



Evaluation of protective and treatment of *Thyme* (*Thymus vulgaris*) oil on *Toxocara vitulorum* infected rats

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ABSTRACT

Toxocara vitulorum (*T.vitulorum*) is a nematode parasite of the small intestine of cattle and water buffalo, particularly buffalo calves between one and three months of age, causing high morbidity and mortality. *Thymus vulgaris* (*Thyme*) oil has used in the Middle East as a traditional medicine for several complaints. This study aimed to evaluate the biochemical, parasitological, histopathological and hematological changes in *Toxocara vitulorum*-infected rat after treatment with *Thymus vulgaris* oil. In the present study, 50 rats divided into 4 groups. The first group: normal control, the second group :animals given with thyme oil, the third group: rats infected with 1500 *T.vitulorum* eggs/rat, the fourth group: rats treated with (42.5 mg/kg body weight) thyme oil for 7 consecutive days pre-infection with *T.vitulorum* eggs, the fifth group: infected with *T.vitulorum* and treated with thyme orally for 7 days starting 1hour from infection. Rats were scarified at 7th and 14th days after last treatment. Blood were collected for hematological and biochemical parameters. Liver, kidney and heart were removed for biochemical and histopathological investigations. Larvae of *Toxocara* were counted in a part of the studied organs tissues. In the present study, *Toxocara* infection resulted in decrease in RBCs count and Hb %, lymphocyte %, and MCHC% while a remarkable increase was observed in WBCs count and monocytes % and granulocytes %. Also, there was increase in lipidperoxidation concentration as malondialdehyde (MDA) accompanied with decrease in superoxide dismutase (SOD) activity and glutathione (GSH) content in organs tissue. Serum biochemical parameters showed a significant increase in the activities, Asparta amino transferase (AST), Alanine amino transferase (ALT), urea, creatinine, albumin and globulin of untreated infected rats in untreated infected rats compared to normal control. Administration of thyme pre

or after infection ameliorate the observed changes occurred by *Toxocara* infection.

It was showed a cytoplasmic vacuolization of hepatocytes and portal infiltration with leucocytes a focal hepatic haemorrhage was shown in liver tissue of the infected group, while in heart tissue it was shown vaculations of cardiac myocytes, intermuscular oedema associated with inflammatory cells infiltration leucocytes cells infiltration between degenerated myocytes. It was observed congestion of glomerular tufts and distension of Bowman' space and dilatation, congestion of renal blood vessels in kidney tissue. Administration of thyme pre or after infection ameliorate the biochemical, and histopathological changes.

These results indicate that *Thymus vulgaris* has a potent effect in protection against organs damage induced by *T. vitulorum* infection.

Keywords: *Thyme oil, Toxocara vitulorum, Liver functions, Kidney functions, antioxidants, Lipid peroxidation, Protein, Albumin, Globulin.*

INTRODUCTION

In Egypt, buffaloes constitute one of the most important features of the live stock as they occupy a prominent position among the farm animals. Buffaloes are indispensable for milk and meat production and therefore, play a great part in the national economy.

T.vitulorum, a nematode parasite in the small intestine of cattle and water buffaloes, causes a high morbidity and mortality of 1-3 months old buffalo calves⁽¹⁾.

Parasitologists have realized that chemoprophylaxis is unsustainable due to increasing drug resistance and the costs of constantly developing new drugs⁽²⁾. The effects of antiparastic drugs on *Toxocara* infection have been studied including albendazole, ivermectin, levamisole and diethylcarbamazine⁽³⁾. Increased use of synthetic drug therapy leads to many side effects and undesirable hazards. Therefore there is a worldwide trend to return to natural resources, which are culturally acceptable and economically viable.

Thyme contains the natural substance and is effective in killing hookworms, roundworms, thread worms and skin parasites⁽⁴⁾. Thymol is considered to be antihelmintic (antiworm) with particular effectiveness against hookworm, and together with carvacrol is both antibacterial and antifungal⁽⁵⁾. Thymol is one of the major components of essential oils of thyme and is a

widely known anti-microbial agent. It has also been tested as a scolicedal agent in some studies and showed an excellent effect⁽⁶⁾. It has the ability to partition in the membrane from an aqueous phase as well as a capacity to affect the membrane organization and the surface electrostatics⁽⁷⁾.

Thyme is a medicinal herb which is native to the Mediterranean region, particularly Italy and Spain, although now it is widely found in Russia, Turkey and Egypt. Thyme (*Thymus vulgaris*) is rich in thymol (20-54% of the plant) and is very antiseptic with extremely penetrating antiparasitic effects. Thymol is a major isolate of this volatile oil that was found to inhibit the growth of a wide range of organisms that cause various diseases^(8,9). Thyme has also, antioxidant properties and it has recently suggested as a natural replacement for synthetic antioxidant⁽¹⁰⁾. Thyme is an excellent source of iron, manganese and vitamin K. It is also a very good source of calcium⁽¹¹⁾. Moreover, thyme, promotes blood circulation and functions as an exciting stimulant for the entire system

The occurrence of parasites or their larvae in the different organs of animals and human generates reactive oxygen species which initiate the oxidative damaging effect⁽¹²⁾. The parasitic infection attenuates the antioxidants such as glutathione and superoxide dismutase in different organs depending on severity of infection⁽¹³⁾. Most of these studies were focused on parasites such as helminthes and protozoa. Little literature was available concerning the effect of helminthes (nematodes) on oxidative stress.

The purpose of this research was to investigate the effect of thyme on liver, kidney and heart of rats infected with *T.vitulorum*.

Material and Methods

Animals

Male albino rats (*Rattus norvegicus*), weighing 100–160 gm each, of the same colony were used. 50 rats were kept in the laboratory at room temperature and housed in cages (10 rats in each cage). Rats received a diet of standard rodent pellets produced by the Cairo Company for Oil and Soap. Water and food available adlibitum.

Parasites

Adult *T. vitulorum* females were obtained post-mortem from the intestinal tract of naturally infected buffaloes. Eggs were recovered by dissecting adult female worms, washed, and then incubated in 2.5% potassium

dichromate at 26–28C° for two weeks. Once embryos were observed, eggs were washed three times in phosphate buffer saline (pH7.0). The average number of infective ova per ml was estimated as described by Oshima (1961)⁽¹⁴⁾.

Plant preparation

The essential oil of the Thyme is obtained from the Egyptian Company for Oils and Soap. Cairo, Egypt. The concentration of the plant oils was prepared by diluting the stock of the oil with few drops of Tween 80 as emulsifier and water were added (42.5 mg/kg body weight)⁽¹⁵⁾.

Experimental design

The experiment was run in five groups; each group consisted of 10 rats, group I: was used as control group (-ve control), group II: normal animals given with thyme oil (42.5 mg/kg body weight), group III: received orally infective embryonated *T. vitulorum* eggs (1500 infective eggs per ml), group IV: rats treated with (42.5 mg/kg body weight) thyme oil orally for 7 consecutive days pre-infection with *T.vitulorum* eggs, group V: rats treated with thyme for 7 days, 1h. after infection. Thyme administrations continued to the end of the experimental time. Five rats were scarified at the 7th and 14th days from the infection. Blood were collected; approximately 2-3 ml blood was collected in Ethylene Diamine Tetra acetic Acid (EDTA) in 5 ml vials for hematological parameters. Serum was separated, from the remaining part of blood, by centrifugation at 3000 rpm for 10-15 minutes and utilized for the measurement of biochemical parameters. Liver, kidneys and heart were removed for biochemical analysis and histopathological studies. The homogenates of organs tissue (10%) were prepared in normal saline for biochemical study. A piece from the studied organs was kept in formalin 10% for histopathological study.

Worm Recovery and Larvae Count

One portion of liver, kidney and heart were finely minced. The larvae were recovered according to the method described by Parsons and Grieve (1990)⁽¹⁶⁾ with little modification. The tissues were weighed and then digested individually in pepsin-HCl solution (pH 1-2, 500 IU pepsin/g tissue) (Sigma-Aldrich, Hyderabad, India) and incubated for 3 hours at 37°C with periodic agitation. Cellular debris was removed after centrifugation. Subsequently, the sedimentation of particles in liquid were poured into a centrifuge tube and stored in a refrigerator at 4°C for 24 hours. The sediment liquid was centrifuged for 2 minutes at 1500 rpm and 2 ml of the sediment was collected and vortexed.

Larvae in the sediment were counted under a light stereomicroscope.

Hematological study

Blood samples were collected in vials contain 0.5 M EDTA (Ethylene diamine tetra acetic acid) .The total number of erythrocytes (RBCs), haemoglobin (Hb)concentration, leukocytes (WBCs), as well as the leukocytes differential counts were estimated by an automatic counter (Coulter Model T-450; Contronics, UK).

Biochemical study

Urea in serum was determined by an enzymatic colorimetric method as described by Patton and Crouch (1977)⁽¹⁷⁾. Serum creatinin was determined by a colorimetric method as described by Henry. (1974)⁽¹⁸⁾. serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were were estimated by the method of Bergmeyer et al. (1978)⁽¹⁹⁾ using Commercial Biochemical kits manufactured by M/s Span Diagnostics Ltd. Surat, Gujarat, India. Alkaline phosphatase (ALP) was measured according to Walter and Schutt (1974)⁽²⁰⁾. Lipid peroxide concentrations were determined by measuring the Malonaldialdehyde (MDA) end as product according to the method of Yoshioka et al., (1979)⁽²¹⁾. Superoxide dismutase (SOD) activity was estimated by Misra & Fridovich (1972)⁽²²⁾ and reduced glutathione (GSH) content were according to Beutler et al., (1963)⁽²³⁾. Protein and albumin were estimated by Begmeyer and Herder (1989)⁽²⁴⁾.

Histopathology

Tissues of liver, kidney, and heart were made into small pieces of 2-3 mm thickness. After proper fixation, the thin tissue pieces were processed in ascending grades of alcohol for dehydration and cleared in xylene. The paraffin embedded tissues were cut into 4-5 micron thick sections and stained with Haematoxyline and Eosin (H&E) as per conventional procedure⁽²⁵⁾.

Statistical analysis

All data were expressed as mean \pm SE (standard error). Data were assessed by using a one- way ANOVA using SPSS 15.0 program and $p < 0.05$ was considered statistically significant.

RESULTS

Effect of thyme on T. vitulorum larvae counts in organs tissue of infected rats

The result of larval counts is shown in Table1. In the present study, the number of larvae in liver of infected group showed a decrease in the second week which indicated the migration of larvae from liver to other organs. This can be confirmed with the increased mean numbers of larvae in kidneys and heart in the second week.

Administration of thyme oil pre infection showed a significant decrease in the numbers of larvae in liver, kidney and heart (-34.1%, -37.3% and -40.8%) at the 7th day (-42.2%, -35.2% and -63.3%) at 14th day respectively as compared to the infected group. Also, a significant decrease in larvae count were shown in liver, kidney and heart of animals treated with thyme after infection (-38.2%, -45.9% and -56%) at the first week and (-48.7%, -58% and -69.3%) at second week respectively. It was observed that administration of thyme oil pre- infection resulted in less numbers of larvae recovered from the studied organs compared to administration of thyme oil after infection, indicating that thyme oil protected the organs against *T.vitulorum* infection.

Table (1): *T.vitulorum* larvae counts in organ tissue (per gram tissue):

Groups	Organs / Time intervals					
	Liver		Kidney		Heart	
	7 th day	14 th day	7 th day	14 th day	7 th day	14 th day
Infected group	794 ±15	714±11.5	34.8±1.7	48.2±1.6	38.2±1.2	67.2±2.0
Thyme+ infection (%)	523 ±15.5 ^b (-34.1%)	412±13.9 ^b (-42.3%)	21.8 ±1.2 ^b (-37.4%)	31.2±1.4 ^b (-35.2%)	22.6±1.5 ^b (-40.8%)	24.6±1.3 ^b (-63.4%)
Infection+ thyme (%)	490±10.0 ^b (-38.2%)	366±10.7 ^b (-48.7%)	18.8±1.1 ^b (-45.9%)	20.2±1.0 ^b (-58.0%)	16.8±1.3 ^b (-56.0%)	20.6±1.1 ^b (-69.3%)

Values are recorded as Mean±SE.

b: significant change from infected group at P<0.05

(%): percentage change from infected group.

Effect of thyme on hematological parameters in infected rats

As shown in Table 2, normal rats given thyme resulted in insignificant changes, which indicated the thyme has no effect on hematological parameters on normal rats. Significant decreases were observed at the first and second week after infection in RBCs (-36.1%, -40.9%) count and hemoglobin percentage (-20.1%, -13.1%) and MCHC% (-25% and -16.1%) respectively, in untreated *T.vitulorum* infected group compared to normal control. Administration of thyme oil pre *Toxocara* infection ameliorates this change of RBCs (-

4.4%, -6%), Hb% (2.1%, -8%) and MCHC% (-16.4 %, -17.6%) at first and second week respectively. While, treatment of thyme after infection showed a significant decrease in RBCs at 7th day after infection (-16.1%) and in Hb% at 7th day post infection (-13.6%) compared to normal control. While the results showed that MCHC% significantly decreased (-19.7% and -11.7%) at (7th and 14th day) respectively in untreated infected group as compared to normal.

Table (2): Variations in some hematological parameters in rats of different groups.

	RBCs (10 ⁶ /mm ³)		Hb(g/dl)		MCHC (%)	
	7 th day	14 th day	7 th day	14 th day	7 th day	14 th day
Control	6.8±0.1	6.6±0.12	13.9±0.4	13.7±0.3	63.4±0.5	61.9±0.2
Thyme group	7.1±0.2 (4.4%)	7.0±0.1 (6.0%)	13.7±0.3 (-1.4%)	13.5±0.4 (-1.4%)	65.5±0.9 (3.3%)	62.8±0.3 (1.4%)
Infected group	4.34±0.2 ^a (-36.1%)	3.9±0.5 ^a (-40.9%)	11.1±0.5 ^a (-20.1%)	11.9±0.2 ^a (-13.1%)	47.5±0.8 ^a (-25.0%)	51.9±0.2 ^a (-16.1%)
Thyme+infection	6.5±0.1 ^b (-4.4%)	6.2±0.1 ^b (-6.0%)	14.2±0.2 ^b (2.1%)	12.6±0. ^{ab} (-8.0%)	53.0±0.9 ^{ab} (-16.4%)	51.0±0.3 ^{ab} (-17.6%)
Infection+thyme	5.7±0.6 ^{ab} (-16.1%)	6.0±0.5 ^b (-6.0%)	12.0±1.2 ^{ab} (-13.6%)	14.0±0.9 ^b (2.1%)	50.9±0.7 ^{ab} (-19.7%)	54.6±1.4 ^{ab} (-11.7%)

Values are recorded as Mean ±SE.

a: significant change from control

b: significant change from infected group at P<0.05 (%) : percentage change from control

As shown in Table (3), a remarkable increases were shown in the count of WBCs, (28.8%, 88.3%), granulocytes % (62%, 61.2%), monocytes% (55.5%, 60.5%) at the 7th and 14th day respectively, compared to control. On the other hand a significant decrease were observed in lymphocytes% (-51.1% and -35.6%) at both interval times, compared to normal control. Administration of thyme oil pre and post infection ameliorate the changes that observed at 7th and 14th day post infection, as results showed a significant increase in WBCs and Gran.% as compared to normal control and significant decreases were observe when, compared to infected group. While a significant decrease and a significant increase were shown in lymphocyte % as compared to normal control and infected group respectively.

Table (3): Variations in leucocytes count and differential percentage in rats of different groups.

	WBCs (10 ³ /cm ³)		Lymphocytes %		Granulocytes%		Monocytes%	
	7 th day	14 th day	7 th day	14 th day	7 th day	14 th day	7 th day	14 th day
Control	4.5±0.2	4.3±0.2	67.8±1.8	68.1±2.1	28.2±0.6	27.9±0.6	3.4±0.2	3.6±0.2
Thyme group	4.7 ±0.4 ^b (4.4%)	4.2±0.31 ^b (-2.3%)	65.9± 0.9 ^b (-2.8%)	66.7±1.3 ^b (-2.0%)	29.1±0.8 ^b (3.1%)	28.6±0.9 ^b (2.5%)	3.6±0.4 (5.0%)	3.8±0.20 (5.5%)
Infected group	5.8±0.7 ^a (28.8%)	8.1±0.4 ^a (88.3%)	33.1±1.1 ^a (-51.1%)	43.8±1.5 ^a (-35.6%)	45.7±1.3 ^a (62.0%)	45.0±0.5 ^a (61.2%)	5.9±0.3 ^a (55.5%)	6.1±0.30 ^a (60.5%)
Thyme+infection	5.0±0.2 ^{ab} (11.1%)	6.9±0.8 ^{ab} (60.0%)	47.7±1.2 ^{ab} (-29.6%)	54.5±1.6 ^{ab} (-19.9%)	30.2±1.8 ^b (7.0%)	35.8±0.7 ^{ab} (28.3%)	3.9±0.3 ^{ab} (2.9%)	4.0 ±0.16 (5.2%)
Infection+thyme	5.1±0.3 ^{ab} (13.3%)	6.3±0.1 ^{ab} (46.5%)	43.2±1.5 ^{ab} (-36.2%)	47.9±1.6 ^{ab} (-29.6%)	36.1±1.7 ^{ab} (28.0%)	40.0±1.1 ^{ab} (43.3%)	3.7±0.3 ^{ab} (8.8%)	4.1. ±0.20 (7.8%)

Legends as in table 2

Effect of thyme on LPO, GSH content and SOD activity in organs tissue of rats of different groups

As shown in Table (4), a remarkable increase in LPO were shown (at 7th and 14th day) in liver tissue (11%, 25.7%), kidney tissue (24%, 28%) and in heart tissue (75.2%, 105.7%) in infected group as compared to normal control. A remarkable protection was observed in organs tissue of animals treated with thyme before *Toxocara* infection. The results showed percentage change of lipid peroxidation from control (at 7th and 14th day) in liver tissue (2.9%, 10.5%), kidney tissue (11.1%, 7.9%) and in heart tissue (26.2%, 49.8%). Also, administration of thyme after infection, resulted in amelioration of lipid peroxidation in liver tissue (3.8%, 9.4%), in kidney tissue (14.6, 9.3%). In heart tissue lipid peroxidation was increased as compared to normal control, although such increase was lower than that of infected group (44.4%, 61.3%) at (7th, 14th day), respectively.

On the other hand, *Toxocara* infection in rats resulted in a highly significant decrease were shown in glutathione contents in liver tissue (-29.5%, -22.9%), in Kidney tissue (-22.4%, -31%) and in heart tissue (-16.4.2%, -29.9%) compared to normal control. While administration of thyme pre- infection showed significant decrease (-9.1%, -14.5%) in GSH in liver tissue at 7th day and 14th day post infection as compared to normal control and significant increase were shown compared to infected group, also in kidney and heart tissue there were a remarkable improvement in GSH content compared to infected group at first and second week. Also, an observed improvement were shown in animals that treated with thyme after *Toxocara* infection, the percentage change from control (at 7th, 14th day) post infection were (-12.7%, -9.4%) in liver tissue, (-8.4%, -18.5%) in kidney tissue and (-14.5%, -8.7%) in heart, respectively.

The present results showed a significant decrease in SOD content in liver (-27.3%, -23.2%), kidneys (-43.5%, -42.3%) and heart tissues (-34%, -44.4%) of untreated infected group at 7th and 14th day respectively, post infection as compared to normal control. While treatment of Thyme pre *T.vitulorum* infection showed insignificant decrease in SOD in liver and heart tissue compared to normal control and a remarkable improvement were shown in kidney tissue as compared to infected group. Also, treatment of infected animals with thyme after infection resulted in insignificant decrease in SOD in liver tissues at 14th day (-6.8%) compared to control and significant increase was shown at 7th day post infection compared to infected group. Also

insignificant change from normal control were observed in kidney tissue at 7th day while significance increases were shown at 14th day post infection in kidney tissue and in heart tissue at 7th and 14th days compared to infected group.

Table (4): LPO activity, GSH contents and SOD activity in organs tissue for all treatment groups:

	Organs	Liver		Kidney		Heart	
	time Groups	7 th day	14 th day	7 th day	14 th day	7 th day	14 th day
LPO (nmol/g issue)	Normal ontrol	82.4±0.6	81.9±0.9	116.4±1.7	117.1±1.7	25.9±0.4	26.1±0.4
	Thyme group	81.7±0.5 (-0.84)	81.3±0.7 (-0.73%)	114.8±1.3 (-1.37%)	116.6±1.2 (-0.42%)	26.1±0.7 (0.77%)	26.8±0.9 (2.68%)
	Infected group	91.5±0.2 ^a (11%)	103.8±0.9 ^a (25.7%)	144.6±2.1 ^a (24.2%)	150.0±1.4 ^a (28.0%)	45.4±0.5 ^a (75.2%)	53.7±0.3 ^a (105.7%)
	Thyme+ infection	84.8±0.6 ^b (2.9%)	90.5±0.8 ^{ab} (10.5%)	129.4±1.6 ^{ab} (11.1%)	126.4±1.2 ^{ab} (7.9%)	32.7±0.4 ^{ab} (26.2%)	39.1±0.3 ^{ab} (49.8%)
	Infection + thyme	85.6±0.5 ^b (3.8%)	89.6±0.8 ^{ab} (9.4%)	133.4±1.9 ^{ab} (14.6%)	128.0±1.3 ^{ab} (9.3%)	37.4±0.7 ^{ab} (44.4%)	42.1±0.6 ^{ab} (61.3%)
GSH (mg /g tissue)	Normal control	27.4±0.2	27.4±0.2	30.8±1.2	31.2±1.5	27.3±1.2	27.3±1.2
	Thyme group	26.9±0.5 (-1.82%)	26.7±0.3 (-2.55%)	31.7±0.6 (2.9%)	31.7±0.8 (1.6%)	26.8±0.7 (-2.19%)	26.2±0.9 (-4.0%)
	Infected group	19.3±0.4 ^a (-29.5)	21.1±0.5 ^a (-22.9%)	23.9±0.4 ^a (-22.4%)	21.5±0.5 ^a (-31.0%)	22.9±0.87 ^a (-16.4%)	19.2±1.2 ^a (-29.9%)
	Thyme+ infection	24.9±0.3 ^{ab} (-9.1%)	23.4±0.4 ^{ab} (-14.5%)	27.2±0.8 ^{ab} (-11.6%)	26.9±0.9 ^{ab} (-13.7%)	26.0±0.8 ^b (5.1%)	26.3±1.3 ^b (4.0%)
	Infection + thyme	23.9±0.4 ^{ab} (-12.7%)	24.8±0.3 ^{ab} (-9.4%)	28.2±1.1 ^{ab} (-8.4%)	25.4±1.0 ^{ab} (-18.5%)	23.4±0.9 ^a (-14.5%)	25.0±0.6 ^b (-8.7%)
SOD (U/g tissue)	Normal control	6.63±0.1	6.59±0.1	6.2±0.3	5.9±0.25	5.0±0.5	5.4±0.43
	Thyme group	6.90±0.12 (4.0%)	6.71±0.2 (1.8%)	6.5±0.15 (4.8%)	6.3±0.25 (6.7%)	5.6±0.8 (12%)	5.9±0.7 (9.25%)
	Infected group	4.82±0.5 ^a (-27.3%)	5.06±0.2 ^a (-23.2%)	3.5±0.25 ^a (-43.5%)	3.4±0.22 ^a (-42.3)	3.3±0.2 ^a (-34.0%)	3.0±0.5 ^a (-44.4%)
	Thyme+ infection	5.96±0.2 ^b (-8.9)	5.92±0.1 ^b (-8.9%)	5.5±0.3 ^{ab} (-11.3%)	5.0±0.28 ^{ab} (-15.2%)	4.6±0.3 ^b (-8.0%)	5.0±0.3 ^b (-7.40%)
	Infection + thyme	5.52±0.2 ^{ab} (-16.7%)	6.14±0.3 ^b (-6.82%)	5.9±0.27 ^b (-4.8%)	6.7±0.23 ^{ab} (13.5%)	4.4±0.5 ^{ab} (-12.0%)	4.7±0.2 ^{ab} (-12.96%)

Legends as in Table 2.

Effect of thyme on some biochemical parameters in serum of different groups

In Table 5, the results of normal rats given thyme oil, showed insignificant change from control, indicating safety treatment of thyme on organs of rats. Rats infected with *Toxocara vitulorum*, showing a significant increase in liver function represented by ALT (35.3%, 12.5%), AST (59.4%, 47.8%) and ALP (31.2%, 47.3%) at (7th day and 14th day) post infection respectively. Also, a remarkable increase in kidney function represented by urea (133.5%, 154.6%) and creatinine (44%, 58.5%). significance increases were observed in protein (24.5%, 13.7%) at 7th and 14th day respectively as compared to the value of control group. While non significant decreases were observed in albumin level at 7th day (-8%) and 14th day (-9%) as compared to control. On

the other hand, a remarkable increases were observed in globulin at 7th day (46.1%) and 14th day (31.5%).

Administration of thyme pre infection showed insignificant decrease in ALT (at 14th day) and ALP, protein, albumin and globulin (at 7th and 14th day) as compared to normal control. While a remarkable improvement were shown in AST, urea and creatinine as compared to infected group. Also, treatment of infected group with thyme after infection showed insignificant change in ALP, protein, albumin and globulin at (7th and 14th day) as compared to normal control. While an observed improvement were shown in ALT, AST and urea as compared to infected group.

Table (5): Effect of thyme on biochemical parameters in serum of different groups.

Groups Parameters		Control	Thyme	Infected group	Thyme+ infection	Infection + Thyme
ALT (U/ml)	7 th day	18.1±0.41	19.8±0.49 ^b (-1.6%)	24.5±0.4 ^a (35.3%)	20.8±0.9 ^{ab} (14.9%)	19.5±0.6 ^{ab} (7.7%)
	14 th day	18.3±0.60	19.2±0.27 ^b (3.2%)	20.6±0.4 ^a (12.5%)	17.9±0.3 ^b (-2.1%)	20.0±0.2 ^{ab} (9.2%)
AST (U/ml)	7 th day	23.7±0.42	24.2±0.21 ^b (-3.3%)	37.8±0.5 ^a (59.4%)	30.0±0.2 ^{ab} (26.5%)	25.2±0.6 ^{ab} (6.3%)
	14 th day	23.4±0.31	23.9±0.65 ^b (-1.7%)	34.6±0.8 ^a (47.8%)	29.7±0.5 ^{ab} (26.9%)	30.6±0.9 ^{ab} (30.7%)
ALP	7 th day	9.6±0.61	10.3±0.2 ^b (-4.1%)	12.6±0.5 ^a (31.2%)	10.2±0.4 ^b (6.2%)	10.6±0.5 ^b (10.4%)
	14 th day	9.3±0.70	9.8±0.42 ^b (5.3%)	13.7±0.6 ^a (47.3%)	10.1±0.2 ^b (8.6%)	10.5±0.5 ^b (7.1%)
Urea (Mg/dl)	7 th day	40.0±2.1	41.3±2.8 ^b (3.2%)	93.4±0.15 ^a (133.5%)	63.2±1.6 ^{ab} (58.0%)	58.4±2.1 ^{ab} (46.0%)
	14 th day	39.5±1.5	38.9±1.2 ^b (-1.5%)	100.6±2.1 ^a (154.6%)	66.7±1.1 ^{ab} (68.8%)	89.5±2.4 ^{ab} (126.5%)
Creatinine (mg/dl)	7 th day	0.84±0.1	0.91±0.15 ^b (8.3%)	1.5±0.12 ^a (44.0%)	0.95±0.09 ^{ab} (13.0%)	1.10±0.14 ^{ab} (30.9%)
	14 th day	0.82±0.12	0.85±0.14 ^b (4.4%)	1.3±0.15 ^a (58.5%)	1.1±0.07 ^{ab} (34.1%)	0.97±0.06 ^{ab} (18.2%)
Total protein (g/dl)	7 th day	6.1±0.56	6.5±0.61 ^b (6.5%)	7.6±0.3 ^a (24.5%)	6.2±0.5 (1.6%)	5.8±0.43 (-4.9%)
	14 th day	5.8±0.31	6.1±0.29 ^b (-3.4%)	6.3±0.15 ^a (13.7%)	6.4±0.3 ^b (10.3%)	6.3±0.3 ^b (8.6%)
Albumin	7 th day	2.5±0.25	2.7±0.19 ^b (8.0%)	2.3±0.12 (-8.0%)	2.6±0.13 (4.0%)	2.3±0.15 (-8.0%)
	14 th day	2.2±0.15	2.42±0.13 ^b (9.0%)	2.0±0.10 ^a (-9.0%)	2.4±0.20 (11.3%)	2.35±0.18 (4.5%)
Globulin	7 th day	3.49±0.51	3.21±0.21 ^b (8.0%)	5.1±0.38 ^a (46.1%)	3.5±0.2 (1.44%)	3.2±0.13 (-8.3%)
	14 th day	3.2±0.48	3.3±0.11 ^b (3.1%)	4.21±0.17 ^a (31.5%)	3.4±0.18 ^b (6.25%)	3.3±0.2 ^b (3.1%)

Legends as in table (2).

Histopathological studies

Histopathological section from liver of rat that infected with egg of *Toxocara* showed a cytoplasmic vacuolization of hepatocytes and portal infiltration with leucocytes at the 7th day, (Fig.1 A). While, a focal hepatic

hemorrhage was shown at the 14th day as shown in (Fig.1,B). liver section of rats that treated with thyme pre-infection showing congestion of central vein and hydropic degeneration of hepatocytes was shown at 7th day (Fig.1 C), While cytoplasmic vacuolizations of hepatocytes at 14th day (Fig.1 D). However, in the group that treated with thyme after *T. vitulorum* infection showing congestion of central vein and vacuolar degeneration of hepatocytes at 7th day (Fig.1 E), while at 14th day showing no histopathological changes.

Fig.2 showed Sections from heart of rats that infected with egg of *Toxocara* showed vaculations of cardiac myocytes, intermuscular oedema associated with inflammatory cells infiltration at the 7th day, (Fig.2 A). While, Leucocytic cells infiltration between degenerated myocytes was shown at the 14th day as shown in (Fig.2,B). Heart section of rats that treated with thyme pre-infection showing Intramuscular inflammatory cells infiltration at 7th day (Fig.2 C), and congestion of myocardial blood vessel at 14th day (Fig.2 D). However, in the group that treated with thyme after *T. vitulorum* infection showing few leucocytes between muscle fibers at 7th day (Fig.2 E), and no histopathological changes at 14th day.

Fig. 3 showed Sections from Kidney of rats that infected with egg of *Toxocara* showed congestion of glomerular tufts and distension of Bowman' space at the 7th day, (Fig.3 A). While, Dilatation and congestion of renal blood vessels was shown at the 14th day as shown in (Fig.3,B). Kidney section of rats that treated with thyme pre-infection showing vacuolations of endothelial lining glomerular tuft and epithelial lining renal tubules at 7th day (Fig.3 C), and no histopathological changes at 14th day (Fig.3 D). However, in the group that treated with thyme after *T. vitulorum* infection showing congestion of glomerular tufts and protein cast in the lumen of some renal tubules at 7th day (Fig.1 E), and no histopathological changes at 14th day.

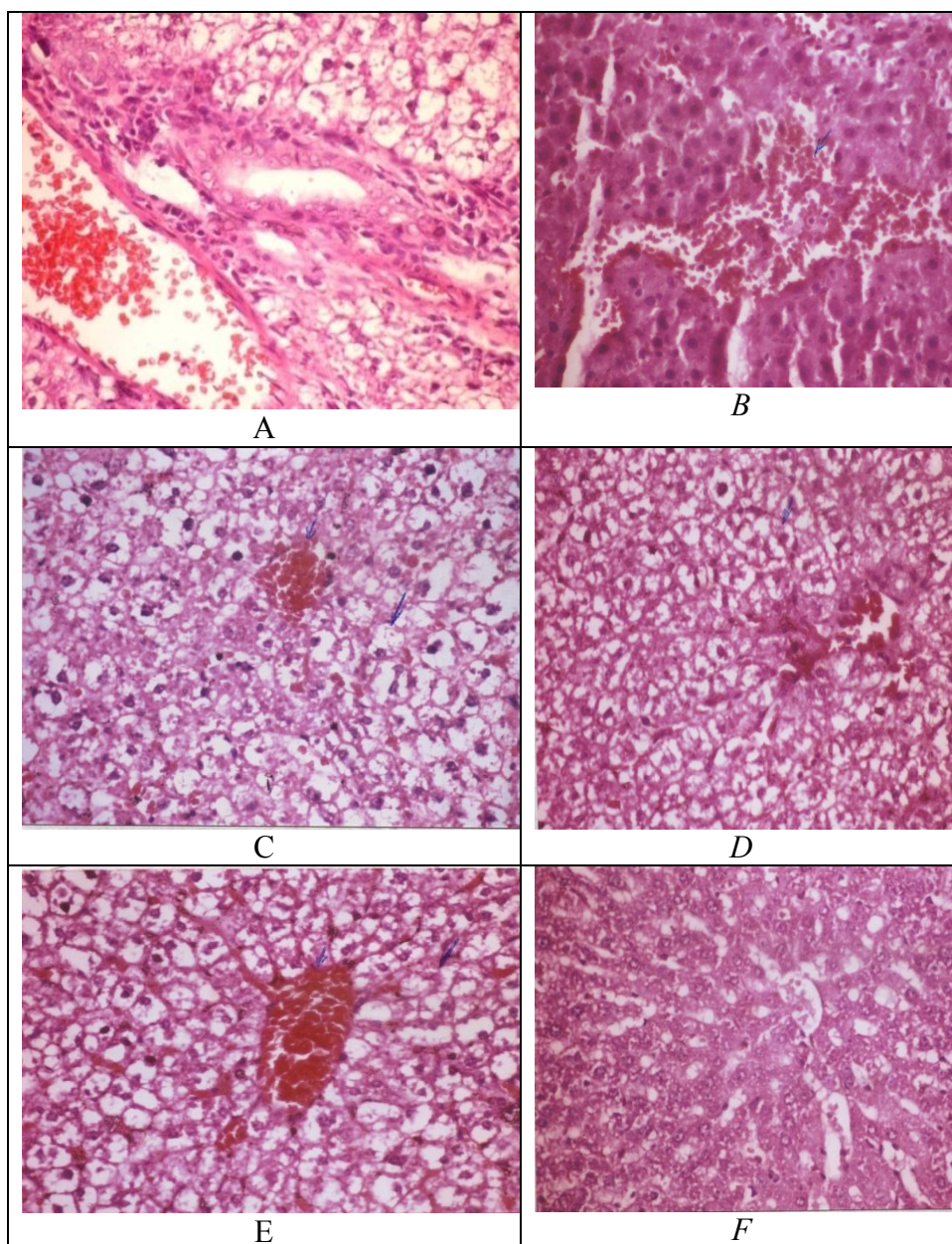
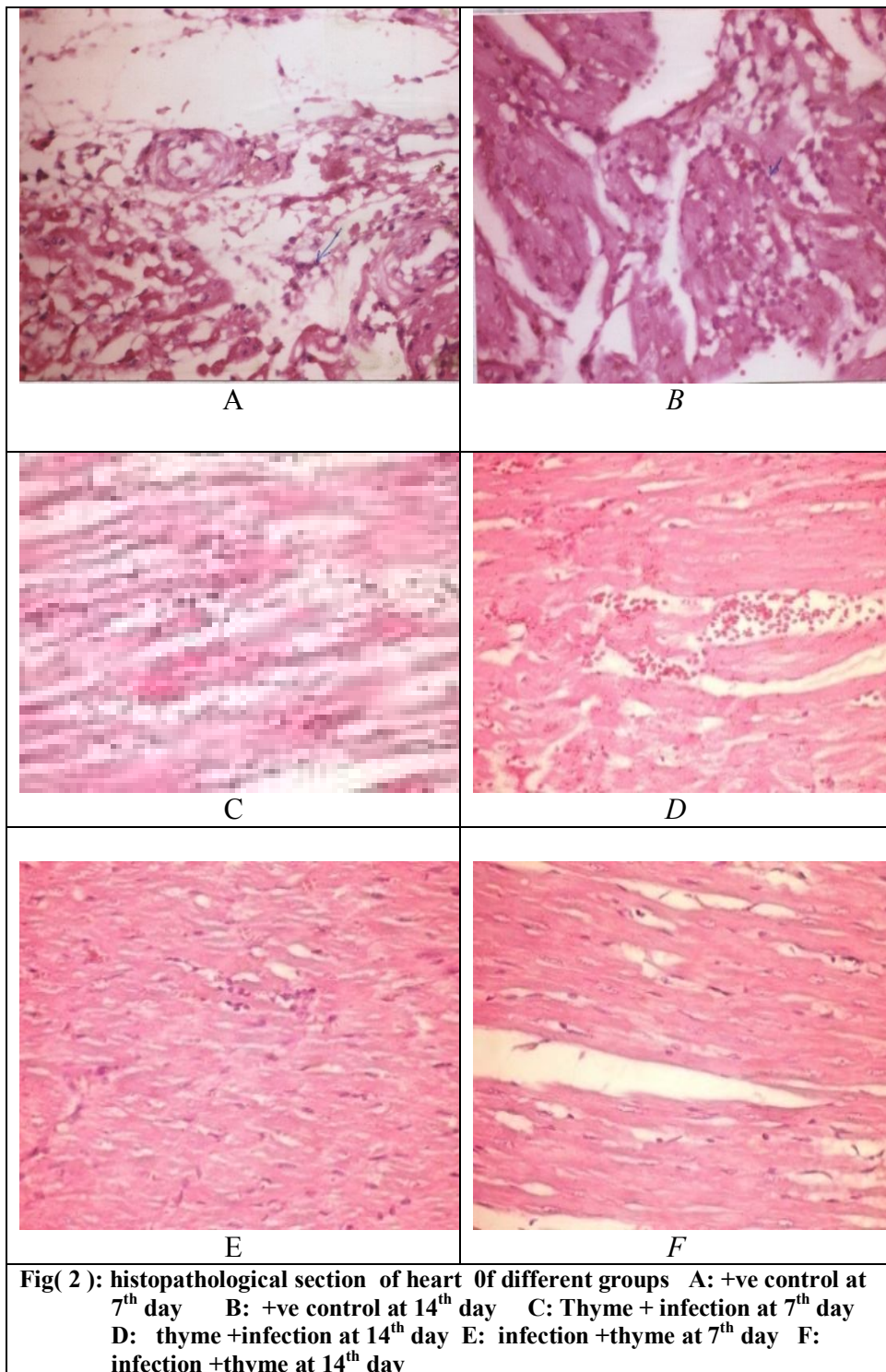
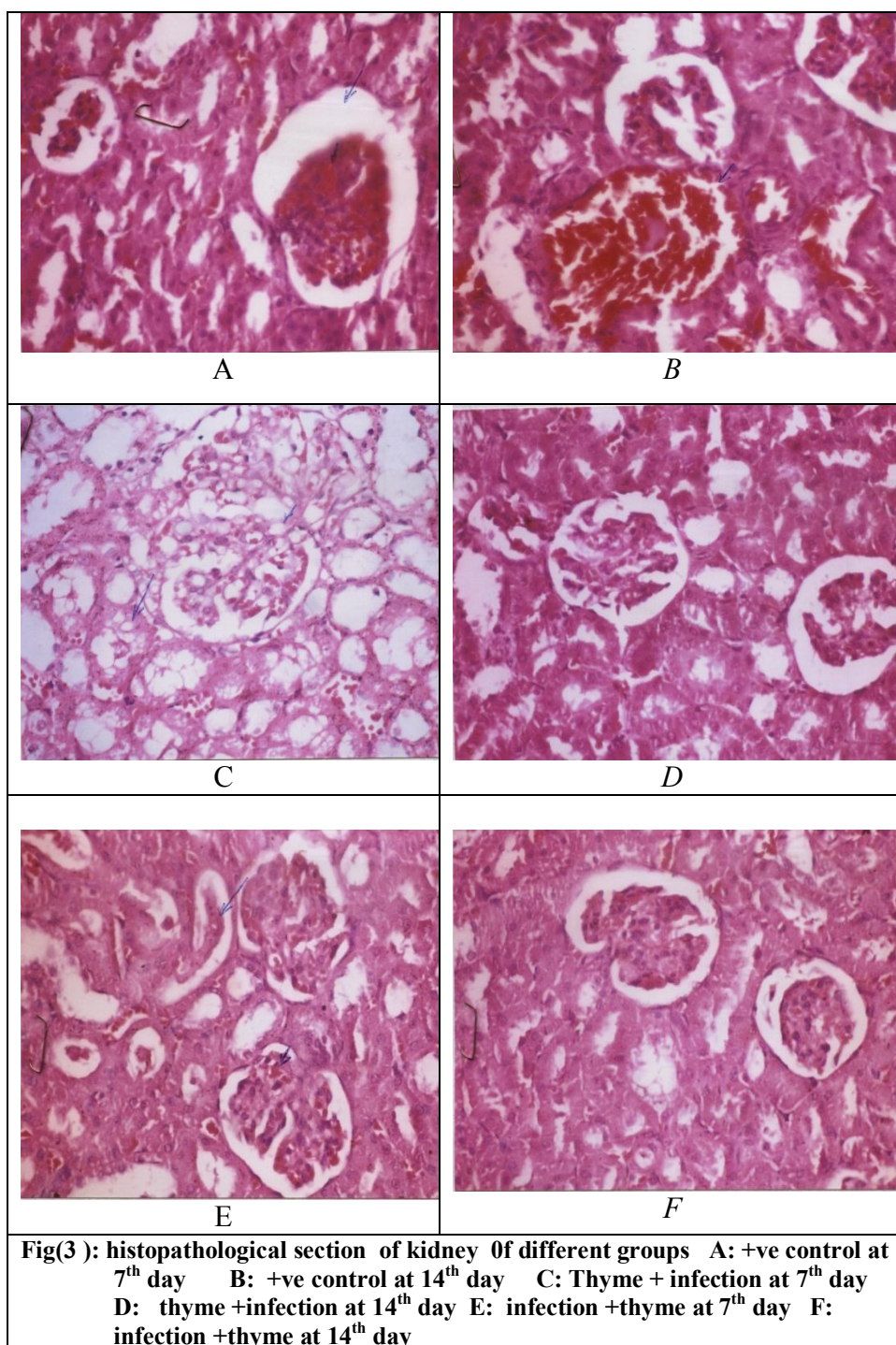


Fig (1): histopathological section of liver of different groups A: +ve control at 7th day B: +ve control at 14th day C: Thyme + infection at 7th day D: thyme +infection at 14th day E: infection +thyme at 7th day F: infection +thyme at 14th day (H & Ex 400)





DISCUSSION

T. vitulorum is considered to be primarily responsible for visceral larva migrans (VLM) and ocular larvae migrans (OLM). The route of larval migration in the experimental rat can be divided into an early hepatopulmonary or visceral phase during the first week after infection, followed by a kidney, heart and kidney phase⁽²⁶⁾. The larvae in the liver, elicit T-lymphocyte responses leading to macrophage activation, which seem to play a role in phagocytic removal of unwanted debris at the site of inflammation⁽²⁷⁾. The severity of the disease is determined by the extent of inflammation and amount of larval debris deposited in the tissues⁽²⁸⁾.

In the present study, the recovery levels in larval toxocariasis were higher in liver and lower in kidney and heart on the 7th day and 14th day after infection, which is in line with a study by Lai *et al.*(2005)⁽²⁷⁾. This result may be attributable to proteolytic activity of enzymes produced by larvae that leads to moderate of severe histopathological changes in liver and mild changes in kidney and heart. While, thyme administration before and after *Toxocara* infection resulted in a significant reduction of larval burden in treatment groups, at the corresponding times, as compared to infected group.

The data revealed that *T.vitulorum* larvae induced a marked anemia manifested in the significant decrease of erythrocyte count, blood haemoglobin level and MCHC% all over interval time. These results are in agreement with the findings obtained from infecting Guinea pigs with *T. vitulorum* and reported by Sinha *et al.* (1981)⁽²⁹⁾. Many helminth infections are characterized by a concomitant anaemia⁽³⁰⁾. This anaemia may be due to shortened half life of red cells⁽³¹⁾. Furthermore, anaemia can be taken as a measure of the toxic effect of helminthic infection⁽³²⁾. The mean levels of these aspects in rats with Thyme oil pre or after infection with infective *T.vitulorum* larvae, were more close to those of the control group which means that such larvae produce the least defects on the haematological aspects tested.

In the present study, the increase of the total WBC of rats infected with infective *T.vitulorum* eggs and the fluctuations found in the percentages of each of lymphocytes, monocytes and granulocytes were not surprising after *T. vitulorum* infection. It is known that *T.vitulorum* migrate during its development in the body through different organs and tissues causing damage of the tissues, haemorrhage, and invasion of other pathogenic organisms and may secrete toxins and antienzymes. However, such responses were in agreement with that

reported by Cheema and Scofield (1985)⁽³²⁾.

In the present study, the elevations in the levels of MDA - the end products of lipid peroxidation- in the liver of rat in infected group suggests that tissue damage and failure of antioxidant defense mechanisms as evidenced by decreased activities of protective enzymes such as SOD and GSH content in liver tissue after infection.

The present study demonstrated that in the infected group resulted in an increase in the renal MDA level paralleled with the decrease in the SOD as well as GSH level when compared with control rats. These parameters were considered the most important antioxidants involved in ameliorating the effects of oxygen metabolism. Also, our results were in agreement with that reported by Kilic *et al.* (2003)⁽¹²⁾ who concluded that the occurrence of parasites or their larvae in the different organs of animals and human generates reactive oxygen species which initiates the oxidative damaging effect. The parasitic infection attenuates the antioxidants such as glutathione and superoxide dismutase in different organs depending on severity of infection⁽¹³⁾. Most of these studies were focused on parasites such as helminthes and protozoa. Little literature was available concerning the effect of helminthes (nematodes) on oxidative stress.

Our data reported increase in the level of GSH in both hepatic and renal tissues of rats treated with thyme alone which may be an evidence for enhancing the antioxidant defense system with thyme. However, the hepatorenoprotective potential of thyme was previously described in alfatoxines-induced liver damage⁽³³⁾.

The alteration of alanine aminotransferase (ALT), alkaline phosphatases (ALP) and aspartate aminotransferase (AST) in sera were significant for monitoring liver function⁽³⁴⁾. In our study, significant increase in serum levels of AST, ALT and ALP were observed in infected rats at all over time in comparison with that of the normal control. This seems to be associated with damage of hepatic tissue and impaired cell membrane permeability or is due to deposition of *T. vitulorum* larvae in tissues. However, these results were in agreement with this reported by Voronkova (1986)⁽³⁵⁾. Certain products of ascarid metabolism (volatile aldehydes of fatty acids), and substances that were formed as a result of the decoposition of dead parasites have reported to be hepatic toxins, also this may be attributed to complications arising from the extensive larval migration inside the body tissues especially the liver

parenchyma and other tissues⁽³⁶⁾. Comparing the results of the groups of animals that were administered with thyme oil pre or after infection with *T.vitulum* with those of the infected group, the activities of the above mentioned enzymes were markedly lower. This may be due to the effect of thyme which retards the development of the larvae. Also, the treatment reduced the degree of damage and inflammation in the liver and the percentage of eosinophils, in comparison to the *T. vitulum*-infected group.

In the present study, *Toxocara* -induced nephrotoxicity was characterized by marked elevations in blood urea and serum creatinine. It has suggested that in nephrotoxicity and renal diseases, the serum urea and creatinine accumulate because the rate of production exceeds the rate of clearance due to the defect in renal function⁽³⁷⁾. The kidneys are involved in the excretion of various xenobiotics, pollutants, toxins and hence they were prone to liberate high quantities of free radicals which contribute to high oxidative stress. This is involved in the pathogenesis of kidney damage⁽³⁸⁾.

Dubey *et al.*, (1994)⁽³⁹⁾ found that, increased total serum protein level in infected rats may be attributed to impaired protein synthesis by damaged liver tissue. Moreover, hepatotoxin impairs the capacity of liver to synthesize albumin.

There have been great efforts to find safe and potent natural antioxidants from various plant sources. Phenolic phytochemicals are thought to promote optimal health through their antioxidant and free radicals scavenging effects⁽⁴⁰⁾. Aqueous extract of thyme are rich in the total phenolic content and have radical scavenging activity⁽³³⁾.

Essential oils are natural products extracted from vegetal materials, which have antibacterial, antifungal, antioxidant⁽⁴¹⁾, or antiparasitic properties⁽⁴²⁾.

Essential oils, or their components have a broad spectrum of pharmacological effects, with antibacterial⁽⁴³⁾, antiviral⁽⁴⁴⁾, antihelminthical⁽⁴⁵⁾, and antiprotozoal⁽⁴⁶⁾ properties. These characteristics are possibly related to the function of these compounds in plants⁽⁴⁷⁾. Thyme is a famous Egyptian plant containing essential oils, previous studies have shown that sage and its constituents have antibacterial actions against various microorganisms⁽⁴⁸⁾.

Thyme is used as an antimicrobial⁽⁴⁹⁾, anti-amoebic⁽⁵⁰⁾, and anti-inflammatory drug⁽⁵¹⁾.

The results of Yones *et al.* (2011)⁽⁵²⁾ showed that the alcoholic extract of both salvia and thyme are effective on the viability of protoscolices. The highest concentration (2,500 µg/ml) of both extracts showed the best results, where all protoscolices died by day 6 post treatment. Other concentrations (1,500, 1,000, and 500 µg/ml) of salvia and thyme extracts caused death of protoscolices also but needed a longer period than the highest concentration. The dose-dependent protoscolicidal effects of these medicinal plant extracts seem to be due to their role in breakdown of biological activities of protoscolices through interference with their metabolism. They may have target sites, such as inhibitors of protein or DNA synthesis, or within cytoplasmic components, like B-lactam antibiotics⁽⁵³⁾.

Treatment of infected rats with thyme oil in the present study, markedly reduced the elevated levels of transaminases, and ALP in serum towards normal indicating its hepatoprotective efficacy and demonstrated membrane stabilizing activity of thyme oil. Also, thyme oil prevented the changes in the total serum protein level. The observed increase of globulin may reflect the ameliorated effect of thyme oil. This further signifies the curative nature of oil against *Toxocara* toxicity. Moreover, administration of thyme alleviates the harmful effects induced by *Toxocara* infection by improvement the kidney functions.

Our data indicate that the treatment with *Thymus vulgaris* oil reduced the degree of inflammation and necrosis in liver, heart and kidney induced by *T. vitulorum* infection, lead to a reduction in the percentage of eosinophils, and markedly decreased the elevated liver enzyme levels. These results are in agreement with Abdel Aziz and El-Badawy (2000)⁽⁵⁴⁾ and Abou El-Nour *et al.* (2005)⁽⁵⁵⁾.

In conclusions, the present study suggests that the thyme oil has a tremendous potential to prevent or reverse the changes induced by *T. vitulorum* infection toxicity back to normal via its antioxidant activity. Hence it is advised that, during infection with *T. vitulorum*, it is preferable to consume thyme oil as a protective agent.

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مجلة البحوث الإشعاعية والعلوم التطبيقية

مجلد ٦ عدد ١ ص ٢٠٩ - ٢٣٢ (٢٠١٣)

التقييم الوقائي والعلاجي لزيت الزعتر على الجرذان المصابة بالتوكسوكارا فيتيولورم

منى محمد أمين - حنان أحمد القباني

قسم البحوث الصحية الإشعاعية ، المركز القومي لبحوث وتكنولوجيا الإشعاع ، ٢٩ مدينة نصر، مصر.

التوكسوكارا فيتيولورم هو طفيلي خيطي في الأمعاء الدقيقة من الأبقار والجاموس والعجول وخاصة الجاموس الذي يتراوح اعمارهم من شهر إلى ثلاث شهور مما تسبب في ارتفاع معدلات المرض و الوفيات. يستخدم زيت الزعتر في الشرق الأوسط كنواء تقليدي لمشاكل عديدة. تهدف هذه الدراسة إلى تقييم التغيرات الكيميائية والطفيلية والهيستوباثولوجية وصورة الدم في الجرذان المصابة بالتوكسوكارا وتأثير زيت الزعتر قبل و بعد العدوى. تم توزيع خمسون من الجرذان في هذه الدراسة إلى خمسة مجموعات :

المجموعة الأولى: المجموعة الضابطة

المجموعة الثانية: مجموعة معاملة بزيت الزعتر

المجموعة الثالثة: مجموعة مصابة ببويضات التوكسوكارا

المجموعة الرابعة: مجموعة معالجة بزيت الزعتر ٤٢.٥ مج / كجم من وزن الجسم لمدة ٧ ايام متتالية قبل الإصابة بالتوكسوكارا فيتيولورم حتى نهاية الأسبوع الثاني

المجموعة الخامسة: مجموعة معالجة بزيت الزعتر ٤٢.٥ مج / كجم من وزن الجسم بعد الإصابة بالتوكسوكارا فيتيولورم وبعد الإصابة لمدة اسبوعين.

تم ذبح الجرذان بعد الأسبوع الأول و الأسبوع الثاني من الإصابة بالتوكسوكارا. تمت ازالة الكبد والكلى والقلب . و جمع الدم للدراسة البيوكيميائية و الأنسجة. كما تم عد اليرقات في أجزاء من الأعضاء التي شملتها الدراسة. و أشارت هذه الدراسة ان زيت الزعتر له تأثير قوي في حماية الأعضاء من التلف الناجم عن عدوي التوكسوكارا.

اوضحت النتائج في هذه الدراسة ان عدوي التوكسوكارا فيتيولورم ادت إلى انخفاض في خلايا الدم الحمراء ومحتوي الهيموجلوبين وزيادة في خلايا الدم البيضاء. ولوحظ ايضاً زيادة تركيز المالونداي الدهيد وانخفاض في كلا من الجلوتاثيون والسوبر اكسيد ديسميوتاز في انسجة الأعضاء. كما أظهرت القياسات في مصل الدم زيادة في معايير وظائف الكبد ووظائف الكلى والألبومين والجلوبيولين. ادت معالجة الحيوانات بزيت الزعتر قبل او بعد العدوي إلي تحسن في التغيرات الناتجة عن عدوي التوكسوكارا . كذلك دلت النتائج المتحصل عليها من الهيستوباثولوجي على تحسن ملحوظ في أنسجة الكبد والكلى والقلب للجرذان التي تم معاملتهم بزيت الزعتر سواء قبل أو بعد العدوى مقارنة بالمجموعة المصابة ببويضات التوكسوكارا فقط.