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THE EFFECT OF DIFFERENT DOSES OF GAMMA IRRADIATION ON THE ECHIS COLORATUS VENOM TOXICITY IN MICE

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Abstract

Snake venoms are complex mixtures of proteins and non proteins components. This study evaluated the toxicity effect of LD_{50} of native *Echis coloratus* venom and the LD_{50} of dry venom irradiated with different doses of gamma radiation (2, 5 & 10 KGy) on Swiss albino mice. Measurement of some biochemical parameters and histological alterations in liver and kidney was investigated. The biochemical parameters showed highly significant increase ($P< 0.001$) in levels of ALT, AST, urea and CK in all groups compared to control group. Levels of serum creatinine showed highly significant increase ($P< 0.001$) in groups treated with LD_{50} of crude venom and 5 times LD_{50} of irradiated venom at 2 and 10 KGy, But, showed significant ($P< 0.05$) with groups treated with 5 times LD_{50} of irradiated venom at 5KGy & LD_{50} of irradiated venom at 10 KGy showed non-significant in group treated with LD_{50} of 5KGy & 2KGy compared to control group. Histological examination showed alterations in liver, kidney in mice injected with crude venom compared to control group. Also, the group of mice which injected with different doses of irradiated venom showed no histopathological alteration in liver and kidney tissues, except the mice group injected with 5 times LD_{50} of irradiated venom at 2 KGy and the group which injected with 5 times LD_{50} of irradiated venom at 10 KGy which showed alterations in liver and kidney tissues.

Key words: *Echis coloratus,* LD_{50}, Ionizing radiation, Biochemical examinations, Histological effect of venom.

Introduction

Snakes are wild animals and some species are venomous. Snakes' bites could cause serious health problems by injecting their modified saliva and venoms into another organism for purpose of self-defense and hunting victim. Also, they use these toxic and complex components for paralyzing and digestion preys (WHO, 2010). Most data suggested that snakes cause in more than 3 million bites/year with approximately about 150,000 deaths, particularly in rural tropical areas and at least 421,000 cases of envenoming and 20,000 deaths occur every year worldwide (Gouda *et al,* 2017). Also, WHO reported that about 5.4 million snake bites occur each year, resulting in 1.8 to 2.7 million cases of envenomings (poisoning from snake bites) and the cases of death between 81, 410 and 137, 880 around three times as many amputations and other permanent disabilities each year (WHO, 2018).

Echis coloratus (the painted saw-scaled viper) belongs to Viperidae family that comprises *Echis carinatus, Echis pyramidum, Cerastes cerastes, Pseudo cerates* and *Bitis arientans* (Al-Asmari *et al,* 2014). *Echis coloratus* is found in north-east Africa and throughout Arabia, and spread largely in the mountainous areas (Babocsay, 2004).

The venoms are mixtures of enzymes, peptides, toxins, nerve growth factors, carbohydrates, lipids, metal ions and organic compounds (Chérifi and Laraba-Djebari, 2013). Since the primary role of venom is to aid prey capture, it is perhaps unsurprising that variation in the protein composition of venom has been associated with significant dietary shifts in a number of genera (Sanz *et al,* 2006). The most numerically abundant venom toxin families in the four *Echis* species were the SVMPs, CTLs, PLA_2s, & SPs that

broadly consistent with viperid venom gland analyses, although considerable intergeneric variations in the EST-inferred expression levels of these toxin families have been reported (Wagstaff and Harrison, 2006). The enzyme leads to protein causing swelling and necrosis. In addition to phospholipase A2 which affects this enzyme effect on normal physiological processes of the victim that could eventually cause death (Harris and Scott-Davey, 2013). Common names: painted saw-scaled viper, painted carpet viper, Burton's carpet viper (Mallow *et al*, 2003). A host of other components in *E. coloratus* venom have a specific biological effect on victim but non-lethal including sever pain, swelling, respiratory failure, blood coagulation, disturbance in blood pressure regulation, arrhythmia, muscular impulse, transmission of the nervous, and circulatory collaps which lead to renal failure. The effected liver by *E. coloratus* venom raise glucose level, glycogen depletion, and raise of alanine aminotransferase (ALT), alkaline phosphatases (ALP), and aspartate aminotransferase (AST) lead to hepatocellular damage (Jarrar, 2011).

Snake poisoning signs and symptoms include numerous clinical abnormalities and development of acute hepato-renal toxicity, organ dystrophy and metabolic complications (Al Asmari *et al,* 2016). Snake venom poisoning is a medical emergency requiring immediate attention. Bites from poisonous European snakes can lead to local tissue damage and systemic symptoms, which is a potentially serious event that requires immediate hospital care. Yet, the majority of victims can be treated successfully with conservative methods (Frangides *et al,* 2006).

Gamma radiation has noticeable effect on lowering toxicity from venom without effect on immunogenicity as it can change the molecular structure of proteins (Rogero and Nascimento, 1995). After exposure proteins or in snake's venom, it has to damage of amino acid occur side chains of protein and produce a new group, form intermolecular and intramolecular cross links and splitting of peptide bonds which this change may be in dry or solution state (Rogero and Nascimento, 1995; Al-Sadoon *et al,* 2013).

Materials and Methods

Venom: *Echis coloratus* lyophilized crude venom was obtained from the Egyptian organization of biological products and vaccines (VACSERA).

Detoxification of venom: Gamma radiation of *E. coloratus* venom was carried out in the National Center for Radiation and Technology (NCRRT, Egypt) using C^{60} gamma radiation source of Indian facility with a dose rate 0.7 KGy/hr at the time of the experiment. Samples of dry venom were irradiated to 2, 5 & 10 KGy respectively.

Male albino Swiss mice weighing between 18-20g from three to five weeks in age were selected from the experimental animal house of the VACSERA. Animals were kept in standard conditions and fed with normal diet and water.

Lethality assay: Toxicity of *E. coloratus* venom was carried out on albino Swiss mice (Theakston and Reid, 1983) by intravenous (i.v) injection of different doses of non irradiated venom and irradiated venom (2, 5 and 10 KGY). LD_{50} was determined by formula:

$M = X_k + ½ d – dr/N$

M= log LD_{50}, X_k = log dose causing 100% mortality (log LD_{100}), d= logarithmic interval of doses, r = Sum of the number of animals dead at each of the individual doses and N= Number of animals in each group.

Tested animals were divided into three groups as following G1: included 10 mice that served as control. G2: included 10 mice were injected intravenous with a dose of LD_{50} of native non- irradiated sample (1.245 mg/g body weight of mouse).

G3: was subdivided into these groups according to the doses of irradiated venom received: 1- Injection with 2 KGy irradiated venom intravenous with LD_{50} dose, (1.245 mg/g body weight of mouse). 2- Injection with 2 KGy irradiated venom intravenous with 5 times LD_{50} dose, (6.225mg/g body

weight of mouse). 3- Injection with 5 KGy irradiated venom intravenous with LD_{50} dose, (1.245mg/g body weight of mouse). 4- Injection with 5 KGy irradiated venom intravenous with 5 times LD_{50} dose, (6.225 mg /g body weight of mouse). 5- Injection with 10KGy irradiated venom intravenous with LD_{50} dose, (1.245 mg/g body weight of mouse). 6- Injection with 10KGy irradiated venom intravenous with 5 times LD_{50} dose, (6.225mg/g body weight of mouse).

Blood was collected from each group of mice 6 hours post-injection from the jugular vein after anesthetizing mice by isoflurane, using disposable syringe to take blood sample according to ethics statement (Hoff and Rlatg, 2000) and collected blood were centrifuged to separate sera at 3000 rpm for 15 min at room temperature and kept at -70°C.

Sodium dodecyle sulfate electrophoresis (SDS-PAGE): The 10% gel was prepared (Laemmli, 1970) to determine the molecular weights of venom in the presence of protein markers. The *E. coloratus* crude of irradiated venom (12µg/ml) and non-irrradiated was subjected to preparative 10% native polyacrylamide gel electrophoresis. The protein samples were mixed with equal volumes of 2X or 5X samples buffers and were heated for 10 min at 100ºC, The comb was slowly removed from the gel to avoid disturbing the well dividers. Each well was filled with tank buffer and the sample was loaded using 50µl or 100µl Hamilton syringe. Electrophoresis was set at 100 to 125 volt. The gel was placed in a container with coomassie stain and shacked for 30 to 60min for 1.5mm thick gel until bands were visible, as placed with de-stain to clear background.

Biochemical examinations: Blood samples were used for estimation of quantitative Creatinine using commercial kit from Vitro Scient serum urea was determined using kit from Vitro Scient (Rock *et al,* 1987), activity of serum ALT by Vitro Scient kit (Zilva and Pannall 1979), activity of serum AST by Vitro Scient kit (Henry, 1974) and activity of serum Creatinine phosphokinase using BioScien kit (CK total) after IFCC (1989).

Statistical analysis: All values were expressed as mean ± standard error and t-test values were calculated using Microsoft Excel 2007, P value <0.01 was considered significant.

Histological studies: Samples were taken from the liver and kidney of mice in different groups and fixed in 10% formol saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24hrs. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by microtome. Tissue sections were collected on glass slides, deparaffinized, stained by hexatoxylin and eosin stain and examined by light electric microscopy (Bancroft *et al,* 1996).

Results

The LD_{50} of native *E. coloratus* venom (non- irradiated), irradiated venom 2, 5 & 10 KGy was 1.245 mg/g body weight of mouse. The LD_{50} of different doses of irradiated venom protected the mice against the venom lethal action. Mice injected with five times of LD_{50} with irradiated venom (6.225mg/g body weight of mouse) showed survive of mice than native venom (non-irradiated).

Table 1: LD_{50} of mice injected with native *E. coloratus* venom and different doses of irradiated venom at 2, 5 & 10 KGy.

Group of mice	Survival	Death
LD_{50} of crude venom	5	5
LD_{50} of irradiated venom (2 KGy)	10	-
5 times LD_{50} of irradiated venom (2 KGy)	9	1
LD_{50} of irradiated venom (5KGy)	10	-
5 times LD_{50} of irradiated venom (5KGy)	10	-
LD_{50} of irradiated venom (10KGy)	10	-
5 times LD_{50} of irradiated venom (10KGy)	8	2

Sodium dodecyle sulfate electrophoresis (SDS-PAGE): *E. coloratus* crude venom and different doses of irradiated venom at 2, 5 and 10 KGy were loading to SDS-polyacrylamide gel electrophoresis along with wide molecular weight protein markers (Fig. 1). The molecular weight of protein bands in the crude venom altered than the molecular weight of protein bands in the irradiated venom.

Biochemical examinations showed highly significant increase ($P< 0.001$) in levels of ALT, AST, urea and CK in all groups compared to control group. Levels of serum creatinine showed highly significant increase ($P< 0.001$) in groups treated with LD_{50} of native venom (non- irradiated), 5 times LD_{50} of irradiated venom at 2 and 10 KGy, But showed significant ($P< 0.05$) with the groups treated with 5 times LD_{50} of irradiated venom at 5KGy and LD_{50} of irradiated venom at 10 KGy and showed non- significant in the group treated with LD_{50} of 5KGy and 2KGy compared to control (Tab. 2).

Table 2: Mean± Std. Dev. and P value of mice serum for Creatinine, Urea, ALT, AST & CK after treating with LD_{50} of native *E. coloratus* venom and different doses of irradiated venom at 2, 5 & 10 KGy.

Group	Parameter	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Urea (mg/dL)	CK(U/L)
Control (N=10)	Mean ± Std. Dev.	36.6 ± 1.14	48 ± 1.58	0.68±0.084	36±1.6	69.8±0.83
LD_{50} of native venom (N=10)	Mean ± Std. Dev. *P*	110 ±1.58 0.000	299± 3.39 0.000	1.7±0.1 0.000	94±3.16 0.000	480.8±1.92 0.000
LD_{50} of 2KGy (N=10)	Mean ± Std. Dev. *P*	74.2 ± 0.837 0.000	183.6 ± 2.3 0.000	0.82±0.13 0.084	44±1.18 0.000	159.2±1.48 0.000
$5LD_{50}$ of 2KGy (N=10)	Mean ± Std. Dev. *P*	131.2 ± 1.3 0.000	226± 3.16 0.000	1.3±0.0707 0.000	79±3.16 0.000	310.2±0.83 0.000
LD_{50} of 5KGy N=10	Mean ± Std. Dev. *P*	48 ± 0.707 0.000	60± 3.16 0.000	0.72±0.0834 0.47	47±1.58 0.000	89.4±1. 52 0.000
$5LD_{50}$ of 5KGy (N=10)	Mean ± Std. Dev. *P*	52.4 ± 1.14 0.000	91± 1.58 0.000	0.82±0.0834 0.039	55±1.58 0.000	100.4±1.14 0.000
LD_{50} of 10KGy (N=10)	Mean ± Std. Dev. *P*	88 ± 1 0.000	251.8± 1.3 0.000	0.82±0.0834 0.029	53±2.55 0.000	210.6±1.52 0.000
$5LD_{50}$ of 10KGy (N=10)	Mean ± Std. Dev. *P*	96 ± 1.58 0.000	273± 4.69 0.000	1.2±0.0707 0.000	74.4±2.88 0.000	369.4±1.51 0.000

$P \leq 0.001$=highly significant, $P \leq 0.05$=significant, $P > 0.05$ = non-significant, P versus control.

Discussion

No doubt, the snake bites represent a public health concern worldwide (Warrell *et al,* 2003). Snake venom is a complex mixture composed of different substances, such as toxins, enzymes, growth factors activators and inhibitors with a variety of biological activities that cause multiple metabolic disorders, changing cellular and enzymatic activities in animals as well as releasing many pharmacological substances (Al-Sadoon *et al,* 2013; Cherifi and Laraba-Djebari, 2013; Tohamy *et al,* 2014). In this study, the dry venom was irradiated with three different doses of gamma radiation (2, 5 & 10KGy). The energy absorbed from ionizing radiation can inactivate biological material as direct effect on the molecule itself (Rogero and Nascimento, 1995). Alexander and Hamilton (1960) showed that irradiation of proteins revealed damage to aminoacids side chains, production of new groups, splitting of peptide bonds and formation of intramolecular and intramolecular cross-links. In the present study, detoxification of dry venom showed survives of mice which injected with LD_{50} and 5 times of LD_{50} of irradiated venom at 2, 5 &10KGy than crude venom, which agreed with Bennacef-Heffar and Laraba-Djebari (2003) reported that injection of the irradiated venom with two doses of gamma rays (1 & 2 KGy) were four and nine times less toxic than native venom. On the other hand, Samy *et al.* (2015) reported that LD_{50} for native and irradiated (1.5 & 3 KGy) *E. coloratus* venom was 2.88 mg/Kg, 18.47mg/Kg & 22.42mg/Kg respe- ctively. So, the doses increase in LD_{50} after irradiation and decrease in toxicity, these finding like the present results, LD_{50} for native and irradiated venom at 2, 5 & 10 KGy was 1.245mg/g and 6.225 mg/g.

The present results showed change in the protein bands after irradiating the crude *Echis coloratus* at 2, 5 and 10 KGy by subjected to SDS-polyacrylamide gel electro-

phoresis, agreed with Samiy *et al.* (2016) reported that the venom components were altered after irradiation, some protein bands gradually disappeared and the intensity of bands decreased. The levels of ALT and AST enzymes are important in evaluating the degree of liver inflammation and hepatic cells necrosis that increase the hepatic cells permeability resulting in the release of these enzymes in blood circulation (Abdel Moniem *et al,* 2013). The present study showed increase in AST and ALT levels after injection with crude venom. This agreed with Asad *et al.* (2014) and Riaz *et al.* (2015) who showed that *Naja naja karachiensis* venom caused significant increase in ALT and AST levels. Also, our groups injected with different doses of irradiated venom at 2, 5, and 10 KGy showed decrease in the rise of ALT and AST than crude venom, agreed with Hayes and Francis (2001) who reported the resulting in a change in the biological activity of venom after exposure to gamma radiation. Also, Al Asmari**,** *et al.* (2016) reported that the administration of Echis coloratus venom to the rats caused significant increase in AST, ALT and ALP and blood serum creatinine levels, when compared with control, which agreed with the present results.

In the current study, the venom was found to induce high significant increase in the concentration of blood urea in both groups treated with dose of venom compared to control group. The group treated with 10 KGy LD_{50} of venom showed more increasing in blood urea level than group treated with 2 & 5 LD_{50} of venom. Also, the concentration of serum creatinine in both injected groups showed high significant elevation compared to control group. These agreed with El-Missiry *et al.* (2010) reported that the LD_{50} of native venom on the kidney and renal functions induced a highly significant increase in urea and creatinine levels compared to the normal control.

The present results revealed that, the mice which injected with different doses of irradiated venom (2, 5 &10 KG) highly significant increase in CK level in compared to control and amount of CK level was less in these groups compared to amount of CK level in group injected with crude venom, agreed with Rogero and Nascimento (1995) who reported that no toxicity no phospholipase activity and no ability to promote CK. Dissanayake *et al.* (2018) reported that creatinine kinase serves as blood serum indicator of tissue destructions associated with skeletal and cardiac muscles and possible renal dysfunction and inflated levels of CK in blood serum of all envenomed mice regardless to toxic involvement of venom. Hemmaid (2010) found severe histopathological changes after 4hr of *Naja haje* crude venom injection. The present study showed histopathological alternation after 6 hr.

In the present study, inflammatory cells infiltration was observed in the portal area as well as in between the hepatocytes associated with diffuse kupffer cells proliferation in the groups injected with LD_{50} of native venom and 5 times $LD_{50}$of irradiated groups (2, 10 KGy). These findings agreed with studies revealed that *Naja haje* envenoming causes cellular swelling, cytoplasmic granulation and vacuolization in addition to intra hepatic hemorrhage, liver necrosis and activation and hyperplasia of the Kupffer cells (Rahmy and Hemmaid, 2000; Nanayakkara *et al,* 2009; Ghani *et al,* 2010; Tohamy *et al,* 2014; Abdou and Ibrahimm 2015).

On the other hand, there was no change in liver and kidney in groups injected with LD_{50} irradiated venom at 2, 5, 10 KGy and 5 times of LD_{50} at 5 KGy agreed with Abib and Laraba-Djebari (2003) reported that the native venom caused severe degenerative changes in the myocardium but, in case of 2 KGy irradiated venom, no tissue alterations were observed.

The current study showed marked histological changes in renal tissue in the form of focal necrosis of the tubules and glomeruli (infarction) associated with perivascular inflammatory cells infiltration surrounding the

cortical blood vessels as well as degeneration in the tubular lining epithelium agreed with Al-Johany *et al.* (2015) who reported that renal failure can be expected after envenomation by *E. pyramidum* venom with a serious course of intoxication.

Conclusion

The results showed the detoxification of dry *Echis coloratus* venom with 2, 5 & 10 KGy of gamma radiation reduced venom toxicity with an optimum safety 5KGy dose.

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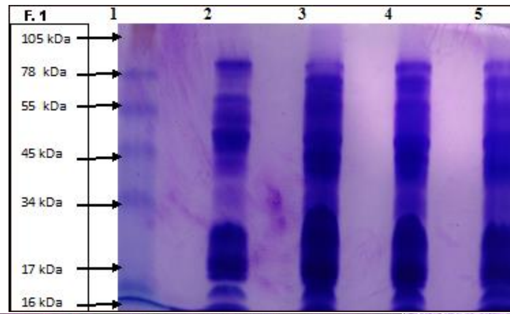
Explanation of figures

Fig. 1: SDS-PAGE of *Echis coloratus* venom. Lane 1 molecular weights of standard proteins, lane 2native non-irradiated venom, lane 3 irradiated venom at 2 KGy, lane 4 irradiated venom at 5 KGy and lane 5irradiated venom at 10 KGy.

Fig. 2: Liver sections of mice a- control shows central vein surrounded by normal hepatocytes b- LD_{50} of native venom shows inflammatory cells infiltration in portal area and between hepatocytes with diffuse kupffer cells proliferation c- LD_{50} 2KGy shows no histological alterations d- 5 LD_{50} 2KGy showing inflammatory cells infiltration with congestion in portal vein and degeneration in hepatocytes e&f- LD_{50}, 5 LD_{50} 5KG & g- LD_{50} 10KGy shows normal h- 5 LD_{50} 10KGy shows massive inflammatory cells aggregation in portal area (H&E x40).

Fig. 3: Kidney sections of mice (A) control showing normal structure of glomeruli and tubules at cortex (B) LD_{50} of native venom showing focal inflammatory cells aggregation in between degenerated renal tubules at cortex (C)) LD_{50} 2KGy showing no histological alterations (D) 5 LD_{50} 2KGy showing degenerative change in lining epithelium of tubules at cortex (E) LD_{50} 5KGy showing no histological alterations (F) 5 LD_{50} 5KGy showing congestion in cortical blood vessels (G) LD_{50} 10KG showing normal renal structure (H) 5 LD_{50} 10KGy showing focal necrosis of tubules and glomeruli (infiltration) at cortical portion.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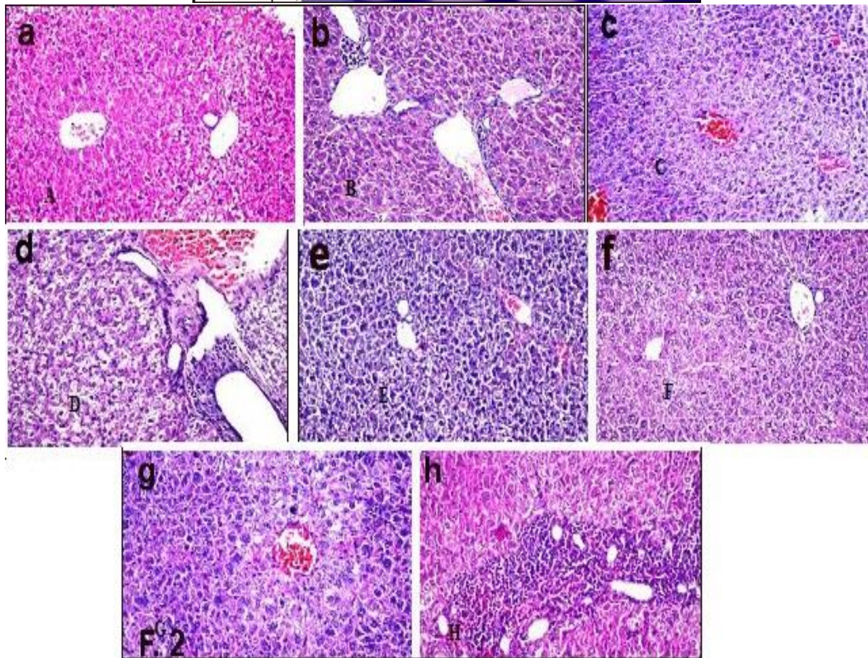
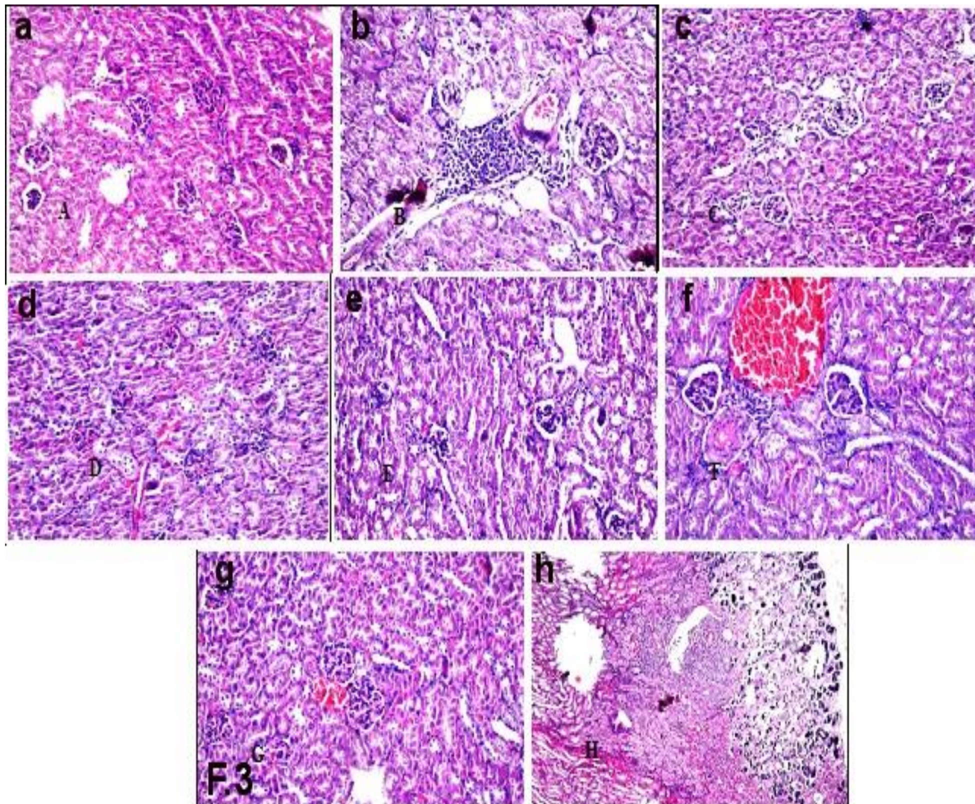
17 kDa
16 kDa


a
b
c
d
e
f
g
h
A
B
C
D
E
F
G
H
F. 2


a
b
c
d
e
f
g
h
A
B
C
D
E
F
G
H
F.3