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Incidence and Evaluation of Anthelmentic Efficacy of *Balanitesegyptiaca* on *Fascioliosis* among Goats in Taif, KSA

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Abstract: This study, reviews a 3 years period (2010-2012), recorded of slaughter house (goats) at Al-Taif, KSA. It aimed the determination prevalence of Fascioliosis affecting the liver of slaughtered animals. A total of 18,925 goats were slaughtered and 593 livers of goats were infected.Of the slaughtered goats were a significantly (p0.05), higher prevalence of Fascioliasis was recorded (3.1%). Fascioliosis considered the most cause of liver condemnation and was responsible for total liver condemnation for goats as 16.9%. The aim of this study also, was to determine the, *in vitro*, effects of plant extracts *B.egyptiaca*and TCBZ on adult *F.gigantica*, through histo-pathological examination. The results concluded that, the *B.aegyptiaca*have a great anthelmintic effect against *F.gigantica*reached nearly to the effect of TCBZ and, it is very low expensive.

Key words: Fascioliosis · Flukes · *Fasciola gigantica* · *Goat* · *B. aegyptiaca* · *TCBZ*

INTRODUCTION

Fasciola infection known as Fascioliosis, or liver rot disease.This disease belongs to the plant-borne trematodezoonosis, which overlap in many areas of Africa and Asia [1]. Fascioliosis is caused by *F.gigantica,* a digenetic trematode which belongs to the family Fasciolidae. They are very important liver parasite of ruminants. The adult inhabits the bile duct and gall bladder of liver in these animals. Inside their host, the liver flukes cause severe damage which may lead to the death of the animals [2].

Fascioliasis has been implicated as the cause of morbidity and mortality in the production of ruminants [3].

Using anthelmintics treatment is a regular practice in enzootic areas, but fails to eradicate the parasite. Allopathic anthelmintics are not completely effective against common flukes [4] and have serious dis-advantages in some developing countries bycost, risk of misuse leading to drug resistance, environmental pollution and food were recorded [5]. In addition, almost all adversely affect milk and meat production of animals during the course of their treatment and even for long after their use [4].

The fasciola infection is distributed all over the world, Kenya [6,7]; Romanians and Australia [8]; around Lahor, Pakistan [9], and South of the State of Esprito Santo [10].

Fasciolosis is a significant live stock problem; yearly an estimated US$ 2 billion are foregone due to weight loss, reduction in milk yield and fertility in production animals. In Egypt, animal fasciolosis is a dangerous disease leading to a huge economic losses in live stock production and causing sever illness in human livers [1,12] Robert and Tolon [13] reported that the prevalence of fasciolosis in Egypt estimated to be 2.17%. Urquhart *et al*. [14] mentioned that in acute form of fasciolosis, there was a massive invasion of immature flukes into the liver which cause sudden death;while in chronic form, there was liver cirrhosis caused by the wandering fluke in which mature fluke lodged into the bile ducts, causing its calcification. Enlargement of gall bladder has been noticed. Submandibular edema frequently occurs. The parasite may cause loss of production during winter season in milking cows [15]. Meaney *et al*. [16] studying the effect of TRICLABENDAZOLE (TCBZ) on adult fluke *in vitro*. Results revealed that posterior region of the fluke are more severely disrupted than the oral cone.

Halferty *et al*. [17] studied the efficacy of TCBZ on sheep experimentally infecte with fasciolosis. Results showed that progressive and time dependent increase in disruption of the tegument, culminating in the death of the fluke. Flukes were still active at 48 hr post treatment and were not severely affected. By 72 hr, all but one of the flukes was inactive and they showed reliable levels of

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disruption. After 96 hr, all the flukes were extremely damaged and dead.

Jennefer *et al*. [18] reported that the tegumental changes in adult *F.hepatica*induced by TCBZ were assessed utilizing SEM *in vitro* by incubation of adult fluke with TCBZ for 24 hours at a concentration of 10 mg/ml led to sloughing, blebbing and eruptions in the tegument.

Al-AShaal *et al*. [19] found that the fixed oil of *B. egyptiaca*fruits hadanti-mutagenic activity against *F. gigatica*induced muta-genicity besidesanthelmentic activity against hepatic worm (*S.mansoni* and *F.gigatica*). In spite of the presence of a number of problems due to fasciolosis there ispaucity of well-documented information on the occurrence of the disease in smallruminants at Al-Taif, KSA. Therefore, the present study was designed with theaims of determining the prevalence of fasciolosisaffecting the liver of slaughteredgoats at Al-Taif abattoir in KSA and aimed to determine the comparative effectsof plant extracts (*B.egyptiaca*) and Triclabendazole on *F.gigantica*infection amonggoat *in vitro* trials, through histo-pathological examination.

MATERIALSAND METHODS

Goats Samples: The present study was conducted at Al-Taif slaughtered house, KSA, from January2010 to December 2012. The study populations were goats of differentage,body conditions, and, local and imported from countries for the purpose of meatproduction. A cross-sectional study was used to determine the prevalence offasciolosis in slaughtered goats using simple random sampling method. A total of18,925 goats livers were collected from the selected animals to determine the prevalenceof fasciolosis in the abattoir. Routine post mortem inspection of liver and gallbladder of each animal was carried out to check the presence of *Fasciola spp.* Livers were inspected by making multiple deep incisions of the lobes and makinga deep cut with a number of small sub-cuts. Gall bladders were opened using a knife and thoroughly investigated for the presence of *Fasciola spp.*

Identification of Fasciola Worms: Sixty adult worms were collected from livers, which had active infection, foridentification of *Fasciola spp*. [2].Theinvestigation and identification of *Fasciolaspp*were done according to their distinct morphological characteristics followingthe standard guidelines [21].

Samples for Histopathology: Adult worms of *F.gigantica*recovered from the bile ducts of slaughtered goats Were used for determination the effect of ethanolic extract Of *B. egyptiaca* and TCBZ on adult flukes.

Drugs: Triclabendazole (TCBZ) "Fasinex®" was purchased from Ciba-Geigy.

Plant: *B.egyptiaca*fruits were purchased from Aswan, Egypt. Ethanolicextract were prepared at Medicinal and Aromatic Plane Research Dept at NationalResearch Center.

In vitro determination of the efficacy of *B.egyptiaca*and TCBZ on adult

***F.gigantica*:** Adult worms of *F. gigantic* recovered from the bile ducts of goats slaughtered ina Taif abattoir. Under sterile conditions in a laminar flow cabinet, flukes were washedin several changes of warm (37.8°C), sterile complete RPMI 1640 culture mediumcontaining antibiotics (penicillin, 50 IU/ml; streptomycin, 50 mg/ml). The flukeswere subsequently transferred to fresh culture medium containing 50% (v/v) heatdenatured rabbit serum, 2% (v/v) rabbit red blood cells[22],and plant extracts of *B.egyptiaca*at five different concentrations 30, 60, 120, 240 and480 µg/ml. Dilutions were made from a stock solution of plant extracts at 10 µg/ml, prepared with 70% (v/v) ethanol. The whole flukes incubated for 24 h at37.8°C in an atmosphere of 5% CO2. A positive control group was prepared byincubating whole flukes for 24 h in RPMI culture medium containing 20 µg/mlTCBZ-SX. This level corresponded to maximum blood levels *in vivo* [23]. TheTCBZ was initially prepared as a stock solution in Dimethyl Sulphoxide (DMSO)and added to the culture medium to give a maximum solvent concentration of0.1% (v/v). Solvent control flukes incubated for 24 h in RPMI 1640 culturemedium containing 0.1% (v/v) DMSO. One fluke examined for eachconcentration.

Specimens Preparation for Light Microscopy Analysis: Middle parts of flukes from each group were prepared for paraffin embedding.They fixed in 10% buffered formaldehyde for 24 h, dehydrated with a series ofethanol and cleared with xylene. They were embedded in paraffin, sectioned at5µm using a rotary microtome (HistoSTAT, Reichert, USA) and stained withhematoxylin and eosin stain. They examined for abnormalities using a NikonE600 light microscope and photographed using a Nikon DXM 1200 digital camera(Tokyo, Japan) [24].

Determination the Efficacy of the Studied Plant and TCBZ Using Light Microscopyexamination: Examination for abnormalities and morphological changes in the tegumentlayer and spines, were observed with plant extracts *B.egyptiaca*at five differentconcentrations 30, 60, 120, 240 and 480 µg/ml after 24 h incubation.

Data Analysis: The data recorded during the study period were entered into Microsoft excel sheet. Data were summarized and analyzed using SPSS version 16 computerprogram. Data were analysed using Epi Info version 6 statistical software [25], and for furthercompared using Chi-square test at critical probability of $p<0.05$.

RESULS

Table 1: showed that the total of 18,925 goats was slaughtered in period (2010-2012) and 593 were suffering from Fascioliosis. A significantly ($p<0.05$) higherprevalence of Fasciolasis was recorded in goats liver (3.1%). Over all table 2 showed liver condemnation due to fascioliosis in goats was 593 out of 3506(16,9%).Liver flukes 60 worm samples examined were found *F.gigantica*.

Table 1: The prevalence of Fascioliasis among slaughtered Goats

Year	No.of Slaughtered goats	No. of Infested goats	%
2010	4,543	128	2,90
2011	7,010	255	3,6
2012	7,371	210	2,8
Total	18,925	593	3.1

Table 2: Nomber of condemned livers due to Fascioliosis among Goats from 2010 to 2012 in Taif abattoir, KSA

No of animals examine	Disease condition	No. of Liver condemned	%
3506	Fascioliasis	593	16,9

Effect of Ethanolic Extract of *B.egyptiaca*on Adult *F.gigantica*using Light Microscopy Examination: All treated flukes with different concentrations (30, 60, 120, 240 and 480 µg/ml)showed tegumental swelling, blebbing, vacuolization and disappearance of spineswhich appear embedded in the swelled tegument compared with intact tegumentand spines in control flukes. The severity of tegumental alterations depends uponthe concentration of extracts. The highest effect on the tegument and spines appearin high concentration of the extract than the lower Fig. 1.

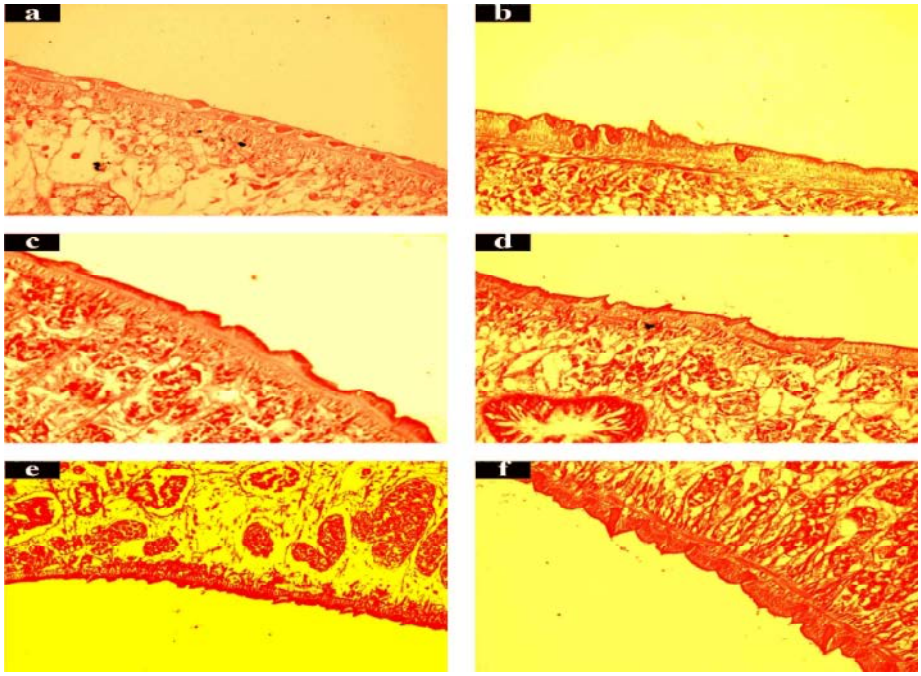

Fig. 1: Light microscopy of the mid-body part of adults *F.gigantica*, after 24h of incubation. (a) Control fluke. (b) *B.aegyptiaca*treated fluke at 30 ìg/mlconc. (c) *B.aegyptiaca*treated fluke at 60 ìg/ml conc. (d) *B.aegyptiaca*treatedfluke at 120 ìg/ml conc. (e) *B.aegyptiaca*treated fluke at 240 ìg/ml conc. (f)*B.aegyptiaca*treated fluke at 480 ìg/ml conc. All treated flukes show tegumentalswelling, blebbing, vacuolization and disappearance of spines compared within tact tegument and spines in control flukes.

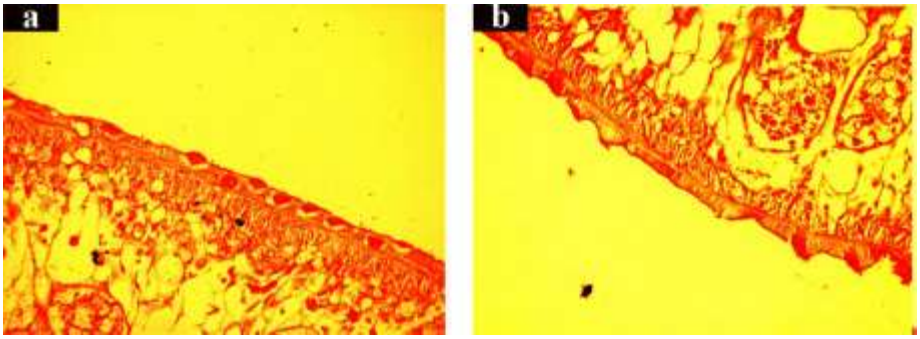

Fig. 2: Light microscope of the mid-body part of adults F.gigantica, after 24 h of incubation. (a) Control fluke. (b) Triclabendazole treated fluke at 20 ìg/ml conc. treated flukes show tegumental swelling, blebbing, vacuolization and disappearance of spines which surrounded by swelled tegument compared with intact tegument and spines in control fluke

Effect of TCBZ on adult *F.gigantica*using Light Microscopy Examination: Treated flukes with different concentrations (20µg/ml) showed tegumental swelling, blebbing, vacuolization and disappearance of spines which appear surrounded by the tegument compared with intact tegument and spines in control flukes Fig. 2.

DISCUSSION

The total infections rate of fasciolosis in the present study (3,1%)was lowercompared with 21.9% in Riyadh governorate abattoir [26]. While the prevalencein Kenya was 0.04%-2.4% [27] and in Egypt was 2,17% [13]. Thisdifference may be due to the big diameter of KSA, so there are different climaticand ecological conditions, also seasons, sources and types of animals involved.Concerning condemnation rate of liver due to fascioliosis was previouslyrecorded in Nigeria [28, 29], in the present study the liver condemnation ratedue to fascioliosis in goats was 16,9 compared to 3.8 % in Bangladesh [29]. The present study revealed that, *Fasciola gigantica* was distinguished among 60 examinedsamples, while *F.hepatica* was difficult to be differentiated from *F.gigantica* on morpho-metricandmorpho-anatomic criteria. This is in agreement with Mas-Coma *et al*. [1] whoreported difficulty to differentiate between the two species.Concerning the *in vitro* studying the effect of ethanolic extract of *B.egyptiaca*different concentration (30, 60, 120, 240 and 480 µg/ml) and TCBZ at 20µg/mlusing light microscope, revealed a tegumental swelling, blebbing, disappearanceof spines and vacuolization of the tegument compared with control flukes. Severeeffect were observed in *B.gyptiaca*and TCBZ depending on the concentration ofextract. These results agreed with that obtained byprevious studies [30,31] that *B.aegyptiaca*showed mild to moderate destruction of tegument with presence of several blebsand swollen nodules were observed showing the underling basal laminar withsome intact spines.The effect of *B.egyptiaca*on adult flukes may be attributed tothe constituent of alkaloids as previously mentioned [32]. In addition, it wasmentioned that *B.egyptiaca*contains 54.53% unsaturated fatty acids and 1.14%sterols which had anti-mutagenic activity against *F.gigantica* [19]. TCBZ is abenzimidazole that binds to tubulin impairing intracellular transport mechanismsand interfering with protein synthesis [33]. *B.egyptiaca*(Balantiaceae), mainly the fruit, is used by traditional healers andherbalists for treating many diseases in Africa and Asia. They investigated the fixedoil composition of fruits and evaluation of its biological activity. Oil content wasidentified using GC and GC/MS. *In vitro* examination of the oil biological activity(including cytotoxicity, antimutagenicity, antiparasitic, antiviral and antimicrobialactivities) was performed. They found that, the oil contained 54.53% unsaturatedfatty acids and 1.14% sterols. The oil exhibited anticancer activity against lung, liver and brain human carcinoma cell lines [19]. It also had anti-mutagenic activityagainst *F.gigantica*induced mutagenicity besides anthelmintic activity againsthepatic worms (*S.mansoni* and *F.gigantica*). Preliminary screening showed thatthe oil had antiviral activity against Herpes simplex virus [19]. It also had anti-microbialactivity against selected strains of Gram-positive bacteria, Gram-negative bacteriaand Candida [19] They showed remarkable biological activity of *B.egyptiaca*fixed oiland proved its importance as natural bioactive source [19].

CONCLUSION

Of the slaughtered goats at Al-Taif, KSA a significantly (p0.05), higherprevalence of Fascioliasis was recorded (3.1%). Fascioliasis considered the mostcause of liver condemnation and was responsible for total liver condemnation for goats as 16.9%.Studing the effects of plant extracts *B.egyptiaca*and TCBZon adult *F.gigantica*, through histopathological examination revealed that, the*B.aegyptiaca*have a great anthilmintic effect against *F.gigantica*reached nearly tothe effect of TCBZ and is very cheaper than the drug.

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