THE EFFECT OF ETHANOL EXTRACT OF *Hypericum triquetrifolium* ON SOME BIOCHEMICAL AND HISTOPATHOLOGICAL PARAMETERS IN MALE RATS EXPOSED TO HYDROGEN PEROXIDE

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ABSTRACT

Hypericum triquetrifolium is an herbaceous perennial plant and has several bioactive compounds which used for various biological functions. This study is carried out to examine the effects of ethanol extract of *Hypericum triquetrifolium* in rats exposed to 1% H_2O_2 in drinking water. Twenty four healthy male wister rats were used. Animals were aged ranged 3-4 months and weighed 190±25 gm which were divided into four groups (6 animals\ each group); Group I (control): Animals were fed on standard ration without additives. Group II: Animals were treated orally with ethanol extract of HT (300 mg/kg, B.W) daily for 30 days. Group III: Animals were treated orally with ethanol extract HT (300 mg/kg, B.W) with exposure to 1% hydrogen peroxide (H_2O_2) along with drinking water for 30 days. Group IV treated only with 1% H_2O_2 in drinking water. The results showed that Group II had a significant decrease in urea concentration in serum and number of RBCs compared to control III &IV groups. The concentration of MCH and MCHC in group II had significantly lower as compared with control group III&IV. Furthermore, the group II had a significant decrease in the number of total WBCs, granulocytes, and lymphocytes compared to control, III &IV groups. This study also showed that HT had an effect on the sperm morphology which decreased abnormal sperms morphology particularly in the head of sperm. Histological section of testis

revealed degeneration in the germinal layer, congestion in the portal vein and hemorrhage in the interstitial renal tubules degenerative changes in the kidney in groups III and IV. In conclusion, HT had an improvement effect on some biochemical and hematological parameters and removes the deleterious effect of H2O2 on some animal treated tissues.

INTRODUCTION

The genus *Hypericum* (Hypericaceae) is regarded as a large group of herbs comprises of approximately 500 different species (1). Among them, sixteen species are found in Iraq (2). One of the most important species of this genus is *Hypericum triquetrifolium* Turra which has been used in traditional medicine to treat various inflammatory diseases (3). *Hypericum triquetrifolium* Turra is native to the Mediterranean areas, Eastern Europe, Asia, and Africa (3). In Iraq, it is distributed in north and north-west of the country, and commonly known as Roja in local Arabic and Rashik or Swrnatik in Kurdish (4).

Although *Hypericum* species is a poisonous to livestock (5), several studies showed that extract of the aerial parts of *Hypericum triquetrifolium* Turra and its related species are used widely as an antidepressant (6), antioxidant (7), anti-inflammatory (8), antibacterial (9), antifungal (10), antiviral (11), antinociceptive (12), Anticancer (13), and hepatoprotective (14). Furthermore, the extract of the *Hypericum* species have been reported to contain a variety of biological active compounds that may be related to their activity like, naphtodianthrones (hypericin and pseudohypericin), phloroglucinol (hyperforin and adhyperforin), flavonoids, Biflavones, phenolic acids, essential oil, xanthones and amino acids (15, 16).

It has been reported that high level of H2O2 causes excessive cellular damage, induce lipid peroxidation, damage to the DNA, proteins, lipids and cell death (17, 18). Herbal natural medicines are being utilized to treat a variety range of diseases among population. Herbal remedies had many advantages including harmless than allopathic medicine, easier availability and limited side effects (19). Many various studies have suggested that *Hypericum triquetrifolium Turra* is used as a treatment for burns and gastroenteritis as anti-inflammatory drug (20). As it is well know that *Hypericum triquetrifolium Turra* act as antioxidant which plays an important role for the prevention of diseases (21). Therefore, *Hypericum triquetrifolium Turra* can be suggested as a plant

for large interest. The purpose of this study is to evaluate the potential effects of ethanol extract of *Hypericum triquetrifolium Turra* on some liver and kidney function parameters in male rats exposed to 1% of hydrogen peroxide in drinking water.

MATERIALS AND METHODS

Experimental Design:

The University of Duhok, College of Veterinary Medicine Ethical Review Committee approved the study in 2016. A total of 24 male Wistar rats aged 3-4 months and weighed 190±25 gm were studied. Animals were divided into four groups (six for each). Group 1(control group) fed with diet only without treatment. Group 2 treated orally with ethanol extract of HT (300 mg/kg, B.W) daily for 30 days. Group 3 treated with daily ethanol extract HT (300 mg/kg, p.o.) with exposure to 1% hydrogen peroxide (H_2O_2) along with drinking water for 30 consecutive days. Group 4 (n=6) just exposed to 1% H_2O_2 along with drinking water without treatment. The animals were housed under standard conditions at an ambient temperature of 20-25°C and 12 hours light/ dark cycle. The animals had free access to commercial pellet food (AL-AMADI AL-AMEEN company, No.2. Amedy, Duhok, Kurdistan, Iraq) and tap water ad libitum and were kept in animal house 7 days before starting treatment.

Preparation of ethanol extracts:

Aerial parts of *H. triquetrifolium* were collected from the Sumel district within Duhok province, Kurdistan regional government, Iraq in June 2016. The aerial parts of the plant were dried in a dark place for ten days at room temperature. The plant material (100 gm) was grounded by an electric blender. Then it was extracted with methanol 100% by the Soxhlet apparatus. The crude extract (23 gm), the dark reddish color was obtained after solvent evaporation. It was kept in the dark glass bottles at 4°C until it was used (22).

Blood collection

Blood samples were collected from orbital venous plexus overnight fasted rats. During blood sampling, the animal was under light ether anesthesia. The serum was obtained by centrifuging blood samples at 2000 rpm for 15 min. Serum samples were stored at -20°C until analysis.

Measurement of biochemical parameters:

Biochemical parameters, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), Alkaline phosphatase (ALP), creatinine, and urea were measured by using a clinical chemistry analyzer, TBA-80FR NEO (Toshiba Medical Systems Co. Ltd., Tokyo, Japan).

Counting of blood

For hematological analyses, 1 ml of blood was collected in a test tube with an anti-coagulant substance (EDTA). Auto hematology analysis (A Coulter Counter, Mindary, Hamburg, Germany) was used for complete blood count. This procedure was performed in the clinical pathology laboratory at college of veterinary medicine, University of Duhok.

Histopathological measures

The animals were sacrificed following slight ether anesthesia. The small portions of the liver, kidney, and testis were removed and were fixed in a 10% buffered formaldehyde solution. Accordingly, the samples at 6 micrometers were stained with hematoxylin and eosin (H&E) and studied under a light microscope for histological analysis (23).

Sperm Analysis

Epididymal sperm suspension was prepared in 2 ml of phosphate-buffered saline (PBS) at the temperature 37 °C and pH 7.2. The caudal part of the epididymis was cut in a sterile Petri dish contained PBS. The motility and abnormalities of the spermatozoa were analysed according to the previous study (24). Thin smears of sperm suspension were prepared from different groups of animals and stained 1 volume was mixed with 2-volume of 1% eosin and nigrosen stain. For each smear about 400 sperms/animals being scored (25).

Statistical analysis

The results were presented as mean ± SD. All data were analysed using Statistical Package for Social Sciences version 25, software (SPSS 25; IBM Corp; USA). The comparison of biochemical and hematological parameters between groups was performed in ANOVA-one way. The pairwise comparison was examined in post-hoc LSD

comparison test. The *P*-value of less than 0.05 was considered a statistically significant difference.

RESULTS

This study found that serum glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) was not significantly different among groups ($P>0.05$, Table 1). Furthermore, the results showed no significant difference of serum alkaline phosphatase (ALP) among groups ($P>0.05$, Table 1). In addition, concentration of serum creatinine was not different among groups ($P>0.05$, Table 1). However, the study showed that concentration of urea increased significantly in group 3 and 4 as compared to group 1 and 2 ($P<0.05$, Table 1).

Table 1: Effects of Ethanol extract of *H.triquetrifolim* on serum liver and kidney function tests (Mean ± SD)

Biochemical Parameter	Group of animals				P-Value (two-sided)
	Group 1 (Control)	Group 2 (Ethanol Extract of HT)	Group 3 (Ethanol Extract of HT and H2O2)	Group 4 (1% H2O2)	
GPT IU/L	35.50±2.88[a]	36.67±2.66[a]	37.33±11.29[a]	40.50±7.06[a]	0.641
GOT IU/L	190.83±7.36[a]	186.67±24.43	190.83±29.05a	218.33±27.87[a]	0.114
ALP IU/L	62.24±5.29[a]	61.60±9.60[a]	59.62±7.34[a]	62.62±8.06[a]	0.910
Urea mg/dl	31.60±4.41[a]	28.58±6.35[a]	52.23±6.17[ab]	49.47±9.72[b]	**<0.001**
Creatinine mg/dl	0.68±0.06[a]	0.65±0.09 [a]	0.65±0.06 [a]	0.65±0.10 [a]	0.878

One-way ANOVA was performed for statistical analyses. Values in the same row with superscripts, a,b,c differed from control (a,b,c $P<0.001$; aa$P>0.05$; bc $P>0.05$).

The study showed that the number of RBCs in group IV were significantly lower than group I and group II ($P<0.05$, Table 2). Concentration of both MCH and MCHC increased significantly groups III as compared to group I, II and IV ($P<0.05$, Table 2). In terms of WBCs, this results found that groups III, IV and III had higher number of WBCs

than group I and II (P <0.05, Table 2). It should be noted that there was no significant difference of Hb, PCV% and MCV found among groups (P >0.05, Table 2).

Table 2: Effects of Ethanol extract of *H.triquetrifolim* on hematological parameters (Mean ± SD)

Hematological Parameters	Group of animals				*P*-Value
	Group I (Control)	Group II (HT)	Group III (HT and H2O2)	Group IV (1% H2O2)	
Hb g/dL	15.90± 1.65[a]	15.63± 1.76[a]	14.82± 1.15[a]	13.60± 2.13[a]	0.120
RBC count 106/mm3	8.62±0. 76 [a]	8.43±1. 02a	7.68±0. 34 [ab]	6.93±1. 49 [b]	**0.031**
PCV %	47.57± 3.99[a]	46.67± 5.17[a]	44.44± 0.90[a]	41.52± 6.25[a]	0.147
MCV fL	55.27± 3.06[a]	55.45± 2.60[a]	58.16± 1.67[a]	56.04± 4.77[a]	0.380
MCH pg	18.30± 0.28 [b]	18.53± 0.41[b]	19.89± 0.19 [a]	18.38± 1.38 [b]	**0.030**
MCHC g/dL	33.35± 1.32 [b]	33.45± 0.99 [b]	34.74± 0.51 [a]	32.70± 0.89 [b]	**0.024**
WBC count 103/mm3	11.53± 1.09 [b]	11.82± 2.48 [b]	13.93± 1.86 [b]	17.82± 4.06[a]	**0.002**

One-way ANOVA was performed for statistical analyses. Values in the same row with superscripts, a,b differed from control (a,b P<0.001; aa$P$ >0.05; ab P >0.05).

The study showed that the number of platelets increased significantly in group IV as compared to group II and III (P <0.05, Table 3). In addition, this study found that the number lymphocytes increased significantly in groups IV compared to groups I and II (P <0.05, Table 3). This results showed that the number granulocytes increased significantly in groups IV compared to groups I, II and III(P <0.05, Table 3).

Table 3: Effects of Ethanol extract of *H.triquetrifolim* on hematological parameters (Mean ± SD)

Parameters	Group of animals				*P*-value
	Group I (Control)	Group II (HT)	Group III (HT and H2O2)	Group IV (1% H2O2)	
Lymphocele (%)	64.62±2.93 [a]	63.00±11.50 [a]	65.63±8.16 [a]	55.83±4.13 [a]	0.133
Monocyte (%)	4.30±0.88 [a]	4.20±1.01 [a]	3.58±0.60 [a]	3.78±0.69 [a]	0.394
Granulocyte (%)	31.08±2.82 [a]	32.80±11.30[a]	30.78±8.21 [a]	40.43±1.24 [a]	0.225
Platelets	559.83±65.72 [a]	486.33±51.65[b]	543.33±72.83 [b]	606.60±43.90 [a]	**0.029**
Lymphocele (no.)	7.42±0.19 [b]	6.56±0.71[b]	9.16±0.56[ab]	9.86±1.81 [a]	**0.001**
Granulocyte (no.)	3.57±0.38 [b]	3.90±1.69 [b]	4.26±1.23 [b]	7.30±2.28 [a]	**0.002**
Monocyte (no.)	0.50±0.13 [a]	0.47±0.01 [a]	0.50±0.09 [a]	0.66±0.16 [a]	**0.052**

One-way ANOVA was performed for statistical analyses. Values in the same row with superscripts, a,b differed from control (a,b $P<0.001$; aa $P>0.05$; ab $P>0.05$)

In terms of sperm abnormalities, this study showed that *H.triquetrifolim* had an effect on the sperm abnormalities (Table 4). The results showed that groups II, III and IV had significantly lower number of abnormal sperm heads (Pin heads and Swollen heads) as compared to control groups (P<0.05, Table 4). The number of double heads was not different between groups (P<0.05, Table 4). This study found that groups II, III and IV had significantly increased number of sperms abnormality including ribon heads and hookless heads as compared to group I (P<0.01, Table 4). The number of sperms with irregular heads decreased significantly in groups II and III, but increased significantly in groups IV as compared to groups I (P<0.001, Table 4). It is interesting to note that the number of sperms with long and broad hook increased significantly in groups II and IV and decreased significantly in groups III as compared to groups I (P<0.001, Table 4). Some of abnormalities of sperms are shown in Fig.1.

Table 4: Effects of Ethanol extract of HT; HT+H_2O_2 and 1% H_2O_2 on sperms morphology (Mean ± SD)

Sperm	Group of animals				P-Value
	Group I (Control)	Group II (HT)	Group III (HT and H2O2)	Group IV (1% H2O2)	
Parameters of the sperm head					
Pin	6.0±0.0 [a]	4.0±1.0 [b]	5.3±0.6 [c]	4.7±0.6 [d]	**0.026**
Swollen	11.0±1.0 [a]	8.0±1.0 [b]	6.3±0.6 [c]	7.0±1.0 [c]	**0.001**
Double	2.0±1.0 [a]	1.0±0.0 [a]	1.3±0.6 [a]	1.7±0.6 [a]	0.330
Ribon	1.3±0.6[a]	4.7±0.6 [b]	4.7±0.6 [c]	7.7±0.6[d]	**<0.001**
Irregular	7.3±0.6 [a]	6.3±0.6 [b]	5.3±0.6[c]	10.3±0.6 [d]	**<0.001**
Hookless	10.3±0.6[a]	13.3±1.2[b]	13.3±0.6[bc]	11.3±0.6[d]	**0.003**
Long and broad hook	3.3±0.6[a]	4.0±0.0 [b]	1.3±0.6 [c]	14.7±0.6 [d]	**<0.001**
Parameters of the sperm tail					
Double	1.0±0.0	0.0±0.0	1.0±0.0	1.0±0.0	n.a.
coiled	9.0±0.0	9.0±0.0	5.0±0.0	22.0±0.0	n.a.
pseudo droplet	4.0±0.0	0.0±0.0	1.0±0.0	7.0±0.0	n.a.
corkscrew	2.0±0.0	0.0±0.0	1.0±0.0	3.0±0.0	n.a.
bend mid piece	5.0±0.0	11.0±0.0	5.0±0.0	24.0±0.0	n.a.

n.a.: The *P*-value was not applicable since there was no difference in the Sta. deviation of the study groups.

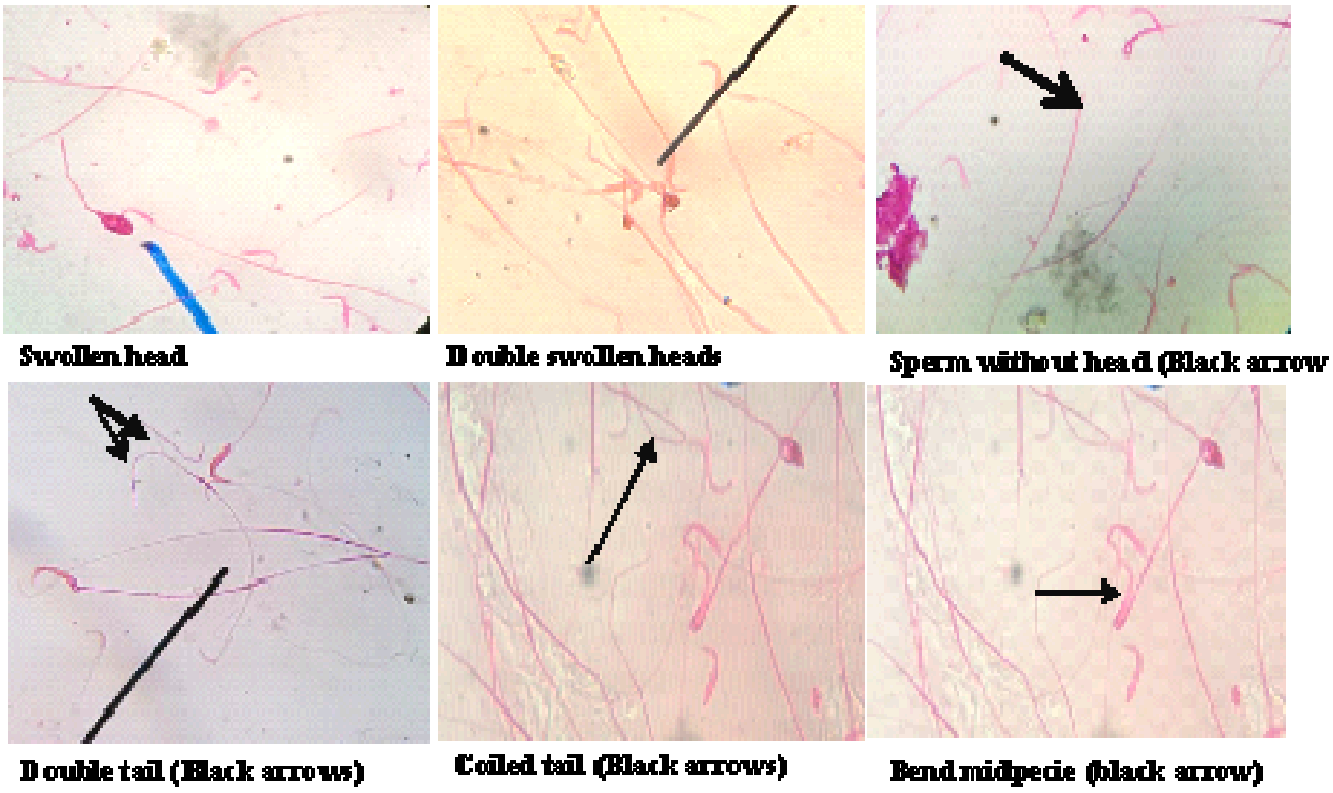

Figure1: Some Sperm abnormalities, based on their morphology (×1000)

Histopathological Study

The histological section of the liver of the control group illustrated with normal hepatocyte, Fig1,A. The results showed that there was a slightly congestions occurred in the portal vein and in the sinusoids as well in animals when treated with *Hypericum triquetrifolium* (300 mg/kg), Fig.1B. It should be noted that the congestion in the portal veins and sinusoids were slightly high in group of animals treated with (*Hypericum triquetrifolium* and 1% H_2O_2), compared to control and groups II. In addition, there was an infiltration in inflammatory cells beside proliferation of Kupffer cells were seen in this group, Fig.1C. Histological section of the liver in rats treated with 1% H_2O_2, showed degenerative necrotic changes of hepatocytes with dilatation and congestion of sinusoids Fig.1D.

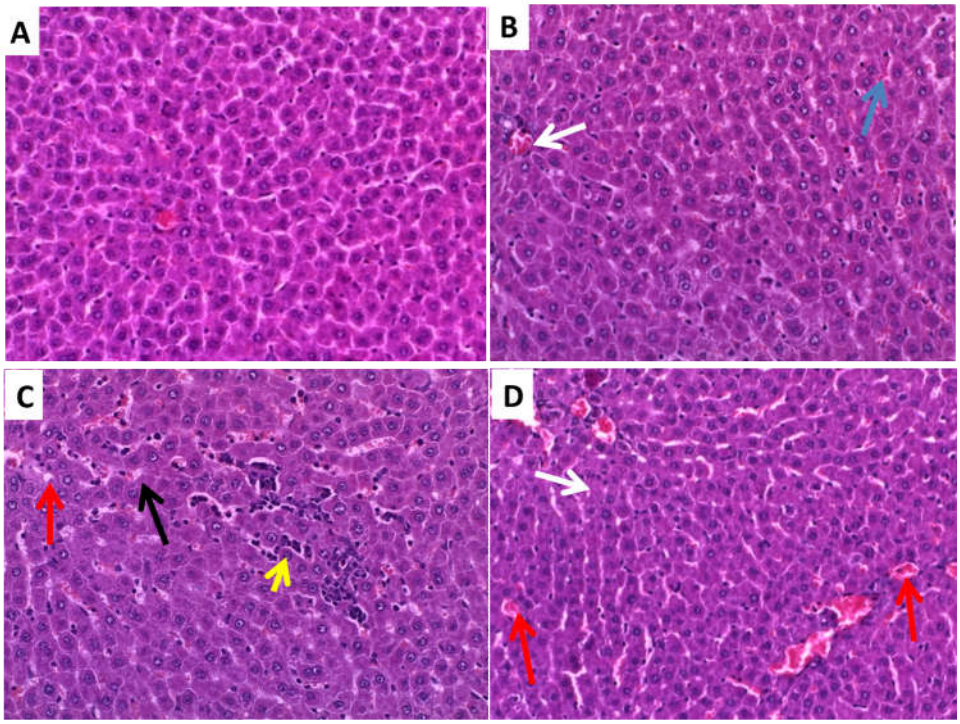

Figure1: Micrograph of rat's liver. A: Section from control liver rats showed normal histological appearance of Hepatocytes H&E10x; B: Section from liver rats received HT showed slight normal structure of hepatocytes with slight congestion of portal vein (white arrow) and sinusoides (blue arrow) H&E 10x; C: Section from liver rats received HT with H_2O_2showed slight normal structure of hepatocytes with slight congestion of portal vein and sinusoids (black arrow), with infiltration in inflammatory cells (yellow arrow) beside proliferation of kpffer cells (red arrow) H&E 10x; D: Section from liver rats received H_2O_2 showed degenerative necrotic changes of hepatocytes (white arrow) with dilatation and congestion of sinusoids (red arrows) H&E 10x.

The section of the Kidney in control group illustrated with normal renal tubules Fig. 2A. Histological section of kidney in rat treated with *Hypericum triquetrifolium* (300 mg/kg) showed mild hemorrhage in the Interstitial renal tubules with mild degenerative changes, Fig.2B. Histological section of kidney form animals treated with (*Hypericum triquetrifolium* and 1% H_2O_2) characterized by mild hemorrhage in the interstitial renal tubules with mild degenerative changes of renal tubules and in glomeruli as well Fig. 2C. Histological section of kidney from animals treated with 1% H_2O_2 showed that there was sever hemorrhage in the interstitial renal tubules and mesenchymal area of glomeruli with infiltration of inflammatory cells, Fig.2D.

The histological section of the tests in control group illustrated with normal structure of the seminiferous tubules and a large number of spermatozoa seen in the lumen of the tubules with normal germinal layers in control group, Fig. 3-A. Section of testis in animals treated with *Hypericum triquetrifolium* showed degeneration in the

germinal layer in the tubules, with wide space between seminiferous tubules were seen between the tubules Fig.3-B. Histological section of the testes in group III and IV denegation in the germinal layer in the tubules and the space between seminiferous tubules were more developed with abnormal between the tubules Fig 3 C&D.

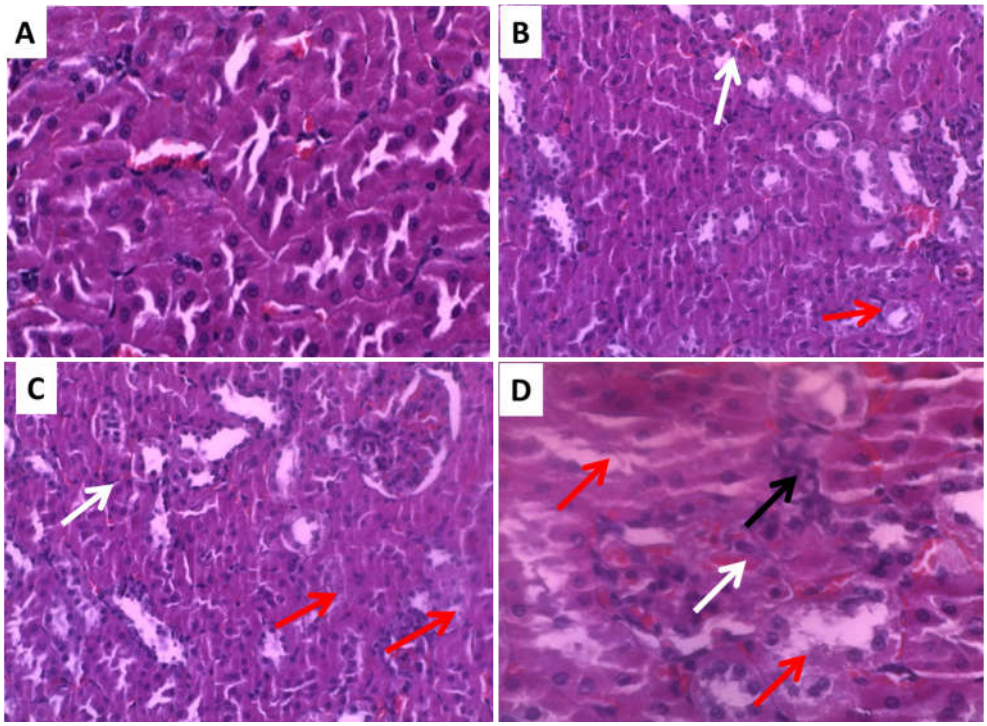

Figure 2: Micrograph of kidney of rat: A: Section from control kidney rats showed normal structure of renal tubules H&E 20x; B: Section from kidney rats treated with HT alone showed mild hemorrhage in the interstial renal tubules (white arrow) and mild degenerative changes (red arrow) H&E 20x; C: Section from kidney rats treated with HT+ H_2O_2 showed mild hemorrhage in the interstial renal tubules (white arrow) and mild degenerative changes of renal tubules and glomerlui (red arrows) H&E 20x;D: Section from kidney rats treated with H_2O_2 showed sever hemorrhage in the interstial renal tubules (white arrow), sever degenerative and necrosis of renal tubules (red arrows), infiltration of inflammatory cells (black arrow) H&E 20x.

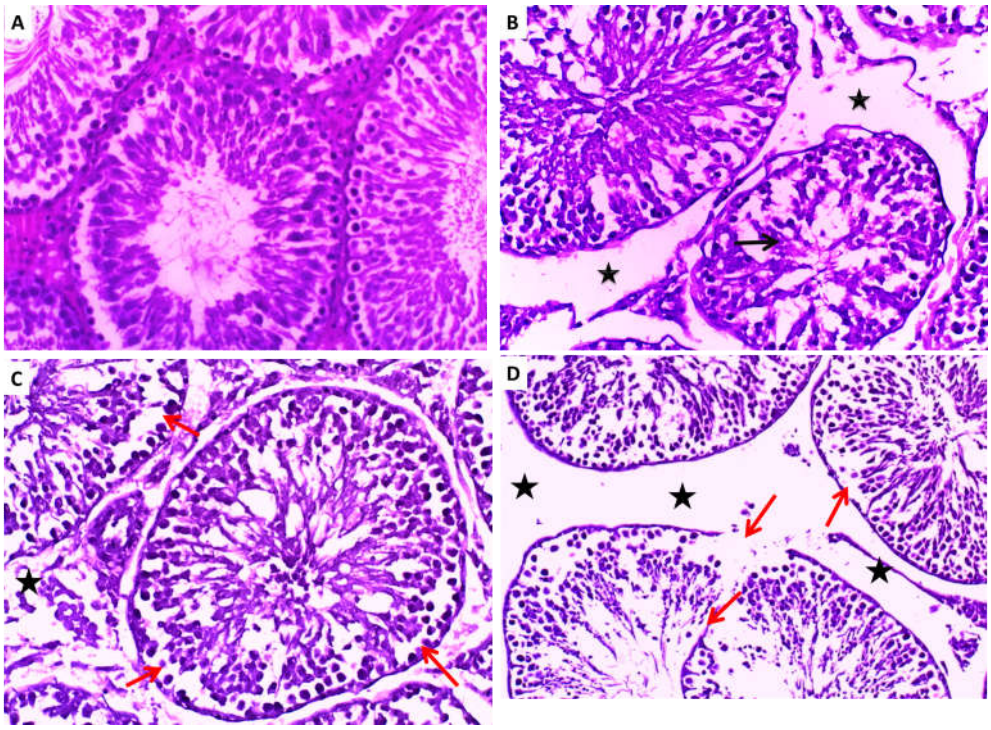

Figure 3: Histo-morphology of the testis in rats. A: Control groups illustrated with normal testis (H&E 20X). B: Group treated with HT (300 mg/kg) showed wide interstitial space between lobules (black star), with low number of spermatozoa in the center of each tubules (black arrow, H&E 20X). C: Group of animals treated with HT (300 mg/kg) + 1% H_2O_2 showed with denegation in the germinal layer in the tubules (red arrows) with space between seminiferous tubules (black star, H&E 20X). D: Animals treated only with 1% H_2O_2 had more space developed between seminiferous tubules (black star), degeneration and sloughing in the germinal layers in the tubules (red arrows, H&E 20X).

DISCUSSION

There are many previous studies supported that *Hypericum triquetrifolium* used as antioxidant supplementation for decreasing the level of oxidative stress in order to prevent the development of complications relating to the diseases (7,26) . However, very few studies have published about how the *Hypericum triquetrifolium* affect the physiological conditions of male rat. Therefore this study aimed to determine the physiological and histological effect of *Hypericum triquetrifolium* ethanol extraction on laboratory male rat.

The study showed that *Hypericum triquetrifolium* had an effect on the physiological conditions in male rats. This study demonstrated that *Hypericum triquetrifolium* decreased concentration of urea compared to treated groups of animals by nearly about one fold. However, this study reported that *Hypericum triquetrifolium* had no effect on the concentration of serum creatinine. The results of this study disagreed with the results

of the previous study (36) which reported that concentration of uric acid and creatinine increased in groups of animal were treated with *Hyperricum*. It has been investigated that *Hypericum* plant act as anti-inflammatory drug (36). The results of the previous study showed that gentamycin induced nephron damage in mice and accompanied by inducible nitric oxide synthase enzyme (iNOS) in the kidney (37,38). The same study recorded that Hypericum perforatum extract decreased the concentrations of iNOS enzyme caused by gentamicin and lead to decreased nephrotoxicity (37). The results of the current study indicated that HT may have protective role in the damage of kidney and liver by restoring antioxidants and followed by reducing lipid peroxidation in renal and hepatic tissue.

Moreover, this study found that *Hypericum triquetrifolium* had no effect on GPT, GOT and ALP as recorded by (27) who used different concentrations (1000, 2000, and 3000 mg/kg body) of an aqueous extract of *Hypericum triquetrifolium*. The results of the present study appeared no significant differences in liver enzymes activity and that may be attributed to the concentrations *Hypericum triquetrifolium* 300 mg/Kg, which are much lower than used in the previous study (27). It is interesting to note that the lower dose of *Hypericum triquetrifolium* used in this study may sufficient to affect the level of urea. In addition, another previous study reported that *Hypericum triquetrifolium* has an effect on the reduction of concentrations of serum liver enzymes compared to the control groups (28).

The present study showed that the number of RBCs in groups, II, III and IV had lower number of RBCs than control groups. The results of the present study was parallel to the results of the previous study (29) which investigated that *Hypericum triquetrifolium* had significantly decreased the number of RBCs and increased in the number of Bone marrow nucleated cells. It has been reported that *Hypericum* triquetrifolium seed extract, especially its biologically active compounds, may be great candidates for alternative adjuvant chemotherapy for decreasing the Cyclophosphamide induced toxicity (29).

This study showed that the number of leukocytes particularly lymphocytes and granulocytes increased in groups of animals treated with *Hypericum triquetrifolium*+ 1% H2O2 and 1% H2O2 compared to control groups. This is could be due to the presence of some active gradients in the extract which stimulate of immunity as marked by significantly increased lymphocytes count. It has been documented that the main

gradients of *Hypericum triquetrifolium* is Hyperforin, hypericin, and pseudohypericin and flavonoids (29). They reported that the flavonoids compounds are kaempferol, rutin, hyperoside, quercitrin, quercetin (29). These results agreed with the study by (30), which reported that *Hypericum triquetrifolium* increased the blood cells count especially lymphocytes. It has reported that *Hypericum triquetrifolium* +cyclophosphamide had an optimum protective on erythrocyte cells. While, using 100 mg/kg dose of *Hypericum triquetrifolium* act as a toxic for erythrocyte cells (30). Because they cyclophosphamide declined the number of bone marrow nucleated cells. However, the number of bone marrow nucleated cells increased significantly when treated with HT+CP. It can be concluded that HT has a protective effect on bone marrow nucleated cells (30). Moreover, another study (31) explored the toxic effect of *Hypericum triquetrifolium* (2, 1, and 0.25 g/kg) on the bone marrow and spermatozoa cells of Swiss albino mice.

Results of the present study appeared that ethanol extract *Hypericum triquetrifolium* decreased the sperm abnormalities especially in the head portion such as Pin, swollen, Irregular, Hookless and long and board hook heads compared to control group. However, this study investigated *Hypericum triquetrifolium* extract had no effect on the sperm abnormalities in the tail defect. This is disagreement with study (31-33) reported that the aqueous *Hypericum triquetrifolium* extract increased head abnormalities but statistically not significant in albino mice. In addition, the same study found that *Hypericum triquetrifolium* increased sperm abnormalities in the tail region such as pseudo-droplet defect, corkscrew midpiece defect, bent midpiece defect, total abnormal midpiece and tail defect. This is disagreement with the present study reported that *Hypericum triquetrifolium* had no effect on sperm tail defect. This may be due to (31,33) used high concentration of *Hypericum triquetrifolium* (0.25-2g/kg).

The histological analysis liver of *Hypericum triquetrifolium* group rats revealed that slight normal structure of hepatocytes with slight congestion was seen in the portal vein and sinusoids. It should be noted that the current study showed moderate degenerative changes, slight hemorrhage, and most tubules appear normal in group of animals when treated with *Hypericum triquetrifolium* and H_2O_2. The similar results have been reported by (34), they reported that the effects ethanol extracted *Hypericum triquetrifolium* on the liver and kidney functions in rabbits. They reported histopathological changes in the liver

and kidney function of the experimental group as compared to the control group. The liver lesions included inflammatory cell infiltration in mononuclear cells of sinusoids, central vein and portal artery. They were associated with necrosis and fatty changes in the hepatocytes.

The present study investigated that *Hypericum triquetrifolium* increased the hemorrhage in the intrestial renal with degenerative changes was seen. Moreover, section of kidney from group of animals treated with *Hypericum triquetrifolium* + H_2O_2 had severed hemorrhage in the renal tubules and glomeruli with an infiltration of inflammatory cells were seen in animals treated with H_2O_2. This means that *Hypericum triquetrifolium*+H_2O_2 may increase local infection due to increase number of leukocytes especially lymphocytes. This is parallel to (27, 35) illustrated that degenerative alterations in the renal tubules, cloudy swelling, with infiltration of lymphocytes, and interstitial edema of rat kidney.

The present study reported that groups of animals treated with Hypericum triquetrifolium (300 mg/kg) illustrated that wide interstitial space between lobules noticed with low number of spermatozoa in the center of each tubules were reported as compared to control groups. Furthermore, this study demonstrated that denegation in the germinal layer in the tubules with space between seminiferous tubules in groups treated with (*Hypericum triquetrifolium* (300 mg/kg) + 1% H2O2, and large space between seminiferous tubules and degeneration and degradation in the germinal layers in the tubules with slightly empty of seminiferous lumen were seen in animals when treated with 1% H2O2 alone. This is in agreement with (27) reported considerable changes in the histology of testis and reported degradation in the germinal layer.

CONCLUSION

The present study concluded that the ethanol extract of *Hypericum triquetrifolium* with its mixture gradients appears to have effect on kidney function test for instant urea, induced the immunity by increasing leukocytes count particularly lymphocytes. In addition, this product seems to induce sperm abnormalities. According to our knowledge there is no data exist on the effect of this product on semen in vivo to be compared. For that reason further experiments must be done in order to identify the mechanism by which *Hypericum triquetrifolium* exerts their activity.

تأثير مستخلص الايثانول لنبات *Hypericum triquetrifolium* على بعض المعايير الكيموحيوية و التغيرات النسيجية في الجرذان المعرضة لبيروكسيد الهيدروجين

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الخلاصة

Hypericum triquetrifolium هو نبات عشبي معمر، يمتلك عدة مركبات حيوية ويستخدم في العديد من الوظائف الحيوية. كان الهدف هذه الدراسة معرفة تأثيرات مستخلص الايثانول لنبات (HT) *Hypericum triquetrifolium* في الجرذان المعامله ببيروكسيد الهيدروجين(H_2O_2) بتركيز ١% مع مياه الشرب. أستخدمت في هذه الدراسة (٢٤) من ذكورالجرذان والتي تتراوح اعمارهم بين ٣-٤ أشهر ومعدل اوزان ١٩٠±٢٥ غرام. تم تقسيم الحيوانات الى أربعة مجاميع (٦ حيوانات / كل مجموعة) ، المجموعة الاولى (مجموعة السيطرة) التي تناولت غذائها القياسية بدون اي إضافة .المجموعة الثانية عوملت عن طريق الفم بمستخلص الايثانول لنبات HT (٣٠٠ ملغم / كغم من وزن الجسم) يومياً لمدة ٣٠ يوماً. المجموعة الثالثة عوملت عن طريق الفم بمستخلص الايثانول لنبات HT (٣٠٠ ملغم /كغم من وزن الجسم) + ١% من بيروكسيد الهيدروجين (H_2O_2) المضاف الى ماء الشرب لمدة ٣٠ يوماً. المجموعة الرابعة عوملت فقط ب ١% من H_2O_2 المضاف الى ماء الشرب. أظهرت النتائج أن المجموعة II وIIIوIV لديها انخفاض معنوي في تركيز اليوريا في المصل مقارنة بالمجموعات السيطرة. انخفض عدد كرات الدم الحمراء في المجموعتين III وIV بشكل ملحوظ مقارنة بالمجموعات الضابطة والثانية. بينما التراكيز هيموغلوبين الكريّة الوسطيّ و متوسط تركيز الهيموجلوبين في المجموعة III أظهرت ازدياد معنوياً بالمقارنة مع مجموعةالتحكم و II و IV . أضافةً الى ذلك، ادت مجموعة III ازدياد معنوياً في اعداد كريات الدم البيض الكلي ، الكريات الحبيبية والخلايا اللمفاوية بالمقارنة مع مجموعة التحكم II و IV. ومن الجدير بالذكر أظهرت هذه الدراسة ان مستخلص *HT له تاثير*على شكل الحيامن خاصة في رأس الحيمن .كذلك لوحظ ضرر في نسيج الخصية حيث ادى مستخلص النبات الى تنكس في الطبقة الجرثومية في الخصية وبعض التغييرات في نسيج كل من الكبد و الكلية.نستنتج هذه الدراسة ان المستخلص الكحولي *Hypericum triquetrifolium* له تأثير معنوي على بعض المعايير الكيموحيوية ومن ضمنها تركيز اليوريا، وبعض المعايير الدموية والمناعية وعلى شكل الحيوانات المنوية والتغييرات النسيجية لكل من تسيج الخصية، الكبد و الكلية في المجموعتين الثالثة والرابعة. في الختام ، كان لـ HT لها تأثير تحسني على بعض المعايير البيوكيميائية والدموية ويزيل التأثير الضار لـ H2O2 على بعض الأنسجة المعالجة الحيوانية.

REFERENCES

1-Crockett SL, Robson NK.(2011). Taxonomy and chemotaxonomy of the genus Hypericum. Medicinal and aromatic plant science and biotechnology. 5(Special Issue 1):1.

2- Al-Mukhtar J.(1975). Hypericum plant. Directorate plant. Bulletin. (231):2-15.

3-Robson NK.(2002). Studies in the genus Hypericum L.(Guttiferae) 4 (2). Section 9. Hypericum sensu lato (part 2): subsection 1. Hypericum series 1. Hypericum. Bulletins of the Natural History Museum: Botany Series. 32(2):61-123.

4-Chakravarty A-RH.(1988). Medicinal plants of Iraq, Ministry of Agriculture and irrigation, State Board for Agricultural and Water Resources Research. National Herbarium of Iraq, Baghdad. 93.

5-Radostits OM, Gay CC, Hinchcliff KW, Constable PD. (2006). Veterinary Medicine E-Book: A textbook of the diseases of cattle, horses, sheep, pigs and goats: Elsevier Health Sciences.

6-Bach-Rojecky L, Kalođera Z, Samaržija I.(2004). The antidepressant activity of Hypericum perforatum L. measured by two experimental methods on mice. Acta pharmaceutica. 54(2):157-62.

7-Kızıl G, Kızıl M, Yavuz M, Emen S, Hakimoğlu F.(2008). Antioxidant Activities of Ethanol Extracts of Hypericum triquetrifolium. and Hypericum scabroides. Pharmaceutical Biology. 46(4):231-42.

8-Saad B, Embaslat W, Abu-Farich B, Mahajna S, Azab M.(2016). Hypericum triquetrifolium extracts modulate IL-6, IL-10 and TNF-α protein and mRNA expression in LPS-activated human peripheral blood mononuclear cells and THP-1-derived macrophages. Med Aromat Plants S. 3:2167-0412.

9-Pistelli L, Bertoli A, Morelli I, Menichini F, Musmanno RA, Maggio TD, Coratza G.(2005). Chemical and antibacterial evaluation of Hypericum triquetrifolium Turra. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 19(9):787-91.

10-Fraternale D, Bertoli A, Giamperi L, Bucchini A, Ricci D, Menichini F, Trinciarelli E, Pistelli L. (2006). Antifungal evaluation of Hypericum triquetrifolium polar extracts against Fusarium spp. Natural Product Communications. 1(12):1934578X0600101209.

11-Rouis Z, Abid N, Koudja S, Yangui T, Elaissi A, Cioni PL, Flamini G, Aouni M.(2013). Evaluation of the cytotoxic effect and antibacterial, antifungal, and antiviral activities of Hypericum triquetrifolium Turra essential oils from Tunisia. BMC complementary and alternative medicine. 13(1):24.

12-Apaydın S, Zeybek U, Ince I, Elgin G, Karamenderes C, Ozturk B, Tuglular I.(1999). Hypericum triquetrifolium Turra. extract exhibits antinociceptive activity in the mouse. Journal of ethnopharmacology. 67(3):307-12.

13-Dongre SH, Badami S, Godavarthi A.(2008). Antitumor activity of Hypericum hookerianum against DLA induced tumor in mice and its possible mechanism of action. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 22(1):23-9.

14-Bayramoglu G, Bayramoglu A, Engur S, Senturk H, Ozturk N, Colak S.(2014). The hepatoprotective effects of Hypericum perforatum L. on hepatic ischemia/reperfusion injury in rats. Cytotechnology. 66(3):443-8.

15-Patocka J.(2003). The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb Hypericum perforatum L. Journal of Applied Biomedicine. 1(2):61-70.

16-Saddiqe Z, Naeem I, Maimoona A.(2010). A review of the antibacterial activity of Hypericum perforatum L. Journal of ethnopharmacology. 131(3):511-21.

17-Ward JF, Blakely WF, Joner EI.(1985). Mammalian cells are not killed by DNA single-strand breaks caused by hydroxyl radicals from hydrogen peroxide. Radiation research. 103(3):383-92.

18-Rodrigo R, González J, Paoletto F.(2011). The role of oxidative stress in the pathophysiology of hypertension. Hypertension Research. 34(4):431-40.

19-Mensah ML, Komlaga G, Forkuo AD, Firempong C, Anning AK, Dickson RA.(2019). Toxicity and Safety Implications of Herbal Medicines Used in Africa. Herbal Medicine, 63.

20-Saad B, Abouatta BS, Basha W, Hmade A, Kmail A, Khasib S, Said O.(2011). Hypericum triquetrifolium-Derived Factors Downregulate the Production Levels of LPS-Induced Nitric Oxide and Tumor Necrosis Factor-α in THP-1 Cells. Evid Based Complement Alternat Med. 13, 24.

21-Okmen G, Balpınar N.(2017). The biological activities of Hypericum perforatum L. African Journal of Traditional, Complementary and Alternative Medicines.14(1):213-8.

22-Aghel NA, Ramezani ZA, Jalalitalab HA.(2012). Isolation and determination of hypericins from different parts of Hypericumtriquetrifolium Turra grown in Khozestan by UV/Visible spectrophotometry. Jundishapur Scientific Medical Journal. 1(3):285-94.

23-Luna, (1968). Manual of histolologic staining methods of the armed forces Institute of pathology, (3 rd ed.) Mc Gran Hill book company. 34, Meruelo, D., Lavie, G. and Lavie, D.

24-Wyrobek A, Bruce W.(1975). Chemical induction of sperm abnormalities in mice. Proceedings of the National Academy of Sciences. 72(11):4425-9.

25-Hamadouche NA, Sadi N, Kharoubi O, Slimani M, Aoues A.(2013). The protective effect of Vitamin E against genotoxicity of Lead Acetate intraperitoneal administration in male rat. Archives of Biological Sciences. 65(4):1435-45.

26-Tandon V, Gupta R.(2005). Effect of Vitex negundo on oxidative stress. Indian journal of pharmacology. 37(1):38.

27-Mustafa YS, Maulood KA.(2019). Protective Effect of Hypericum Triquetrifolium Aqueous Extract on Biochemical and Histopathological Parameters in Hyperlipidemic Male Rats. Kirkuk University Journal /Scientific Studies (KUJSS). 14(4):63-88.

28-Yildiz SC, Keskin C, Ayhanci A.(2018). Investigation of invitro Antioxidant and invivo Protective Effects of Hypericum triquetrifolium Seed Methanol Extracts against

29-Roz N, Rehavi M.(2004). Hyperforin depletes synaptic vesicles content and induces compartmental redistribution of nerve ending monoamines. Life sciences,75(23), 50.

30-Fidan I, Ozkan S, Gurbuz I, Yesilyurt E, Erdal B, Yolbakan S, Imir T. (2008). The efficiency of Viscum album ssp. album and Hypericum perforatum on human immune cells in vitro. Immunopharmacology and immunotoxicology. 30(3):519-28.

31-Mohammed BM, Kheravii SK.(2011). Evaluation of genotoxic potential of Hypericum triquetrifolium extract in somatic and germ cells of male albino mice. Research Opinions in Animal & Veterinary Sciences.

32-Aleisa A. (2008). Cytological and biochemical effects of St. John's Wort supplement (a complex mixture of St. John's Wort, Rosemary and Spirulina) on somatic and germ cells of Swiss Albino mice. International journal of environmental research and public health. 5(5):408-17.

33-Raouf CG, Ahmad MO.(2019). Suspected Protective Effect of Eruca Sativa Leaves Extract on Sperm Abnormalities in Male Albino Mice Treated with Hypericum triqutrifolium aqueous crude extract. UHD Journal of Science and Technology. 3(2):87-92.

34-Suleiman SD, Jubrail AMS, Abdullah MA.(2014). Histopathological effects of hypericum triquetrifolium on liver and kidney in local rabbits. Journal of University of Duhok. 17(1):17-22.

35-Yildiz SC, Keskin C, Sahintürk V, Ayhanci A.(2019). A Histopathological, Immunohistochemical and Biochemical Investigation on the in vitro Antioxidant, Myeloprotective, Hematoprotective and Hepatoprotective Effects of Hypericum triquetrifolium Seed Extract Against Cyclophosphamide-Induced Toxicity. Brazilian Archives of Biology and Technology. 62.

36-Sentürk H, Kabay S, Ozden H, Bayramoglu G, Ustüner MC, Oztürk N, Güven G, Kutlu A, Bilgi G, Ustüner D, Günes V.(2013). The protective effect of Hypericum origanifolium in experimental renal ischemia/reperfusion injury in rats. African Journal of Pharmacy and Pharmacology.7(33):2306-12.

37-Kaplan HM, Izol V, Aridogan IA, Olgan E, Yegani AA, Pazarci P, Singirik E.(2016). Protective effect of Hypericum perforatum extract on gentamicin induced nephrotoxicity in mice. Int. J. Pharmacol.12:663-8.

38-Kang BY, Chung SW, Kim TS.(2001). Inhibition of interleukin-12 production in lipopolysaccharide-activated mouse macrophages by parthenolide, a predominant sesquiterpene lactone in Tanacetum parthenium: involvement of nuclear factor-κB. Immunology letters.77(3):159-63.