at freezer in −20,after that the abdominal lumen was open and kidney was removed and then placed in formalin (10%) as a fixative for histological preparation.

Estimation Of Biochemical Renal Function Tests :

Estimation of Serum Creatinine

Estimation of Creatinine was done calorimetrically by Jaffe's method by use a kit provided by SYRBIO company (S.A.R) [43,44,45]

Estimation Of BUN

Urea in the sample produced, by means of the coupled reactions which lead to form complex which is colored that can be measured by spectrophotometry using a kit provided by BioSystems S.A. company (Spain , COD 11536) [46,47,48].

ELISA Methods

Estimation . rat CPP marker, Elabscience company(code E-EL-R1440).

Estimation. Rat KIM-1 marker ,Elabscience company(code E-EL- R0575).

Estimation. Rat NAGL marker ,Elabscience company(code E-EL- R0662).

Histological study

The histological preparation achieved in histological section unit in faculty of sciences and medical genetic laboratory/ Kufa University in standard histological processing are prepared for the kidneys of the rats to study the histopathological changing that may be found in the experimental groups in compared with the animals of the control group. The rats were sacrificed and the organs were excised immediately. The preparation of the microscopic slides and staining procedures were done according to [49]. (for hematoxylin and eosin , and for trichome stain (modified masson's) according to [50].

Statistical analyses

Data were analyzed by using windows software packages Graphpad prism v6, data were offered as the mean, ±standard deviation and ±standard error (SE). Statistical analysis of variance to compare between treated and control groups were tested by one way anova (F-test).a level of statistically significant determination by P-value < 0.01 [51].

RESULTS

Phytochemical analysis of *Eruca sativa* leaves Methanolic extract

TABLE (1) Phytochemical analysis of *Eruca sativa* leaves Methanolic extract

Constitutes	
Phenolic compounds	+
Tannins	+
Glycosides	+
Flavonoids	+
Alkaloids	+
Saponins	-
Coumarins	+
Fixed oils	+

RESULT OF SERUM

Creatinine mg/dl assessment in rats groups of induced acute renal failure; cisplatin , Control , treated with oil extract at doses 1000 , 750 and 500 mg/kg.

Creatinine concentration significant elevated in Cisplatin group by comparison with the control group as shown in figure (1) . mean ,standard deviation, standard error and LSD were (31.6667,2.5819,1.0540 and 1.33) respectively as shown in table (2). there is highly significant decrease in Creatinine concentration in treatment group at dose 1000 mg/kg when compared with Cisplatin group as shown in figure (1) .mean ,standard deviation, standard error and LSD were (10.8333,±1.4719,±0.6009 and 2.22) respectively as shown in table (2). there is significant decrease in concentration of Cr. In treatment group at dose 750 mg/kg by comparison with cisplatin group as shown in figure (1) . mean ,standard deviation, standard error and LSD were (16.3333,±1.6329,±0.6666 and 1.11) respectively as shown in table (2).The concentration of Cr. In treated group at dose 500mg/kg is significant decrease as compared with Cisplatin group as shown in figure (1). mean ,standard deviation, standard error and LSD were (27.0000,±2.3664,±0.9660 and 3.24) respectively as shown in table (2).

Blood urea nitrogen mg/dl assessment in rats groups of induced acute renal failure cisplatin , Control , treatment with oil extract at doses 1000 , 750 and 500 mg/kg.

Concentration of BUN. is highly significant increase in Cisplatin group by comparison with Control group as shown in figure (2) . mean ,standard deviation, standard error and LSD were (36.8333,±1.4719,±0.6009 and 1.44) respectively as shown in table (3). there is highly significant decrease in BUN. concentration in treatment group at dose 1000 mg/kg when compared with Cisplatin group as shown in figure (2) .mean ,standard deviation ,standard error and LSD were (16.3333,±1.7511,±0.7149 and2.65) respectively as shown in table (3). The concentration of BUN in treated group with dose 750 mg/kg is significant decrease by comparison with Cisplatin group as shown in figure (2). Mean ,standard deviation ,standard error and LSD were (19.8333,±1.3291,±0.5426 and 1.34) respectively as shown in table (3). there is significant decrease in BUN. concentration in treatment

group at dose 500 mg/kg when compared with Cisplatin group as shown in figure (2). mean, standard deviation, standard error and LSD were (28.0000, ±3.5777,±1.4605 and 1.97) respectively as shown in table (3).the figure (2), shown BUN. Concentration In group which treated with oil extract at dose 1000 mg/kg was lower than the concentration of BUN. In groups which treatment with oil extract at dose 750 and 500 mg/kg.

Copeptin pg/ml concentration in serum of male rats induced acute renal failure treated with extract oil 1000,750 and 500 mg/kg.

Concentration of the CPP is statically significant increase in Cisplatin group when compared with control group as showed in figure(3)The mean , standard deviation , standard error and LSD were (920.2313 , ±54.4744 , ±22.2390 and 31.11) respectively as shown in table (4). The concentration of CPP. After treatment with oil extract at dose 1000 mg/kg statically highly decrease significant when compared with cisplatin group as shown in the figure (3).The mean , standard deviation , standard error and LSD were (170.7170, ±6.6343, ±2.7084 and 9.55) respectively as shown in table (4).The concentration of CPP. After treatment in oil extract at dose 750mg/kg statically decrease significant when compared with Cisplatin group as shown in the figure (3).The mean , standard deviation , standard error and LSD were (483.1918, ±41.5968, ±16.9818 and 15.27) respectively as shown in table (4).The concentration of CPP. After treatment in oil extract at dose 500mg/kg statically decrease significant when compared with Cisplatin group as shown in the figure (3).The mean , standard deviation , standard error and LSD were (715.5503, ±48.6955, ±19.8798 and 22.12) respectively as shown in table (4).

kidney injury molecule -1 pg/ml concentration in serum of male rats induced acute renal failure treated with extract oil 1000,750 and 500 mg/mg.

Concentration of KIM-1 in Cisplatin group statically increase significance when compared with control group as showed in figure(4). The mean , standard deviation , standard error and LSD were (913.7345, ±59.3683, ±24.2370 and 28.63) respectively as shown in table (5). The concentration of KIM-1. After treatment with oil extract at dose 1000 mg/kg statically highly decrease significant when compared with cisplatin group as shown in the figure (4).The mean , standard deviation , standard error and LSD were (162.0165, ±20.0830, ±8.1988 and 5.76) respectively as shown in table (5). The concentration of KIM-1. After treatment in oil extract at dose 750mg/kg statically decrease significant when compared with Cisplatin group as shown in the figure (4).The mean , standard deviation , standard error and LSD were (483.7347, ±67.5172, ±27.5638 and 11.58) respectively as shown in table (5). KIM-1 concentration after treatment in oil extract at dose 500mg/kg statically decrease significant when compared with Cisplatin group as shown in the figure (4).The mean , standard deviation , standard error and LSD were (753.2520, ±86.2465, ±35.2099 and 18.31) respectively as shown in table (5). the figure (4), shown KIM-1. Concentration In group which treatment with oil extract at dose 1000 mg/kg was lower than the concentration of KIM-1. In groups which treatment with oil extract at dose 750 and 500 mg/kg.

Neutrophile associated gelatinase lipocallin pg/ml concentration in serum of male rats induced acute renal failure treated with the extract oil 1000,750 and 500 mg/kg.

Concentration of NAGL in Cisplatin group statically increase significance when compared with control group as showed in figure(5). The mean , standard deviation , standard error and LSD were (5066.9173, ±102.2230, ±41.7324 and 133.88) respectively as shown in table (6). NAGL concentration After treatment with oil extract at dose 1000 mg/kg statically highly decrease significant when compared with cisplatin group as shown in the figure (5).The mean , standard deviation , standard error and LSD were (1719.2427, ±184.6154, ±75.3689 and 52.76) respectively as shown in table (6). The concentration of NAGL After treatment in oil extract at dose 750mg/kg statically decrease significant when compared with Cisplatin group as shown in the figure (5).The mean , standard deviation , standard error and LSD were (2320.2055, ±116.5335, ±47.5746 and 72.09) respectively as shown in table (6). NAGL concentration after treatment in oil extract at dose 500mg/kg statically decrease significant when compared with Cisplatin group as shown in the figure (5).The mean, stand deviation , standard error and LSD were (4556.5047, ±155.3181, ±63.4083and 98.24) respectively as shown in table (6).

Table (2) view mean ,standard deviation, standard error and LSD to Creatinine mg/dl concentration of rats groups induced ARF.

n=6	Mean	±Std. Deviation	±Std. Error	F	LSD
Cisplatin + oil 500 mg/kg	27.0000	±2.3664	±0.9660	164.503	3.24
Cisplatin + oil 750 mg/kg	16.3333	±1.6329	±0.6666		1.11
Cisplatin + oil 1000 mg/kg	10.8333	±1.4719	±0.6009		2.22
Cisplatin	31.6667	±2.5819	±1.0540		1.33
Control	7.8333	±1.4719	±0.6009		0.78

Table(3) View mean ,standard deviation, standard error and LSD to BUN mg/dl concentration of rats groups induced ARF.

n=6	Mean	±Std. Deviation	±Std. Error	F	LSD
Cisplatin+ oil 500 mg/kg	28.0000	±3.5777	±1.4605	117.310	1.97
Cisplatin+ oil 750 mg/kg	19.8333	±1.3291	±0.5426		1.34
Cisplatin+ oil 1000 mg/kg	16.3333	±1.7511	±0.7149		2.65
Cisplatin	36.8333	±1.4719	±0.6009		1.44
Control	12.0000	±2.2803	±0.9309		0.88

Table (4): the mean,standard deviation,standard error and LSD in control, Cisplatin, treatment group 500, treatment group 750 and treatment group 1000 mg/kg for copeptin pg/ml. Biomarker in induced ARF.

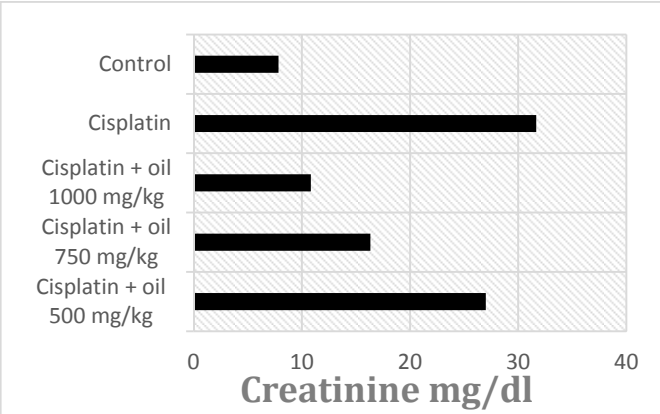
n=6	Mean	±Std. Deviation	±Std. Error	F	LSD
Cisplatin+oil 500 mg/kg	715.5503	±48.6955	±19.8798	542.915	22.12
Cisplatin+ oil 750 mg/kg	483.1918	±41.5968	±16.9818		15.27
Cisplatin+ oil 1000 mg/kg	170.7170	±6.6343	±2.7084		9.55
Cisplatin	920.2313	±54.4744	±22.2390		31.11
Control	66.0533	±3.7371	±1.5256		1.51

Table (5): view the mean , standard deviation , standard error and LSD in control, Cisplatin, treatment group 500, treatment group 750 and treatment group 1000 mg/kg for KIM-1 pg/ml biomarker in induced ARF.

n=6	Mean	±Std. Deviation	±Std. Error	F	LSD
Cisplatin+ oil 500 mg/kg	753.2520	±86.2465	±35.2099	246.410	18.31
Cisplatin+ oil 750 mg/kg	483.7347	±67.5172	±27.5638		11.58
Cisplatin+ oil 1000 mg/kg	162.0165	±20.0830	±8.1988		5.76
Cisplatin	913.7345	±59.3683	±24.2370		28.63
Control	79.5480	±5.6812	±2.3193		1.37

Table (6): the mean , standard deviation , standard error and LSD in control, Cisplatin, treatment group 500, treatment group 750 and treatment group 1000 mg/kg for NAGL pg/ml biomarker in induced ARF.

n=6	Mean	±Std. Deviation	±Std. Error	F	LSD
Cisplatin+ oil 500 mg/kg	4556.5047	±155.3181	±63.4083	900.7051	98.24
Cisplatin+ oil 750 mg/kg	2320.2055	±116.5335	±47.5746		72.09
Cisplatin+ oil 1000 mg/kg	1719.2427	±184.6154	±75.3689		52.76
Cisplatin	5066.9173	±102.2230	±41.7324		133.8
Control	1234.5212	±130.2065	±53.1566		11.37



Figure(1): show Creatinine mg/dl concentration in rats groups; adenine, control , treated with oil extract at 500 ,750 and 1000 mg/kg in induced CRF. n = 6 for each group.

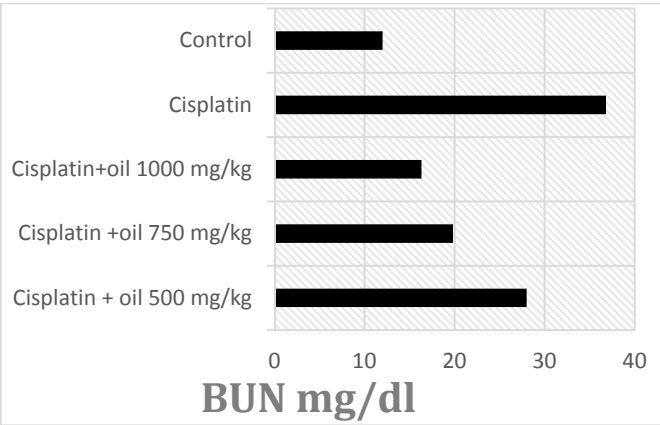


Figure (2) : show blood urea nitrogen mg/dl concentration in rats groups; Cispaltin, control , treated with oil extract at 500 ,750 and 1000 mg/kg in induced ARF. n = 6 for each group.

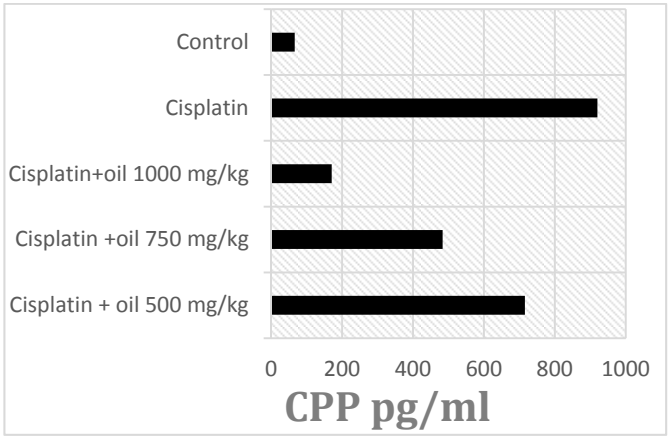


Figure (3) : Show Copeptin pg/ml concentration in control group , Cisplatin group, treatment group 1000, treatment group 750 and treatment group 500 mg/kg in induced ARF, n = 6 for each group.

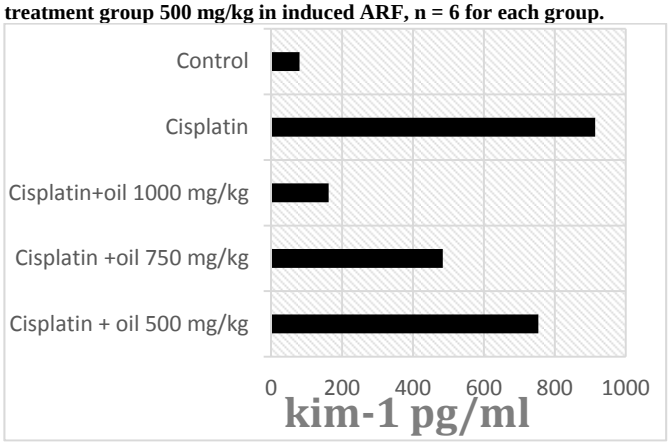


Figure (4) : show KIM-1 pg/ml concentration in control group , Cisplatin group, treatment group 1000, treatment group 750 and treatment group 500 mg/kg in induced ARF. n = 6 for each group.

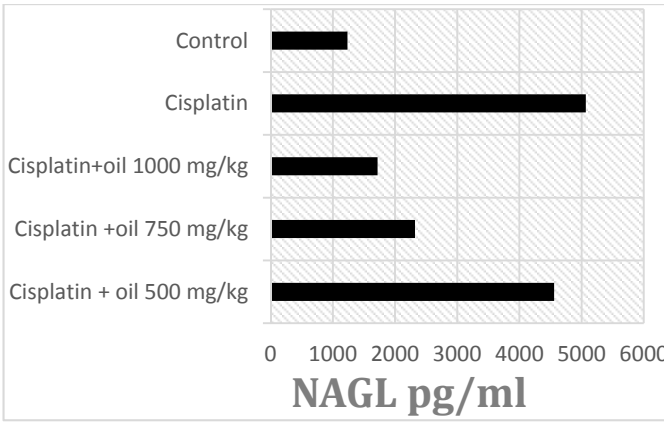


Figure (5) : show NAGL pg/ml concentration in control group , Cisplatin group, treatment group 1000, treatment group 750 and treatment group 500 mg/kg in induced ARF. n = 6 for each group.

TISSUE RESULTS

Histopathological change of kidney

The histopathlogical changes of kidney in rats group of cisplatin induced ARF for 14 days as shown in figures (8,9,10) included interstitial inflammation , tubular cellular swelling, diffuse cellular swelling, tubular necrosis , mild inflammation and Fibrosis as blue color respectively. kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and treated with oil extract 1000 mg/kg stained with H&E figure (11) show normal histological features and there is no fibrosis in MT stain figure(12) . after treated with oil extract 750 mg/kg kidney sections which stained with H & E show diffuse cellular swelling , no inflammation figure (13), and no fibrosis in figure (14). kidney in male rat intraperitoneal injected with Cisplatin 7.5 mg/kg and treated with oil extract 500 mg/kg stained with H & E .Show tubular swelling, no inflammation figure (15) and No Fibrosis in MT stain figure (16).

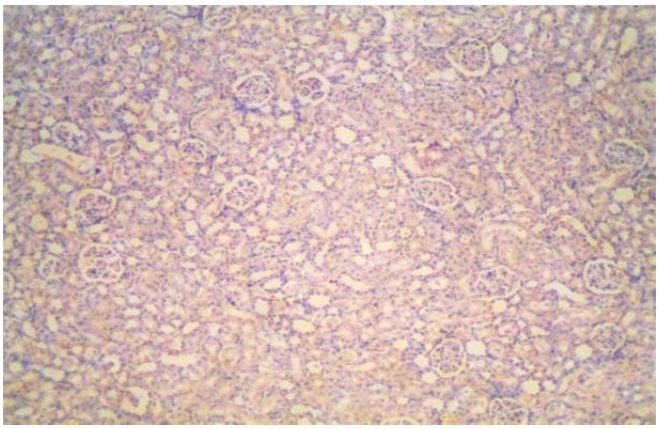


Figure (6): kidney in male rat administered standard diet and acatia as control group stained with H & E (X10x10). show no swelling, no inflammation , no necrosis and no fibrosis.

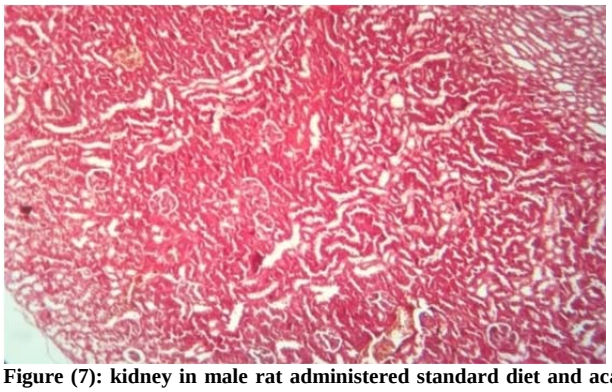


Figure (7): kidney in male rat administered standard diet and acatia as control group stained with masons trichrome (X10x10). show no fibrosis.

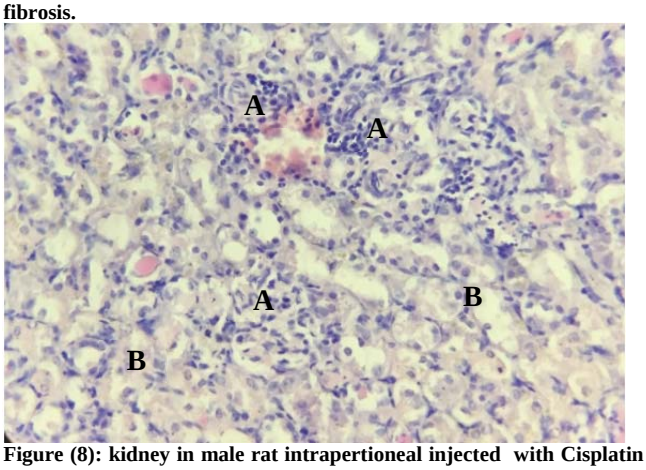


Figure (8): kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and acatia stained with H & E (X10x20). show interstitial inflammation(A) and tubular cellular swelling(B).

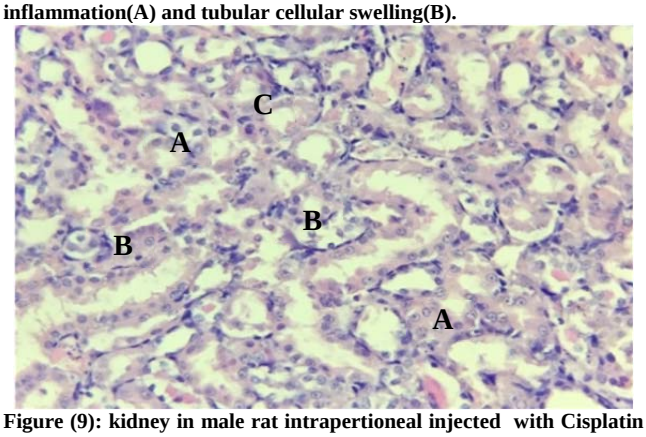


Figure (9): kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and acatia stained with H & E (X10x20). Show diffuse cellular swelling (A) and tubular necrosis (B)mild inflammation (C).

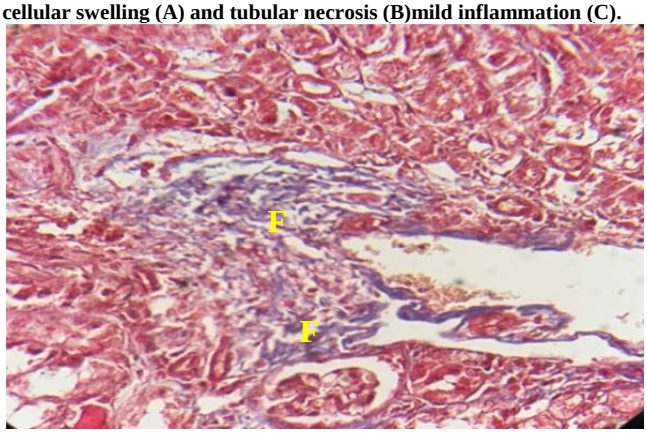


Figure (10): kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and acatia stained with masons trichrome (X10x10). Fibrosis (F) (blue stain).

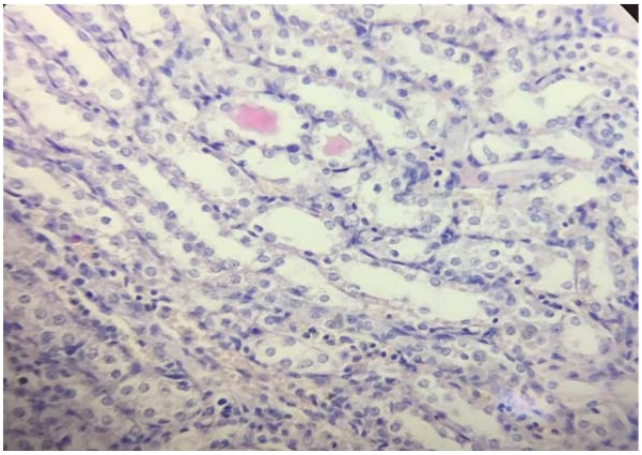


Figure (11): kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and treated with oil extract 1000 mg/kg stained with H & E (X10x20). Show normal histological features.

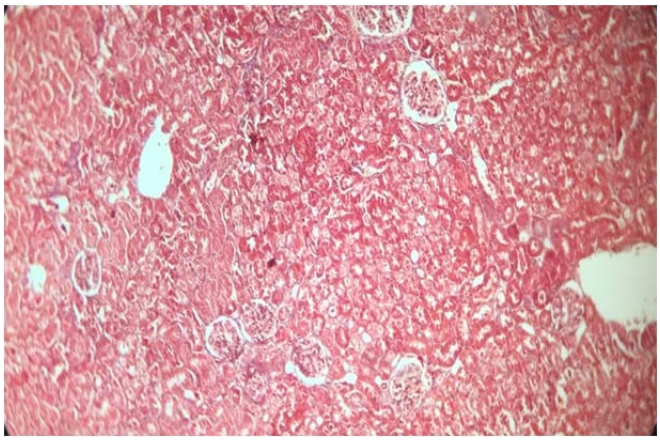


Figure (12) kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and treated with extract of oil with 1000 mg/kg stained with masons trichrome (X10x10). No Fibrosis.

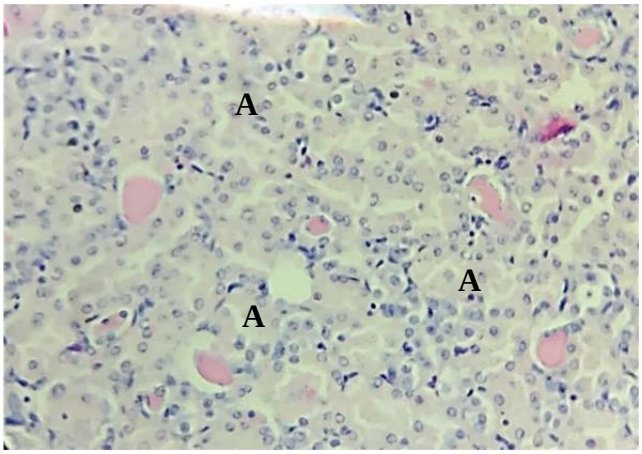


Figure (13): kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and treated with oil extract 750 mg/kg stained with H & E (X10x20). Show diffuse cellular swelling(A), no inflammation

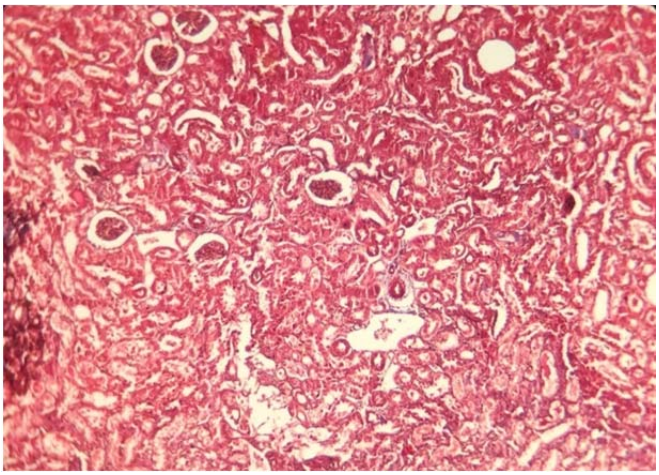


Figure (14): kidney in male rat intrapertioneal injected with Cisplatin 7.5 mg/kg and treated with extract of oil with 750 mg/kg stained with masons trichrome (X10x10). No Fibrosis.

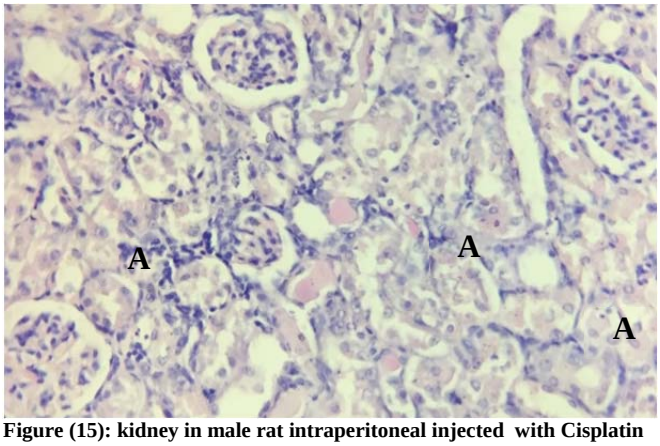


Figure (15): kidney in male rat intraperitoneal injected with Cisplatin 7.5 mg/kg and treated with oil extract 500 mg/kg stained with H & E (X10x20). Show tubular swelling(A), no inflammation.

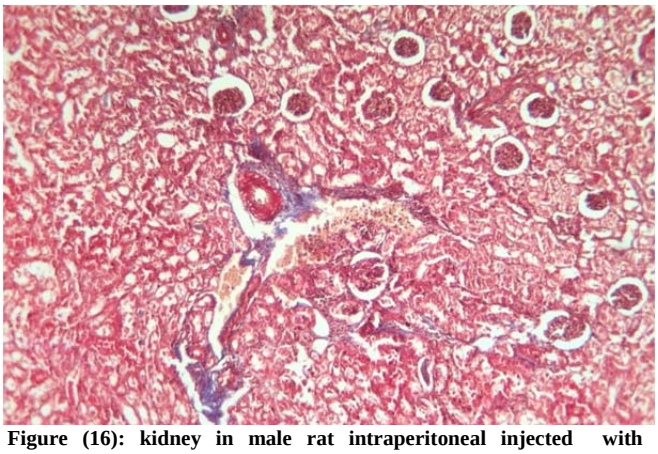


Figure (16): kidney in male rat intraperitoneal injected with Cisplatin 7.5 mg/kg and treated with extract of oil with 500 mg/kg stained with masons trichrome (X10x10). No Fibrosis.

DISCUSSION

Discussion of serum result.

Creatinine assessment in rats groups of induced acute renal failure; Cisplatin , Control , treated with oil extract at doses 1000 , 750 and 500 mg/kg.

Creatinine concentration significant elevated in Cisplatin group by comparing with the control group as viewed in figure (1). [52] found there is significant increase in creatinine concentration when compared with control group and this result agrees with Current study. Another study agrees with present study done by [53]which found elevation of Creatinine level in serum of ARF, were cisplatin accumulated in renal tubular cells so lead to disturbance of substance transport through renal tubular cells [54] whereas concentration of creatinine reveal a significant decrease in groups of treatment at doses 1000 ,750 and 500 mg/kg when compared with CP. group as shown in figure (1),study done by[55]. which include the relationship between *E.Sativa* seeds extract and serum creatinine level and he found there is significant decrease of serum creatinine in treated group with *E.Sativa* seeds extract by comparison with control group. There are no studies include of rats treated with oil extract of leaves of *E.Sative* with different doses, the dose which associated with lower level of creatinine concentration by comparison with other doses was 1000mg/kg ,because of the content of antioxidant in oil extract of *E.Sative* leaves at dose 1000mg/kg was sufficient to protect the nephron against the oxidant stress therefor the level of creatinine concentration in treated group with oil extract 1000 mg/kg was low when compared with other doses and this current study agrees with the study done by [56].

Blood urea nitrogen assessment in rats groups of acute induced renal failure; Cisplatin , Control , treated with oil extract at doses 1000 , 750 and 500 mg/kg .

Concentration of BUN. is highly significant increase in Cisplatin group by comparison with Control group as shown in figure (2) .Current study agrees with study done by [57]. Which approved that there is significant increase in induced ARF group when compared with control , also [58]. found there is significant increase of BUN in Cisplatin inducer ARF group by comparison with control group and this current study. The result reveal significant decrease in BUN concentration in treatment groups at doses 1000,750 and 500 mg/kg when compared with Cisplatin group as shown in figure (2) . the current study agrees with study done by [59].which found there significant decrease in BUN concentration in treated group of oil extract of *E.sativa* seeds by comparison with induced ARF groups. The content of antioxidant in oil extract of *E.Sative* leaves at dose 1000mg/kg was sufficient to protect the nephron against the oxidant stress therefor the level of BUN concentration in treated group with oil extract 1000 mg/kg was low when compared with other doses and the current study agrees with the study done by [56] .

Copeptine concentration in serum of male rats induced acute renal failure treated with the extract oil 1000,750 and 500 mg/kg.

Concentration of the Copeptine is statically significant increase in Cisplatin group when compared with control group as showed in figure(3). current study agrees with study done by [60]. which found all patients with ARF had higher concentration of copeptin ,also There is study done by [61]. found relationship between elevation copeptin concentration with any kidney damage because of the disturbance in blood osmolarity and any stress stimulate the hypothalamus to release AVP and the copeptin is the precursor of AVP and more stable therefore its concentration is increase and can be used as diagnostic biomarker for acute renal failure [62]. The present study show the concentration of CPP. After treatment with oil extract at doses 1000 , 750 and 500 mg/kg statically decrease significant when compared with cisplatin group as shown in the figure (3). There is study done by [55]. found when treated the rats with oil of *E. Sativa* seeds there is a protective effect for histological damage and nephron functions . another study done by [63]. found the protective role of *E. Sativa* seeds to nephron .*E. Sativa* seeds had protective activity and potential antioxidant for kidney [64]. three doses of treatment 1000 ,750 and 500 mg/kg and found the most protective dose was 1000 mg/kg by comparison with 750 and 500 mg/kg respectively . the good outcome from 1000 mg/kg when compared with 750 and 500 mg/kg may result from the sufficient amount of oil compounds which act as antioxidants .

kidney injury molecule -1 concentration in serum of male rats induced acute renal failure treated with the extract oil 1000,750 and 500 mg/kg.

Concentration of KIM-1 in Cisplatin group statically increase significance when compared with control group as showed in figure(4). Present study agrees with studu done by[65]. which found the KIM-1 concentration is higher in acute kidney injury when compared with normal. Another study done by [66]. agrees with present study which found the elevation of KIM-1 associated with ARF. The concentration of KIM-1. After treatment with oil extract at dose 1000 , 750 and 500 mg/kg statically decrease significant when compared with cisplatin group as shown in the figure (4). In current study found the good result associated with 1000 mg/kg of oil extract of *E. Sative* leaves when compared with 750 and 500 mg/kg , this occur may be due to high amounts of oil compounds and antioxidants in treated group with dose 1000 mg/kg . KIM-1 is transmembrane glycoprotein in epithelial cells of proximal tubules, any damage to this cells lead to loss there polarity and increase transepithelial permeability that finally cause leak back of KIM-1 to the circulation [67,68].

Neutrophile associated gelatinase lipocallin concentration in serum of male rats induced acute renal failure treated the extract oil 1000,750 and 500 mg/kg.

Concentration of NAGL in Cisplatin group statically increase significance when compared with control group as showed in figure(5). This result agrees with the study done by [69].which confirm the elevation of NAGL concentration associated with ARF, also [70]. found that NAGL is good biomarker for diagnosis of ARF which induced by drugs and this result agrees with current study. Another study done by [71]. confirm that NAGL have good sensitivity and specificity for the detection of ARF . NAGL concentration After treatment with oil extract at doses

1000,750 and 500 mg/kg statically decrease significant when compared with cisplatin group as shown in the figure (5). In current study found the more effective dose to protective the nephron was 1000 mg/kg when compared with 750 and 500 mg/kg, kidney damage lead to the elevation of NAGL that associated with inflammatory processes and immune response.as explaining to the results the 1000 mg/kg of oil extract have sufficient amounts of antioxidants therefore more better results and this agree with the study done by [72]. NAGL expression in neutrophils and renal epithelial cells, it have important anti-inflammatory role due to have the ability to covalently bound with matrix metalloproteinase 9 which release from neutrophils and act directly against inflammation, therefore any damage to renal epithelial cells or inflammation lead to release NAGL to interstitial tissue and finally pass to circulation [73].

Discussion of histopathological changes

According to slides of control group, the kidney is appear normally ,there are no changes in tublules, the epithelial cells is normal in shape and arrangement ,there is no inflammation ,no fibrosis and no necrosis. In slides of cisplatin group there are obvious changes in kidney such as interstitial inflammation and tubular cellular swelling, diffuse cellular swelling , tubular necrosis (showed by H &E) and fibrosis (showed by M.T stain) this result agrees with study done by [41] Histological changes that appear in rat kidney after treatment with cisplatin showed acute tubular necrosis due to oxidative stress via free radical formation that agree with study done by [74].and also infiltration of inflammatory cells in the interstitium due to cisplatin stimulating inflammatory, apoptotic pathways by generating reactive oxygen species[75].The pathological fibrillar matrix, Deposition of with high fibrillar collagens I and III, in the potential space between tubules and peritubular capillaries is one of the most feature of acute renal fibrosis and that Agree with [76].

Infiltrating of injured tissues Macrophages that lead to form peptide growth factors like transforming growth factor-1 (TGF-_1) and platelet-derived growth factor, these factors induces myofibroblastic cells development that have the ability to produce extracellular matrix (ECM), like collagens and fibronection, which lead for formation of fibrotic lesions. Macrophages and myofibroblasttic cells are the principle cells in fibrogenesis. interstitial fibrosis of kidney, after tubular injury fibrosis is the common final pathway in it [77]. In slides of groups of induced ARF that treated with oil extract of *E.sativa* leaves 1000 ,750 and 500 mg/kg show obvious repair of tissue damage , no inflammation , no fibrosis and with mild cellular swelling when compared with cisplatin group, this result agree with study done by [55,78] .according to the compounds of oil leaves extract such as trace elements (Cr,Cu,Fe,Mn and Zn) for example Copper (Cu) has important role for the function of immune system that due to Cu metabolism in body effects on the function of some immune system cells specially that participate in the antibodies production. it also has important role on the activity of an enzyme which responsible for removing toxic free radicals from the body (Cu-Zn superoxide desmotase) also it is important for phagocytes activity [79].

CONCLUSION

From current study conclude that The biomarkers CPP, KIM-1 and NAGL may be good predictive indicator of renal failure, plant extract may be has a highly effect for treating the renal failure and this was shown through the sections of the tissue in addition to biomarkers tests which reveal the induced ARF groups showed high response to treatment .

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