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NEPHROPROTECTIVE EFFECT OF FLAX SEED OIL, L-CARNITINE, DEFEROXAMINE, LABETALOL IN INDUCED MYOGLOBINURIC ACUTE RENAL FAILURE

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ABSTRACT

Objective: The present study was designed to determine the nephroprotective effect of flax seed oil, L-carnitine, deferoxamine & labetalol in rabbit animal model with myoglobinuric acute renal failure (ARF).

Materials & Methods: The normal serum values of blood urea nitrogen (BUN), creatinine, K+ & Na + were determined in 48 healthy domestic rabbits before glycerol administration and at two occasions 24, 168 hrs after ARF induction by glycerol and treatment with these tested agents that lasted seven successive days.

Results: Revealed significant decrease in serum BUN, creatinine, and K+ levels of all the tested agents compared with control group measured at 24 & 168 hrs.

Conclusion: All the tested agents (flax seed oil, L-carnitine, deferoxamine & labetalol have moderate nephroprotective activity in rabbit model of ARF. The histopathological examination showed a clear improvement in the sections of renal tissue that supports the effect of these agents in the kidney.

Keywords: Flax seed oil, L- carnitine, deferoxamine, labetalol, acute renal failure

INTRODUCTION:

Acute renal failure (ARF) is a clinical syndrome characterized by a rapid decline in glomerular filtration , retention of nitrogenous waste products & perturbation of extracellular fluid volume , electrolyte and acid-base hemostasis (1). There are different setting of ARF with different etiology & pathology. Myoglobinuric ARF is a model of intrinsic type of ARF(2) related to rhabdomyolysis which results from skeletal muscle cell injury that leads to the release of intracellular contents notably myoglobin into system circulation (3).

Glycerol (50%) was used practically for induction of Myoglobin ARF in the rabbits. However, cellular release of myoglobin leads to uncontrolled leakage of reactive oxygen species free radicals causing cellular injury. Renal vasoconstriction is a characteristic feature of rhabdomyolysis induced ARF and is the result of a different mechanism (4). A number of familiar drugs & medicinal herbs have been proved to have nephroprotective activity (5) in glycerol induced myoglobinuric ARF. The present study was performed to explore the possible nephroprotective activity of flax seed oil, L-carnitine, deferoxamine & labetalol in experimental model of ARF in rabbits.

Flax seed oil (*Linum usitatissimum*) is one of the oldest crops known to mankind & is cultivated for fibers and oil (6). It is a source of polyunsaturated fatty acids such as alpha-linolenic acid has antioxidant activity is used as hypoglycemic and hypolipidemicagent in diabetes mellitus type-2 (7).

L-carnitine is an amino acid that is vital for mitochondrial use. It is present in the diet and can be synthesized. It is used in pediatric cardiomyopathy(8)and male infertility (9), also in thalassemia major (10),and in prevention of pilocarpine induced seizure (11).

Deferoxamine is isolated from Streptomyces pilosus and binds with free iron & indirectly affecting hemoglobin and hemosiderin used for acute and chronic iron toxicity (12).

MATERIALS & METHODS:

Chemicals: All the chemicals used in the present study are of analytical grade. L-carnitine was supplied by APO Company-Canada as tablet. Deferoxamine vial (Desferol® Novartis- Switzerland). Labetalol (Trandeta®) tablet Glaxosmith-England. Flax seed oil purity 100% bottle Alzaymcomp-Syria. Glycerol 50% BDH-England.

Animals: Forty-eight healthy domestic rabbits weighing 800-1000 gm were used in

the present study. They were supplied by animal house of Al-Nahrain College of Medicine. Animals were kept in separated cages under good conditions at 28 c◦ and were fed standard oxoid pellets and were given water ad libitum. The rabbits were allocated to six groups (each containing 8 rabbits). The tested agents were given at 8 a.m. After two hours at 10 a.m. Glycerol was administered to animals of all groups except group 1. The tested agents were used for seven successive days as the following:

Group 1: received distilled water 2 ml/min daily.

Group 2: received distilled water 2ml/min daily prior to glycerol injection 50% 10 ml/kg/1 min.

Group 3: received flax seed oil 100% 2ml/kg orally prior to glycerol injection.

Group 4: received L-carnitine 500 mg/kg orally prior to glycerol injection.

Group 5: received deferoxamine 50mg/kg/i.m. prior to glycerol injection.

Group 6: received labetalol 1mg/kg orally prior to glycerol injection.

The doses of the tested agents have been chosen using many doses in pilot study. Glycerol was given at 10a.m. of the first day to the all groups except group-1 as 1:1 (v/v) single dose for the induction of ARF. Blood samples were obtained by cardiac puncture in which the needle of the syringe was inserted between the ribs to get 3 ml of blood in order to estimate the serum BUN, creatinine, potassium, sodium, at two occasions 24 & 168 hrs using spectrophotometer method. Later on, all the rabbits were sacrificed under light anesthesia of ether to take kidney specimens. The histopathological examination was carried out to check the microscopic changes of the renal tissue using polarized microscope after fixating the sections in 10% formalin for 48 hrs and staining with hematoxylin and eosin (13).

Statistical analysis:

Data are expressed as means±SEM. The differences among means have been analyzed by student t-test using SPSS software version 12. P values <0.05 was considered to be statistically significant.

RESULTS:

The obtained results in this study revealed that glycerol produced a marked significant increase in serum BUN, creatinine and K+ levels with a decrease in Na+ levels compared with normal control group levels at 24, 168 hrs.

All the tested agents: flax seed oil, L-carnitine, deferoxamine & labetalol (Groups 3,4,5,& 6) produced significant reduction in serum BUN, creatinine & K+ levels with

increase in Na+ levels measured at 24, 168 hrs.

Labetalol was more potent than others in reducing s.BUN levels from 12.68±2.55 to 7.09±1.76 followed by L-carnitine from 12.68±2.55 to 7.21±1.81 level at 24hrs. While s.BUN level reduced from 11.95±1.94 to 5.45±1.15 & 6.0±1.41 at 168hrs for L-carnitine & flax seed oil respectively. The creatinine level significantly decreased from 152.04±35.36 to 88.4±11.5, 89.28±17.08 respectively for labetalol and flax seed oil at 24 hrwhereas deferoxamine was coming in the lead to reduce creatinine level from 162.8±36.6 to 68.15±9.45 at 168 hrs. Deferoxamine also decrease K+ level significantly than the other agents. The histopathological examination of the kidney sections in glycerol group (Group 2) demonstrated severe functional and morphological renal deterioration with large numbers of obstructive renal tubules mainly with proteinaceous materials also renal vascular congestion & ischemia were seen (Figures 3,4) . But in using the tested agents, they showed a clear improvement with normal glomerulus and reduced tubular obstruction & vascular congestion. This improvement supports the nephroprotective effect of these agents against glycerol induced ARF (Figures 5,6,7& 8).

Table -1: serum levels of BUN, creatinine, K, Na of tested agents flax seed oil, L-carnitine , deferoxamine , labetalol measured at 24 hr& 168 hr after ARF induction by glycerol. P<0.05

Group	Dose	Duration	S.BUNmmol/L	S.creatinine mmol/L	S. K mmol/L	S. Na mmol/L
Normal control			5-64±1.13	72.31±13.7	4.63±0.29	142.08±2.04
Glycerol alone	10 ml/Kg	24 hr	*12.68±2.55	*152.04±35.36	*5.54 ± 0.58	*134.75±2.12
		168 hr	*11.95±1.94	*162.8±36.6	* 5.57±0.18	*137.14±4.52
Flax seed oil	2 ml/kg	24 hr	*7.28±2.93	*89.28±17.08	*4.81±0.26	*143.57±2.37
		168 hr	6±1.41	74.34±13.17	4.58±0.15	144.17±2.31
L-creatinine	500 mg/kg	24 hr	*7.21±1.81	*100.33±16.79	*4.97±0.38	*142.86±3.76
		168 hr	5.45±1.15	71.33±11.5	4.63±0.28	143.85±1.86
Deferoxamine	50 mg/kg	24 hr	*7.78±1.04	*96.53±15.02	*4.78±0.3	*141.5±2.67
		168 hr	6.83±0.6	68.15±9.45	4.51±0.23	144.14±2.27
Labetalol	1 mg/ kg	24 hr	*7.09±1.76	*88.4±11.5	*4.8±0.28	*141.0±5.16
		168 hr	6.04±1.23	71.98±8.3	4.64±0.17	144.13±1.34

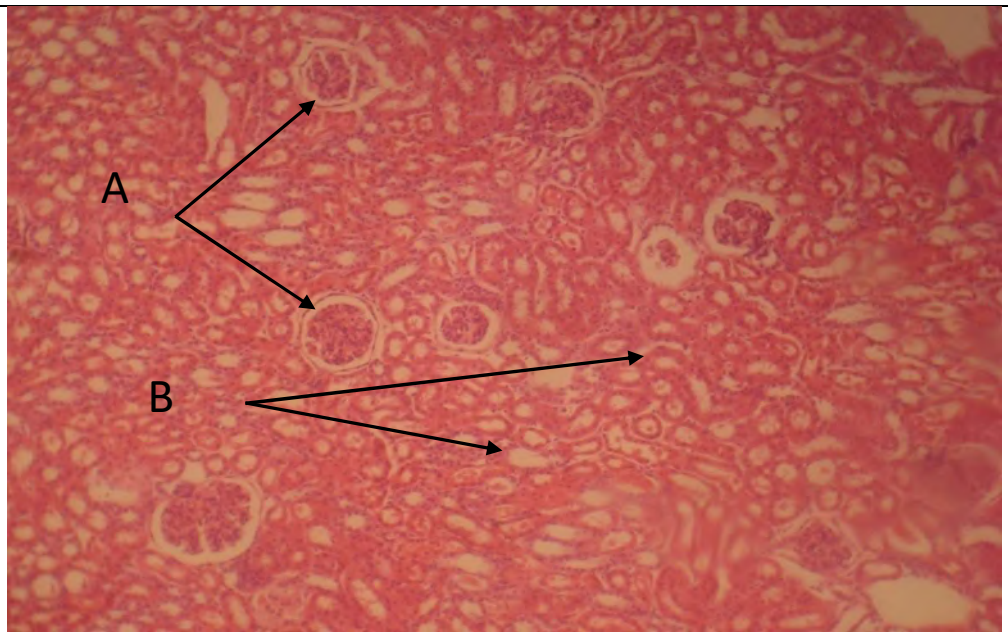

Fig. (1):The cortical region of the normal kidney shows(A) normal glomeruli (B) normal renal tubules. 125X , H&E.

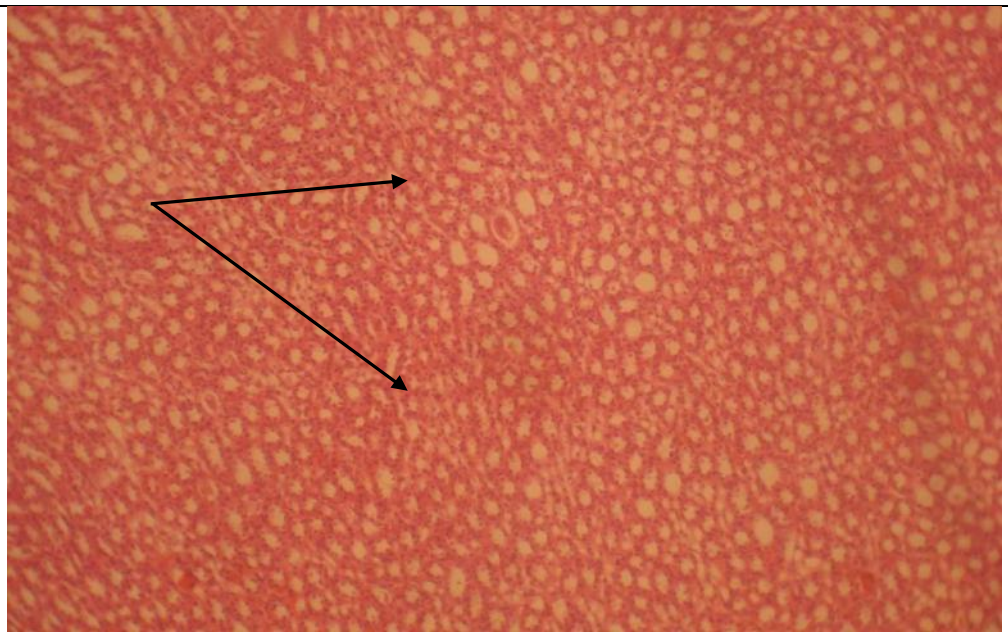

Fig. (2): The medullary region of the normal kidney shows normal renal tubules. 125XH&E

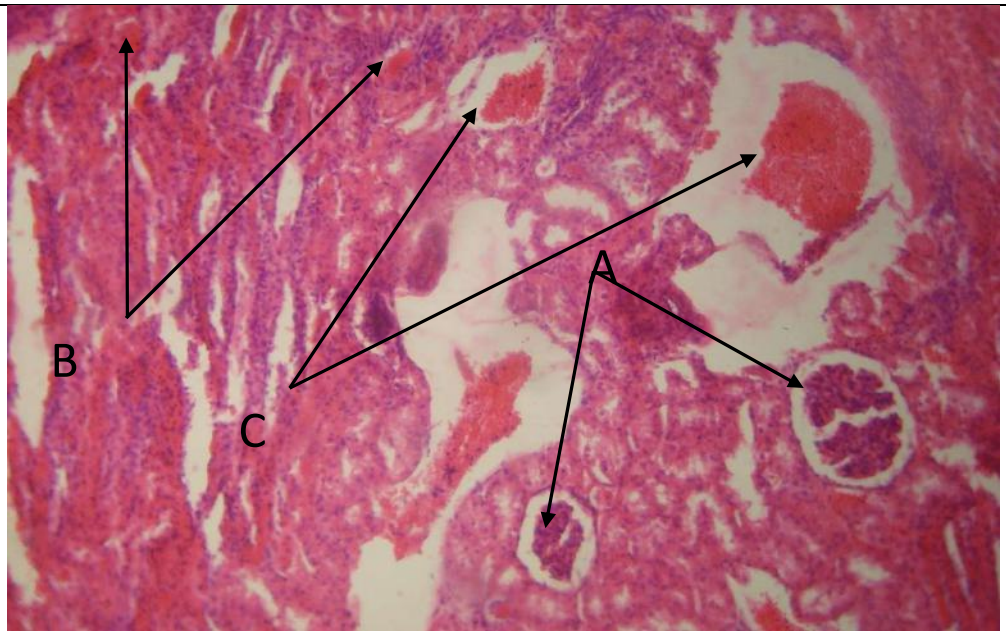

Fig. (3): The cortical region of the kidney of glycerol group shows (A) atrophied glomeruli(B) obstructive renal tubules (C) interstitial hemorrhage.125X .H&E

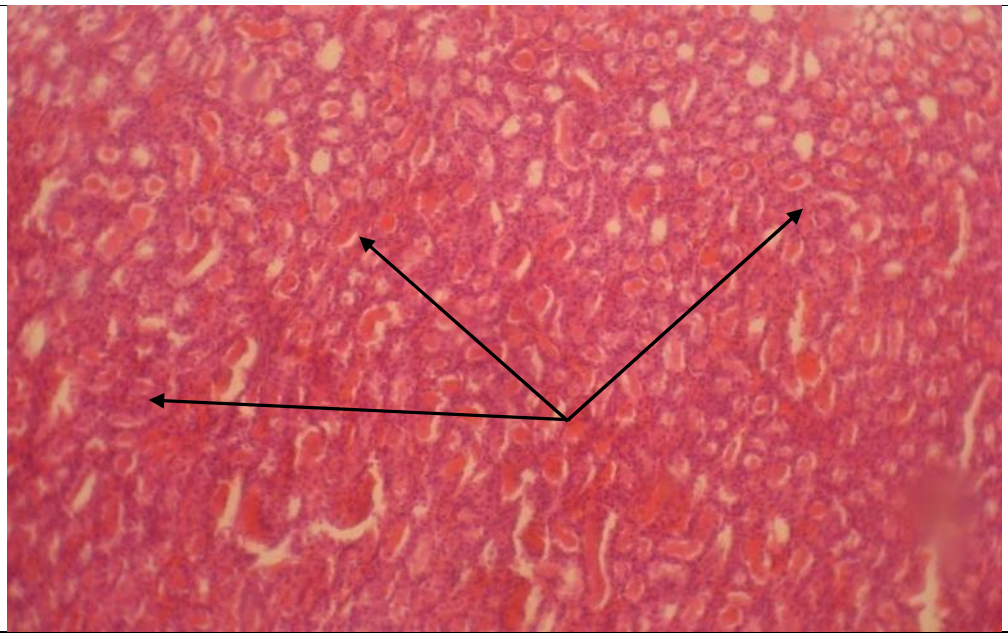

Fig (4): The medullary region of the kidney of glycerol group shows heavy number of obstructive renal tubules which is filled by proteinaceous material. 125X. H&E.

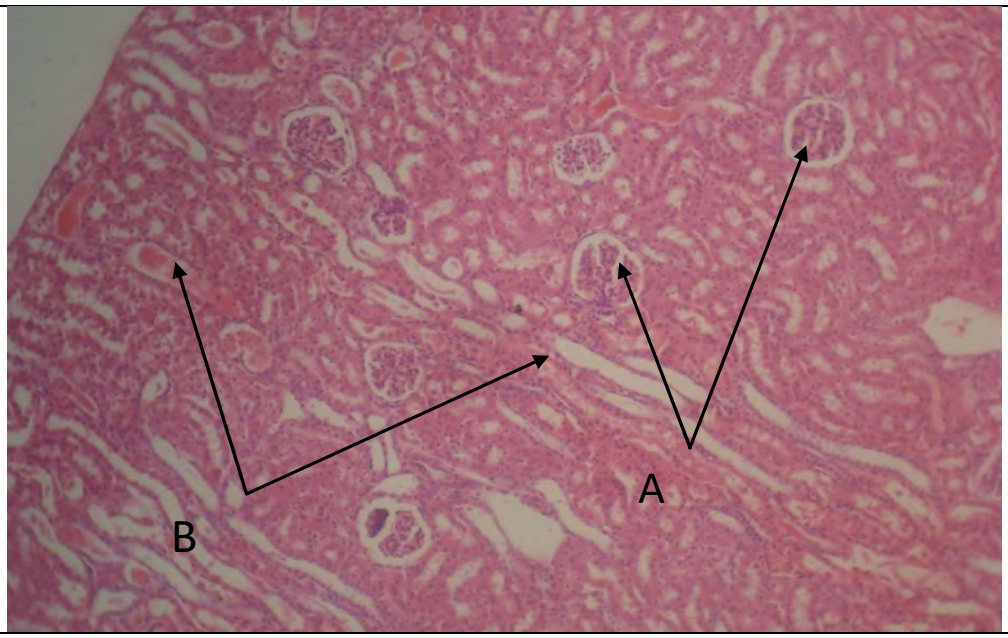

Fig.(5): The cortical region of the kidney of Flax seed oil group shows (A): normal glomeruli (B): few renal tubules contain proteinaceous material while theothers appear normal. 125X. H&E

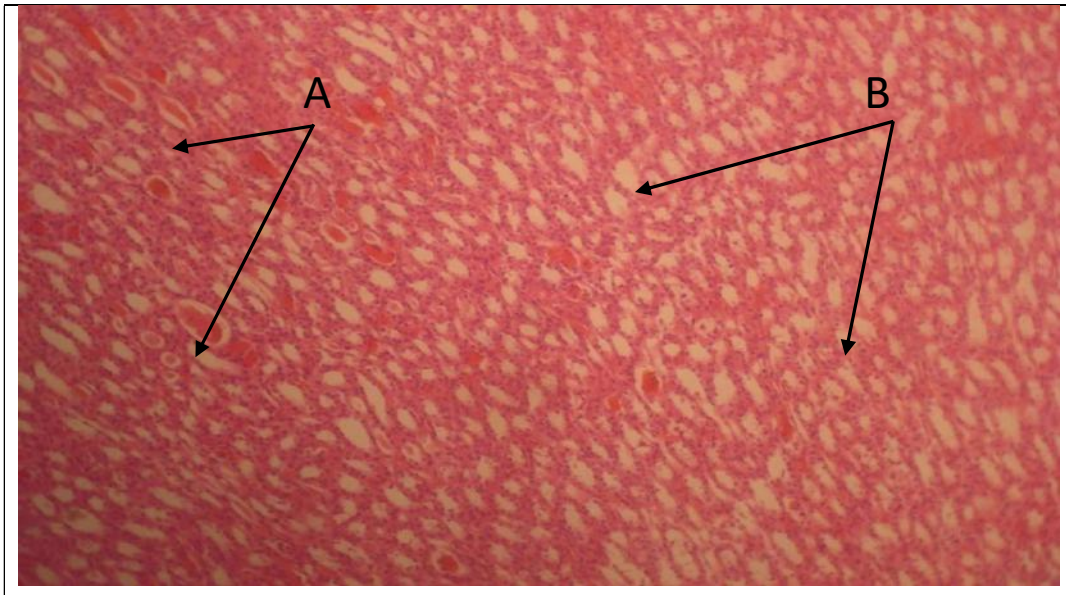

Fig.(6) The medullary region of the kidney of L- Carnitine group shows (A) few number of obstructive renal tubules (B) heavy number of normal renal tubules. 125X. H&E

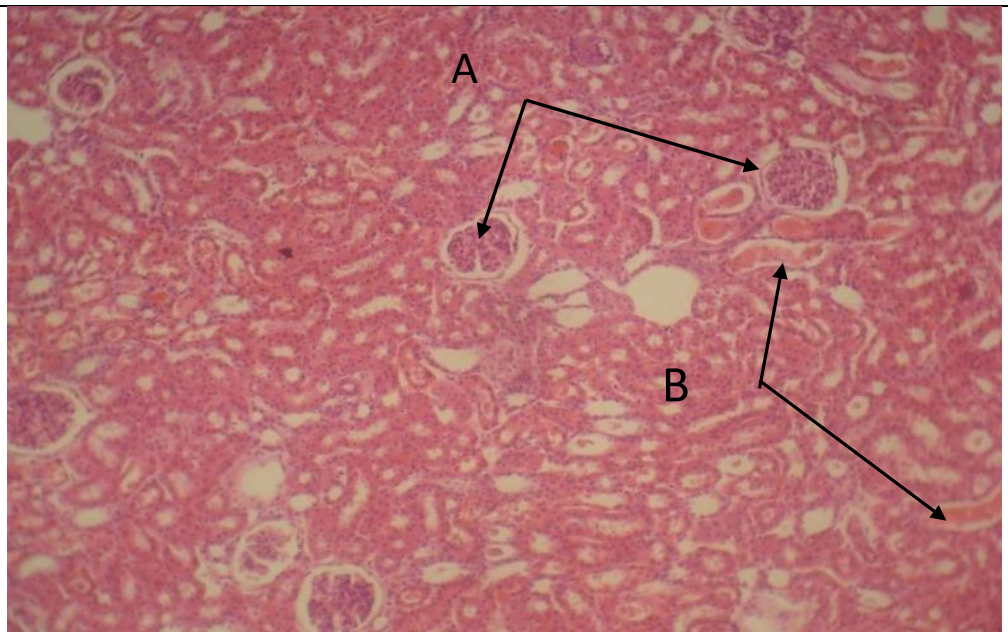

Fig. (7): The cortical region of the kidney of Deferoxamine group shows (A) normal glomeruli (B)few renal tubules contain proteinaceous material. 125XH&E

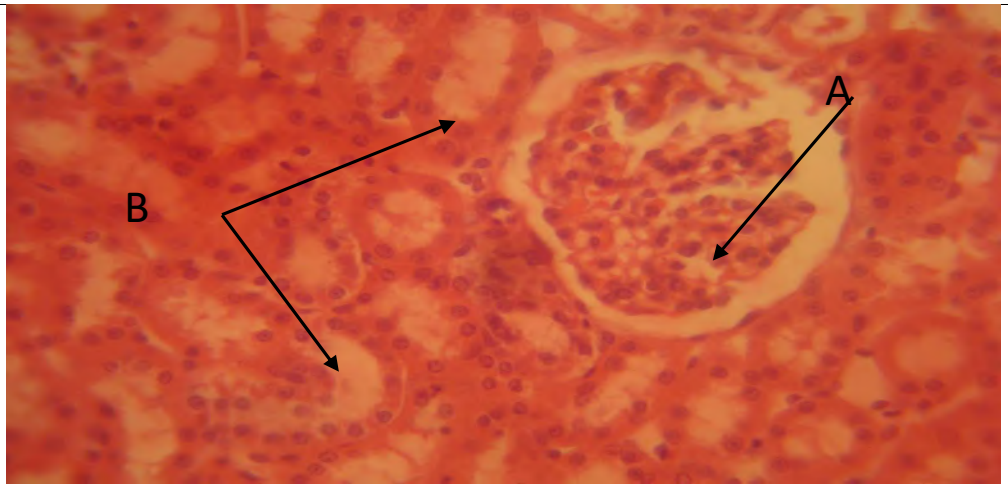

Fig. (8): The cortical region of the kidney of Labetalol group shows (A) normal glomerulus (B) some renal tubules contain acidophilic fluid. 500XH&E

DISCUSSION:

Administration of glycerol which is a well-known agent to be used for induction of myoglobinuric ARF in experimental animal model (2). It depends on the role of vasoconstriction in the development of this syndrome (14). The nephrotoxicity of myoglobin was attributed to different oxidative stress in which oxygen free radicals and the decrease in antioxidant defense system which found to be the main events (15) who reported that the total antioxidant level decreased within 24 hrs of induction with spontaneous recuperation after 72 hrs.

It is important to identify that iron play an essential role in tissue injury involving reactive oxygen species with pathologic blood disorders as a result of iron deficiency (16). The marked significant increase in s.BUN, creatinine & K+ with decrease in Na+ levels after administration of glycerol (group -2) are compatible with results of others (17).

The protective effect of flax seed oil (group 3) is related to its antioxidant effect which is demonstrated by (18). Results also showed significant nephroprotective effects which are compatible with results obtained by (19). This effect reduces tubular obstruction by myoglobin that occurs by myoglobinuric ARF and protect kidney tissue from damage.

L-carnitine showed a significant nephroprotective effect (group-4) through its antioxidant effect reported by(20). L-carnitine deficiency can increase carboplatin induced nephrotoxicity. So, L-carnitine supplementation ahead of carboplatin challenge ameliorated all the biochemical changes & also mitigated the injurious effects of cytotoxic drugs (21). Deferoxamine which is an iron chelating agent (group 5) that produce nephroprotective effect by preventing free radical generation which useiron as a co-factor. These results corresponding the results of others (22) who reported that deferoxamine interacts with Haber-Weiss reaction to protect the kidney against the oxidative stress as a result of renal dysfunction produced by glycerol.

A researcher (23) had shown the direct antioxidant effect of deferoxamine in rat with Adriamycin induced nephrotic syndrome. The antioxidant effect of this agent may be attributed to its direct scavenging activity of oxygen free radicals rather than its well-known chelating effect.

Labetalol is a combined selective and non-selective B-adrenoceptor blocker (24) (group 6). Labetalol decreased the systemic vascular resistance without reducing total peripheral blood flow. It has antioxidant and vasodilator

effect (25) that protects renal tissue from effect of glycerol. The same results obtained by (2) in using Carvedilol on the same model of ARF.

Conclusion: All the tested agents flax seed oil, L-carnitine, deferoxamine & labetalol have nephroprotective activity & possibility to be used clinically at the mentioned doses after clinical trials.

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