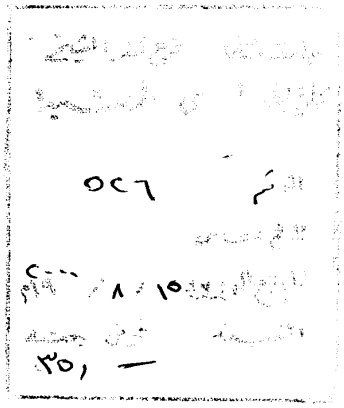
# التاثيرات السمية للتراى كلا بندازول على وظائف الكبد و الكلي

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بكالوريوس العلوم الطبية البيطرية

جامعة طنطا- فرع كفرالشيخ (١٩٩٦)


## أشراف


*الدكتور*

**فتحى رضوان على**

أستاذ الطب الشرعى و السموم

و وكيل الكلية لشئون الطلاب و التعليم

كلية الطب البيطرى- جامعة المنصورة

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استاذ و رئيس قسم الفارماكولوجى و الطب الشرعى و السموم

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رسالة مقدمة ألى جامعة طنطا للحصول على درجة الماجستير

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## List of abbreviations

ALT : alanine amino-transferase
AP : alkaline phosphatase
AST : aspartate amino-transferase
DCHBS: 3,5- dichloro-2- hydroxy benzene sulfonic acid
E.D.T.A: ethylene diamine tetra-acetic acid
*F.* : fasciola
FAD : food and drug administration
GGT : gamma glutamyl transferase
Hb. : haemoglobin
LCAT : lecithin cholestrol acyl transferase
MCH : mean corpascular haemoglobin
MCHC : mean corpascular haemoglobin concentration
MCV : mean corpascular volume
NOEL : non observable effective level
PAB : 4- aminophenazone
P.C.V. : packed cell volume
R.B.C.s : red blood cells
TCBZ : triclabendazole
W.B.C.s: white blood cells

# CONTENTS

## List of tables

## List of figures

# INTRODUCTION

# Introduction

Fascioliasis is wide spread disease caused by liver fluke fasciola (*F. hepatica, F. gigantica and F. magna*). The disease causes reduced growth rate and milk yeild, as well as losses of liver condemend at slaughter and reduction of up to 39% in wool production **(Chick et al.,1980)** .

Anthelmintics are commonly used in veterinary practice especially fasciolicidal drugs, among recent fasciolicidal drugs is triclabendazole (TCBZ ) which is a novel benzimidazole compound (6-chloro-5-2,3 dichlorophenoxy-2-methylthio ) benzimidazole **(Britt,1986)** and highly effective against fluckes as young as one week old onwards but the exact mode of action of TCBZ is unknown ;the fact that it does not affect nematodes suggests a different mechanism of action than other anthelmintic drugs that affect the neuromuscular system .

In vitro experiments demonstrate that TCBZ penetrates through tegument, decreasing parasite motility due to affecting on microtubuli **(Werner and Ximena,1995).**

The drug has been tried in human fascioliasis with promising results **(Wessely et al.,1988 and Ripert,1990)** .

There are few reports on the side effects of TCBZ in farm animals which motivated for the present study . The aim of the present work is to study the effect of TCBZ (200 and 400 mg/kg b.w twice weekly orally for one. to 6 weeks) on:-

1- Liver and kidney functions.

2- Blood picture.

3- Parenchymatous organs (liver, spleen, kidney and heart) and muscles of treated albino rats.

# REVIEW OF LITERATURE

# *Review of literature*

## Antitrematodal drugs

**Fowler,(1971)** mentioned that on the basis of chemical structure, the fasciolicidal drugs can be classified into :

(1)The bisphenolic compounds (hexachlorophene, oxyclozanide).

(2) The halogenated hydrocarbons (carbon tetrachloride, hexa chloro-ethane).

(3) The nitrophenolic compounds ( niclofolan, disphenol).

(4) Salicylanilides (closantel, brotianide).

(5) Sulphonamides (clorsulphon).

(6) Benzimidazoles (albendazole, triclabendazole).

A common feature of the drugs of the first 3 groups is presence of halogen atoms. Whether the halogen atom presents a common mechanism for fasciolicidal activity of all these drugs is not known but is suggested to be unlikely.

**Losson,(1988) and Richards,(1990)** tested the efficacies of many of the current antitrematodal drugs against immature and mature stages of *F. hepatica* in cattle. The drugs tested were triclabendazole (TCBZ), albendazole, clorsulon, nitroxynil, oxyclozanid and rafoxanide. None of these drugs, however, have FDA approval in the USA for use against flukes except albendazole and clorsulon.

## Benzimidazoles

**Knight and Colglazier,(1977)** reported that among benzimidazoles, albendazole is unique in having therapeutic activity against adult forms of *F.hepatica* (76-100%) at single doses of 10 mg/kg in cattle or 5-7 mg/kg in sheep. At these doses, the activity for immature (3-week-old) *fasciola* is only 25% but can be increased to 75% with a high doses of 50 mg/kg in cattle.

**Van Den Bossche,(1980)** reported that there are two major biochemical effects of benzimidazoles on helminths, these are inhibition of fumarate reductase by compounds such as thiobendazole, and interaction with tubulin and microtubules by newer benzimidazoles such as mebendazole, parbendazole and albendazole.

**Watts et al.,(1982)** recorded that microtubules were thought to be essential for enzyme secretion ( acetylcholine estrase) by parasites, which would be inhibited by benzimidazoles.

**Strote et al.,(1989,1990)** recorded that research on parasites of veterinary importance has mainly focused on lesions caused by benzimidazoles in the intestine of adult worms.

**Ali and Hennessy,(1993)** reported that the activity of benzimidazoles had been influenced by a number of factors, including the effect of feed intake. The same results were recorded by **Hennesy et al.,(1993)** in addition to concurrent parasite burdens.

**Lanusse and prichard(1993a)** reported that benzimidazoles had a limited water solubility and small differences in solubility may substantially influence their gastrointestinal absorption and their resultant systemic bio- availability and clinical efficacy.

**Richard,(1995)** reported that at the higher or lower body temperature of mammals, benzimidazoles have a much higher affinity (as indicated by a much slower rate of dissociation of the benzimidazole-tubulin complex) for nematode than mammalian tubulin, hence its selectivity. Also he added that in most tissues of treated animals, residues of benzimidazoles approach the low limit of detection (0.05 mg/kg) in 2 days post administration; however, residual quantities are detectable by radioactivity in the liver at weeks after dosing and occur in range of 3 microgram or less/ gm tissue.

## Triclabendazole (Fasinex®)

**Bennett and Thompson, (1986); Boray, (1986); Fetterer, (1986); Bennett and Kohler, (1987); Prichard,(1987); Campbell, (1990) and Townsend and Wise,(1990)** mentioned that TCBZ is recently add to the list of potent, orally active benzimidazole anthlmintics due to it has neither a methyl carbamate nor a thiazolyl substituent in 2-position and is narrow spectrum .

## Mode of action

**Behm and Bryant,(1979)** recorded that benzimidazoles interfere with purine-like groups and it interact with enzymes in parasites causing a decline in ATP available to the parasites.

**Stitt and fairweather,(1994)** reported that TCBZ in its active sulphoxide metabolite form affects on the tegumental ultrastructure of *F. hepatica*. Also **Stitt,(1995)** showed that active sulphoxide metabolite of TCBZ had a significant inhibition effect on protein synthesis by *F. hepatica*. Further more **Stitt and fairweather,(1996)** determined the effects of active sulphoxide metabolite of TCBZ on the vetilline cells of *F. hepatica in vitro* by transmission electron microscopy. He found that sulphoxide metabolite cause degeneration of vetilline cells of *F. hepatica*.

## Efficacy

**Coles,(1986)** tested TCBZ *in vitro* and *in vivo* against a range of helminths. *In vitro* activity was found against *hymenolepis diminuta* (0.5 microgram/ml), *F. hepatica* (2.5 microgram/ml), *taenia crassiceps* and *schisto-soma mansoni* (50 microgram / ml). On the other hand, *in vivo* activity was only found against *F. hepatica* , a single oral dose of 40 mg / kg killing 99% of adult flukes in the rat. This spectrum of activity suggests a mechanism of action unlike that of other benzimidazoles, in addition **Negro,(1991)** reported that TCBZ exhibit high activity against immature and mature stages of *F. hepatica* , *F. gigantica* in sheep and cattle, he added that TCBZ undergoes rapid and extensive

metabolism, first to its sulphoxide and sulphone, with the parent compound remaining undetectable in plasma. Further more **Hammouda,(1995)** found that following administration of TCBZ orally in a single dose of 10 mg / kg b.w. to 10 human cases with a variability of clinical manifestation suggesting fascioliasis, the general condition and all sings were ameliorated with drop in the eosinophilic count, which returned to normal after 2-3 months. Also **Richard,(1995)** recorded that TCBZ has also been used successfully in treatment of *F. hepatica* in horses (12 mg / kg), *fascioloides magna* in white tailed deer(10 mg / kg) and wapiti (50-60 mg / kg). **Yilmaz et al.,(1998) and El-Karaksy et al.,(1999)** reported that, children were treated with TCBZ in a dose of 10 mg/kg b.w. as a single oral dose. Within 2 months, 78% of children were cured as evidenced by clinical well-being, normalization of eosinophil counts, fasciola antibody titres, and absence of *F. hepatica* eggs in stools. No side effects and the drug easy to administer.

## Pharmacokinetics

**Muecke,(1981) and Hambock,(1983)** found that following single oral doses of 0.5 or 25 mg / kg b.w. of C14-labeled TCBZ to rats, excretion was rapid with approximately 93% of the dose eliminated in 48 hours. At the end of 144 hours, total recovery amounted to 98% of which 88 to 95% was in faeces, 4 to 10% in urine, less than 0.05% in expired air and up to 1% in tissues. There no differences related to sex or dosage. In a bile canulated rat given 5 mg/kg b.w. by gavage, biliary excretion in 49 hours was 34% of administered dose.

**Alvinerie And Galtier,(1986)** mentioned that TCBZ is probably metabolized in liver. In addition, **Hennessy et al.,(1987) and Kinabo & Bogan,(1988)** reported that when TCBZ given orally or intrarumenally, is metabolized rapidly to its sulphoxide and sulphone derivatives which reach the

maximum plasma concentration in approximately 12-36 hours. The derivatives bound to albumin and persist at measurable concentration in plasma up to 7 days. Excretion is principally via bile (approximately 50%). The binding to plasma albumin likely influences the duration of exposure of flukes to this drug.

**Souhaili-El-Amri et al.,(1987); Short et al.,(1988) and Lanusse & Prichard,(1993a)** recorded that for commercially available benzimidazole thioethers and sulphoxides, liver microsomal sulphoxidation was a common metabolic pathway. **Lanusse et al.,(1995)** added that a portion of the sulphoxide undergoes a second, slower oxidative step which forms the sulphone metabolite ; this latter metabolic step was irreversible.

**Mckellar et al., (1995)** mentioned that the metabolism of benzimidazoles was associated with hepatic cytochrome P450 and flavin mono-oxygenase system.

## Toxicity

**Patton,(1976)** reported that toxic effect of benzimidazoles may be related to their antimicrotubular activity. In addition, they reduce milk secretion.

**Hoebeke et al.,(1976)** suggested that the benzimidazoles antimitotic activity was due to binding to brain tubulin which cause inhibition of microtubules formation leading to disruption of cell division.

**Ullmann and Sachsse, (1979c)** examined the skin irritating effect of TCBZ suspended in propylene glycol/saline and applied for 24 hours in Newzeeland white rabbits. The test material was judged to be slight irritant to skin.

**Ullmann and Sachsse, (1979d)** recorded that when rabbits received 0.1 gram of TCBZ instilled into conjunctival sac of the eye, the subsequent examination failed to reveal any eye irritation .

**Ullmann and Sachsse,(1979e)** found that 10 intracutaneous injections of 0.1% (w/v) suspension of TCBZ in propylene glycol/saline in Pirbright guinea-pigs produced no reaction in epidermal challenge but an intradermal challenge resulted in a positive sensitization reaction.

**Tupin,(1981)** recorded that, groups of 6 male and 6 female Beagle dogs were fed diets containing 0,10,100,1000 p.p.m. TCBZ (purity 97.6%) for 13 weeks. The calculated intake of the test material was 0.35, 3.4 or 37 mg/kg b.w./day in males and 0.35, 3.5 or 39 mg/kg b.w./day in females. Food intake was unaffected but body weight gain was markedly depressed in 1000 p.p.m. dogs. ECG's revealed prolongation of QT interval and QTC value in 1000 p.p.m. males and females at weeks 5 and 9 but not at week 13. Haematological studies and blood chemistry were examined before treatment and at weeks 5, 9 and 13. Erythrocytes, hemoglobin and haematocrite value were markedly reduced at 1000 p.p.m. throughout the study, with evidence of reticulocytosis at week 9 only .Alkaline phosphatase was increased in 100 and 1000 p.p.m. groups; serum Alanine aminotransferase (ALT) and cholestrol were increased in 1000 p.p.m. dogs. Histopathology showed increased liver weight, centrilobular hepatocellular pigment granules accompanied by cytoplasmic basophilia, glycogen depletion and foci of pigmented macrophages at 1000 p.p.m. Also ovaries and testes were immature with lower organs weight. Females failed to reach estrous . males showed incomplete spermatogenesis. The NOEL was 10 p.p.m., equal to 0.35 mg /kg b.w./day.

**Giese et al.,(1981b)** mentioned that, groups of 20 pregnant *Chinchilla rabbits* were given gavage doses of 0.3,10 or 20 mg/kg body weight/day of TCBZ (purity unknown) as a suspension in 0.5% (w\v) carboxy methyl cellulose. Treatment was on gestation days 6 to 18 and does were killed on gestation day 28. Three low-dose female died and diarrhea was noted in a few animals from each treated group. Maternal body-weight gain was similar in all groups but when 'corrected" by substracting the weight of uterus and contents, there was slight non-dose related decrease in treated groups. The number of implantation, resorptions and fetal deaths were similar in each group and fetal body weight was not affected. Fetal examination showed unossified phalanxes of fore and hind limbs at 10,20 mg/kg body weight/day. One 20 mg / kg body weight/day fetus show omphalocele which is rare in this strain of rabbits. The NOEL was 3mg/kg body weight/day.

**Strong and Ryan,(1981)** studied the teratogenic effect of TCBZ in *Merino ewes* drenched with a single dose of 0, 5 or 10 mg / kg b.w. TCBZ in the third trimester. There were no effects on lambing or on the morphology of offspring. Also **Strong,(1981)** studied the lambing performance of ewes following drenching of multiple dosing with 0 or 30 mg/kg b.w./day TCBZ during first trimester of pregnancy. There were no clinical signs of toxicity in ewes and no effects on lambing or development of lambs.

**Hunter et al.,(1982)** recorded that, groups of 20 male and 20 female *Charles River CD rats* were fed diets containing 0, 10, 100, 1000 p.p.m. TCBZ (purity 97%) for 13 weeks. The calculated intake of test material was 0.7, 6.6 and 68.5 mg/kg b.w./day in males and 0.8, 7.9 and 87.3 mg/ kg b.w./day in females.

Food and water intake were reduced in 1000 p.p.m. males while body weight gain was depressed in 100 p.p.m. males and 1000 p.p.m. males and females. Hematology, blood chemistry and urinanalysis were performed before treatment and at weeks 5 and 12. Number of red blood cells, hemoglobin and haematocrite were reduced in 1000 p.p.m. males and females. Lymphocytes and therefore total leucocytic count were reduced in 1000 p.p.m. females. Serum alkaline phosphatase was increased in 1000 p.p.m. groups. Cholestrol, albumin and total protein were increased at 1000 p.p.m. particularly in females. Urine volume was reduced in 1000 p.p.m. groups. Gross examination showed increase in pale livers and kidneys at 100, 1000 p.p.m. and congested lungs at 1000 p.p.m. The NOEL was 10 p.p.m., equal to 0.7 mg/kg b.w./day.

**Sarasin,(1982 a.b)** reported that the oral $LD_{50}$ in male and female rats of TCBZ was greater than 8000 and for sulfoxide, sulfone metabolites of TCBZ was greater than 5000 mg/kg body weight. Toxic signs were similar to those induced by TCBZ including sedation, dyspnea, exophthalmus, ruffled fur and curved body position after oral, dermal or inhalation exposure. Ataxia was also noted following intraperitoneal dosing.

**Strong and Steiger,(1983)** reported that, groups of 27 to 28 pregnant *Merino ewes* were drenched with a single dose of 0 or 50 mg/kg b.w. of TCBZ. Drug administration was on days 12, 17, 24 or 28 days after mating. There were no effects on lambing performance or development of lambs.

**Turner et al.,(1984)** reported that, lambs were dosed orally at 2,6 and 12 weeks after infection with a 5% drench suspension of TCBZ at dose rates ranging 2.5 to 10 mg/kg body weight. The lambs were observed daily for 14 weeks. No adverse signs were noticed.

**Fritz et al.,(1984)** studied 2-generation toxicity of TCBZ in groups of 20 male and 20 female Tif:RAIf rats at dose rates of 0, 3, 15 or 75 p.p.m.(purity 97.6%) in diets. He found that initial generation and second one revealed no overt sings of toxicity, no effects on body-weight gain and no influence on reproductive parameters. Examination of pups showed no meaningful changes in the second generation offspring but in third generation pups, mortality was increased in 15 and 75 p.p.m. groups during lactation. Also body weights at weaning were lower at 15 and 75 p.p.m. The NOEL was 75 p.p.m., equal to 5.5 mg / kg b.w./day.

**Bowen and Ryan,(1985a)** mentioned that, *Hereford* cows were drenched with single dose of 24 mg/kg body weight TCBZ during first month of pregnancy. Calving and uterine development of offspring were unaffected .

**Bowen and Ryan,(1985b)** reported that, *Hereford* or *black poll* cows were drenched with a single dose of 0 or 24 mg / kg b.w./ day TCBZ during the second, third, fourth and sixth to seventh month of pregnancy. There were no difficulties at calving or abnormalities in the calves.

**Robinson,(1985)** reported the same results recorded by **Hunter,(1982)** in addition to that following doses of 10 mg/kg to sheep and 12 mg/kg to cattle, residues of TCBZ were below 0.4 mg/kg in muscles and other edible products at day 28 in sheep and day 14 in cattle. further more, in dairy cows given 6 mg/kg, at 7 days, total TCBZ and metabolites averaged (mg/kg) 0.06 in whole milk, 0.03 in skim milk, 0.16 in cheese and 0.77 in butter so he added that TCBZ should not be used in lactating animals whose milk products are to be consumed by humans. He added that, dairy cattle should be given TCBZ only when dry prior to calving.

**Charnley et al.,(1986)** recorded that, groups of 60 male and 60 female Charles River CD rats were fed diets containing 0, 3, 15, 30 or 100 p.p.m. TCBZ (purity 99.5%) for 2 years. The calculated intake of the test material was 0.1, 0.6, 1.2 or 4.0 mg/kg b.w./day for males and 0.2, 0.7, 1.5 or 5.2 mg / kg b.w./day for females. There were depressed body-weight gain at 100 p.p.m., significantly only in females. Clinical laboratory parameters were examined pre-test and at weeks 13, 26, 51, 78 and 104. Lymphocyte counts were reduced at termination in 30 and 100 p.p.m. groups. Also plasma chloride was decreased but calcium and proteins were increased in 100 p.p.m. males and in all female groups. The necropsy showed lower kidney weights in 100 p.p.m. males. At pathology, pancreatic islet cell adenomas were increased in 15 and 100 p.p.m. males. The NOEL was 30 p.p.m., equal to 1.2 mg/kg b.w./day.

**Seiler,(1975) and Delatour&Parish,(1986)** stated that, benzimidazoles were suspected of having a mutagenic effect due to their antimicrotubular effect causing disruption of the mitotic spindle, which could lead to non disjunction of chromosomes during cell replication. In mammals, the changes in the number of chromosomes usually lead to cell death. This may be the reason for the absence of correlation between the antimitotic activity and the mutagenic effect of benzimidazoles .

**Doroshina and Bragina,(1987)** noticed that, TCBZ showed moderate toxicity ($LD_{50}$ = 980 mg/kg b.w. in white mice). The toxicity coefficient for cattle was 80 and for small ruminant was 98. The drug was neither embryotoxic nor teratogenic in white rats .

**Lacey et al.,(1987)** claimed that, benzimidazole carbamates had strong inhibitory activity against mammalian tubulin.

**Yoshimura,(1987)** tested the teratogenic effect of TCBZ when administered by gavage to pregnant rats at 0 (control),10, 25, 50, 100 or 200 mg on days 8 through 15 of pregnancy. The dams were killed on day 21 of pregnancy and fetuses were examined by routine teratological method. As the result, no increase in the incidence of resorption even at 200 mg/kg corresponding to 20 times the recommended therapeutic dose. There was a dose-related decrease in fetal body weights which was statistically significant at 100 mg/kg or more.

**Basler et al.,(1988a)** mentioned that, groups of 80 male and 80 female albino Tif:MAGF mice were fed diets containing 0, 3, 15, 60 or 300 p.p.m. TCBZ (purity 99.5%) for 2 years . The calculated intake of test material was 0.29, 1.44, 5.7 or 29.6 mg/kg b.w./day in male and 0.27, 1.39, 5.35 or 28.7 mg/kg b.w./day in female. Hematology, blood chemistry and urine analysis were performed at 6 month intervals. Serum levels of alkaline phosphatase (AP), ALT, AST were increased in the 300 p.p.m. groups during the first year and in all treated groups in the second year. At the end of the study, absolute and relative liver weights were increased at 15 p.p.m. and above. Hepatomas were increased in all treated female groups, but significance was not reached at the 99% level. The incidence of carcinomas and time to appearance of tumors were unaffected . The NOEL was 3 p.p.m., equal to 0.27 mg/kg b.w./day.

**Fayek et.al.,(1989)** reported that TCBZ at dose of 10 mg/kg b.w. had no effect on liver tissue as the flactuation in transaminases activity in treated group and also in infected and normal control groups.

**Counotte et.al.,(1990)** recorded that TCBZ was administered at 12 mg/kg to cows. The maximum concentration of the main metabolite of TCBZ (TCBZ-sulphone) measured in milk was 1.415 mg/L. Metabolites could be detected in

milk during a period of 10 days. Only 1.5% (maximum) of the administered TCBZ was eliminated as TCBZ-sulphone via the milk.

**Steffan M.J.,(1990)** mentioned that a single oral dose of 150-200 mg TCBZ/kg b.w. for sheep and cattle may lead to side effects such as unsteady gait, stiffness of hind quarter, dullness and reduced appetite. These effects are slight and last for 1 to 3 days. An antidote is not known. The minimum lethal dose lies between 200 and 500 mg/kg b.w. Lethal intoxication is characterized by sever respiratory insufficiency due to serious effusion in the thorax, pericardium and abdomen as well as to distinct pulmonary edema . In cattle; the situation similar to that in sheep.

**Lipkowitz and McCracken, (1991)** recorded that any structural modification of TCBZ and/or its sulfoxide and sulfone metabolites to reduce plasma protein binding (in an attempt to attain better distribution to parasites other than *F. hepatica*) may result in higher host toxicity.

**Russel et al.,(1992)** stated that, benzimidazoles are potent inhibitor of the tubulin microtubule equilibrium in many species.

**Stephen and Elain, (1992)** claimed that many benzimidazoles were embryotoxic or teratogenic in rodent models.

**Carr et al.,(1993)** determined the uncoupling activity of TCBZ using rat liver mitochondria. With glutamate or succinate as mitochondrial substrate, and the respiratory control index (RCI) as indicator of uncoupling activity. They found that TCBZ and its 2 main metabolites were uncouplers of oxidative phosphorylation at micromolar concentration.The rank order of in vitro activity was TCBZ sulphone >

TCBZ sulphoxide >TCBZ. So TCBZ and its metabolites are lipophilic protonophoric uncouplers of rat liver mitochondrial oxidative phosphrylation.

**Hamed,(1993)** studied that oral administration of TCBZ at the therapeutic dose (35 mg/kg b.w.) for rats induced significant increase in serum ALT, AST levels till 4 weeks post treatment which return to normal 60 days post treatment . Further more there was non significant changes in serum urea and creatinine levels These findings are supported by histopathology where liver showed after first day, moderate lymphocytic infiltration in portal tract associated with cloudy swelling of some hepatocytes. After the first week, liver showed pronounced thickening of portal triads with leucocytes on the expense of the hepatic lobules and bile duct proliferation, the newly formed bile ductules either possessed a lumen or appeared elongated and trabecular with very narrow lumen. On the other hand, renal tissues showed no histological changes. He added that rats showed tachycardia and tachypenia followed by depression and no mortalities occurred .

**Martin et al.,(1993)** recorded that skin lesion resembling photose nsetization were first seen in some cows following the 6 weekly TCBZ treatment used in the 1987-1989 eradication program for liver fluke out breaks in 2 herds in west Australia. When a similar problem was encountered in a second eradication program in 1992, four black and white fresian herds, including 2 treated and 2 control herds matched on the basis of location and feeding practices were monitored during the treatment period. One control herd had experienced skin inflammation in the earlier eradication program. The udder and teat skin of all lactating dairy cows (average number of cows per herd 59-116) in each of the herds were scored for inflammation once a week for 7 weeks. In 2 of the matched herds a significantly higher proportion of cows in the treated herd with normal teat skin 4 days before treatment developed inflammation by day 3 after treatment (27.1%) compared with the control herd (4.4%). Similar figures 16.07% vs 2.7%

were observed for udder skin in these 2 herds . The proportion of cows in the 2 treated herds (combined ) with teat skin inflammation rope from 6.5% before treatment to 31.4% on day 3 and 33.2% on day 10 after treatment , while in untreated cows in the control herds it was 2.8% and 7.5% on day 3 and 10, respectively . The results suggest that TCBZ treatment was not the only factor giving rise to skin inflammation during the study period.

**Stokol et al.,(1997)** detected bone marrow toxicosis in dog and cat following albendazole administration . Both animals were admitted with pancytopenia . In the dog, pancytopenia was attributed to sever pan-marrow hypoplasia, whereas the cat had hypoplasia of erythroid and megakaryocytic series, but with a left-shifted granulocytic hyperplasia. Results of cytological examination of bone marrow from both animals were compatible with acute injury. Both animals had been treated with albendazole for giardiasis befor the oncet of clinical signs. Bone marrow toxicosis was attributed to albendazole administration for the following reasons: this was only or most recent drug administered, other causes of bone marrow toxicosis were not found, and the both animals recovered rapidly with supportive care that consisted of fluid and antibiotic administration. Albendazole induced toxicosis appeared to be dose related in the dog and idiosyncratic in the cat.

# MATERIALS AND METHODS

# Material

## I-Chemicals

### 1-Drug

Tricalbendazole (Fasinex 10% solution w/v, CIBA-GEIGY)

***Chemical name:*** 6- chloro-5-(2,3- dichloro phenoxy)-2- methyl- thio benzimidazole.

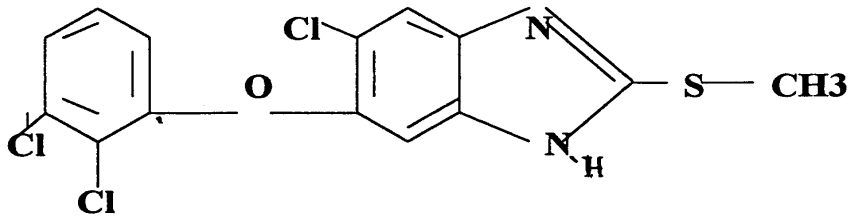
It is white crystalline substance .

***Melting point:*** 173-176 C°.

***Solubility:*** 0.1 p.p.m. at 20C in water; soluble to a varying degree in usual organic solvents ( as methanol ) ; density of suspension 1.53, vapour pressure of suspension 1.3 X $10^{-12}$.

***Source:*** water based drench suspension formulation of CGA 89317 ( code number ) containing 100 gm / litre active ingredient was used in all experiments.

Triclabendazole has the following structural formula, **Eckert et al.,(1984)**




***Empirical formula*** $C_{14}H_9CL_3N_2HOS$

### 2) Chemicals for haematological examination

*Disodium ethylene diamine tetra-acetic acid (E.D.T.A) was used in a concentration of 15%, where 1.5 gm of E.D.T.A powder were dissolved in 10 ml. distilled water. One drop was used to prevent clotting of 0.5 ml blood.

### 3) Kits for biochemical examination

1) Kits from RANDOX were used for deterimination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin and creatinine.

2) Kit from QCA was used for deterimination of serum gamma glutamyl transferase enzyme activity.

3) Kits from Merckotest® were used for deterimination of total serum proteins, bilirubin and conjugated bilirubin.

4) Kits from bioMerieux were used for deterimination of serum alkaline phosphatase, cholestrol, glucose and urea.

5) Kit from Spinereact was used for deterimination of serum uric acid.

### 4) Chemicals for histopathological examination

1) Ethanol $C_2H_5OH$, (Prolabo)

2) Formaldehyde $CH_2O$, (Prolabo)

3) Haematoxylin and eosin stain

4) Hard paraffin

5) Periodic acid Schiff reaction (PAS)

6) Xylol, (Chemico, Egypt)

## II- Equipments

1- Automatic cell counter (MS9 cell counter analyser, Melet schloesing laboratories)

2- Centrifuge (modle 80-2, shanghai surgical instruments factory) and centrifuge tubes.

3- Decanting racks, forceps.

4- Electrical sensetive balance (OHAUS Ls 200 balance).

5- Insulin syringe, microhaematocrite tubes.

6- Pipettes, rotatory microtome ERMA, Japan.

7- Scalp, scissors.

8- Spectrophotometer ( Clema jr spectrophotometer, Ral Co., Spain )

9- Water bath ( Kutermann thermostatic controlled water bath )

## III- Experimental animals

Albino rats from both sex weighed from 100- 150 gm were obtained from Experimental Unit, College of Veterinary Medicine, Zagazig university. Animals were clinically healthy, and housed in stainless-steel cages with hard wood shavings as bedding. Animals were accommodated to the laboratory conditions for one month befor being experimented. They were maintained on balanced ration composed of barley, milk and green fodder. Water and feed were given ad libitum throughout the experimental period.

# Methods

## Grouping and experimental design:

Ninty mature albino rats were used to study the effect of TCBZ at a dose rate of 1/40 $LD_{50}$ (200 mg/kg b.w.) and 1/20 $LD_{50}$ (400 mg/kg b.w.) twice weekly orally for one to six weeks on liver, kidney functions, blood picture and histopathology of some organs. The animals were divided into 3 equal main groups 30 rats each .

-First (control) group was given 5 ml saline orally, and kept as control where it was subdivided into 6 subgroups each one contains 5 rats .

-Second (1/40 $LD_{50}$) group was given the drug orally at 200 mg/kg b.w. twice weekly using stomach tube connected with insulin syring.

-Third (1/20 $LD_{50}$) group was treated with the same manner at a dose level of 400 mg/kg b.w.

Five rats from each group were killed per week.

## Blood sampling

Blood samples from control and treated rats were taken before sacrificing them from the orbital plexus using microhaematocrite tubes. The blood allowed to flow freely and gently on the inner wall of clean dry centrifuge tubes, for biochemical examination, another blood samples were collected in centrifuge tubes containing E.D.T.A for haematological examination.

## Preparation of serum

Blood samples for biochemical examination were left to clot at room temprature, then kept in refrigerator till the morning and centrifuged for 10 minutes at 3000 r.p.m. to obtain clear serum. The sera were identified and stored in deep freezer at -20 C until examined.

## Biochemical studies

The collected sera were used to investigate the effect of TCBZ on hepatic and renal functions.

a) Determination of the activity of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Serum AST and ALT activities were determined colorimetrically according to the method described by **Reitman and Frankel, (1957) .**

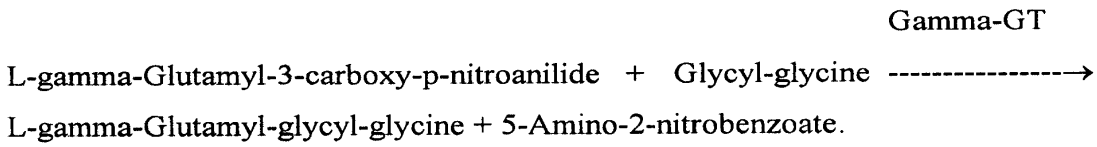
Principle:

The oxalacetate formed in the reaction with AST was decarboxylated spontaneously to pyruvate which measured by hydrazone formation. The pyruvate produced by ALT transamination reacts with 2,4-dinitro phenyl hydrazine (DNPH) giving a brown colored hydrazone measured colorimetrically at 520 nm. In both estimations the concentration of the substrate was suboptimal to reduce the back ground color given by α- keto glutarate in the reaction with 2,4 dinitro phenyl hydrazine.

b) Determination of serum gamma glutamyl aminotransferase(GGT) activity.

Serum GGT activity was determined according to the method described by **Szasz,(1969)** at wave length of 405 nm.

Principle:




c) Determination of total serum proteins

Total proteins were determined colorimetrically according to the method described by **Weichselbaum,(1964), Josephson et al.,(1957), Sundermann,**

**(1958) and Henry,(1964).** The method used called Biuret method where the peptide bonds of protein (NH-Co) react with an alkaline cupper solution to give violet coloration and measured at wave length 530 nm.

d) Determination of serum albumin

Serum albumin was determined colorimetrically by dye binding method as described by **Doumas et al.,(1971)** where the measurement is based on quantitative binding of albumin to the indicator 3,3,5,5-tetrabromo-m cresol sulphonaphthalein (bromocresol green, BCG). The albumin-BCG-complex is absorbed maximally at 578 nm.

e) Determination of serum globulins

Globulins value was determined by substracting the albumin value from the total proteins in the same sample according to **Coles,(1974).**

f) Determination of serum bilirubin

Serum total and conjugated bilirubin were determined colorimetrically according to the method described by **Jendrassik & Grof,(1938) and Schellong & Wende,(1960).**

Principle:

For the measurment of conjugated bilirubin , the serum is acidified with dilute HCL and then mixed with diazotized sulfanilic acid to produce azobilirubin. .Only the conjugated form will react with the diazo reagent in the absence of accelerator caffeine benzoate. The reaction is stopped by the addition of an ascorbic acid solution. Then an alkaline tartrate solution is added to the reaction mixture, followed by the addition of an aliquot of caffeine reagent. The latter shifts the absorbance peak of azobilirubin to 578nm. at which the absorbance is measured, while the tartrate reagant provides an alkaline PH to

produce the blue and more intensive color of azobilirubin. The measurment of total bilirubin is achieved by adding caffeine reagant (accelerator) to the specimen followed by the addition of diazotized sulfanilic acid. During the incubation period both conjugated and unconjugated bilirubin react with the diazo reagant to produce azobilirubin. Ten minutes after the addition of diazotized sulfanilic acid, solutions of ascorbic acid, alkaline tartrate and dilute HCL acid are added to the reaction mixture. The absorbance of the resulting blue azobilirubin solution is measured at 546 nm.

(g) Determination of serum alkaline phosphatase

Serum alkaline phosphatase was determined colorimetrically according to the method described by **Kind & King,(1954) and Belfield & Goldberg,(1971)**

Principle:

Alkaline phosphatase (pH 10)

Phenylphosphate----------→ phenol + phosphate

The phenol liberated by alkaline phosphatase is measured in the presence of amino-4-antipyrine and potassium ferricyanide at wave length of 510 nm. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

(h) Determination of cholesterol

Cholesterol was determined enzymatically according to the method described by **Richmond and Flegg,(1973), Allain,(1974) and Arcol,(1989).**

Principle:

cholesterol esterase

Cholesterol ester----------→ cholesterol + fatty acids

Cholesterol oxidase

Cholesterol + $O_2$ ----------> cholestene-3-one + $H_2O_2$

Peroxidase

2 $H_2O_2$ + phenol + 4-amino-antiphenazone----------> quinoneimine + $4H_2O$

(I) Determination of glucose

Glucose was determined enzymatically according to the method described by **Trinder,(1969), Siest et al.,(1981)** . Glucose is determined at wave length of 505 nm.

Principle:

Glucose oxidase

Glucose----------> gluconic acid + $H_2O_2$

Peroxidase

$2H_2O_2$ + phenol + amino-4-antipyrine----------> quinoneimine + $4H_2O$.

(j) Determination of urea

Serum urea was determined according to the method described by **Fawcett & Scott,(1960) and Patton & Crouch,(1977)**. This method based on enzymatic degradation of urea by urease enzyme..

Principle:

Urease

Urea + $H_2O$----------> $2NH_3$ + $CO_2$

Ammonium ions react with salicylate and hypochlorite in alkaline medium to form a green colored indophenol which measured colorimetrically at 580 nm.

(k) Determination of uric acid

Serum uric acid was determined according to the method described by **Barham & Trinder,(1972) and Fossatti & Prencipe,(1980).** In this method uric acid is oxidized by uricase to allantion and hydrogen peroxide, which under the influence of POD oxidizes DCHBS and PAB . to form a red quinoneimine compouned.

Principle:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow[\text{Uricase}]{} \text{Allantion} + CO_2 + 2\,H_2O_2$$

$$2H_2O_2 + \text{PAB} + \text{DCHBS} \xrightarrow[\text{POD}]{} \text{Quinoneimine} + 4\,H_2O$$

The quantity of this red quinoneimine formed is propotional to the uric acid concentration.

(l) Determination of creatinine

Serum creatinine was determined according to the method described by **Henry,(1974)**. In this method creatinine in alkaline solution reacts with picrate to form an umber yellow color that is measured colorimetrically after 20 minutes at 546 nm.

**Histopathological studies**

Livers, kidneys, spleens, lungs, hearts, brains and gastrocinemius muscles were taken from rats in treated and non treated groups, dissecated out, grossly examined. All specimens were perserved in 10% buffer neutral formalin solution and processed through the paraffin embedding technique. Sections of about 5-7 microns thickness were cut then stained with Haematoxylin and Eosin stain **(Culling, 1983)**.

**Haematological studies**

Every week, blood samples were collected from both treated and control groups on EDTA containing tubes, blood parameters including total white, red blood cells counts, haemoglobin, packed cell volume and differential leucocytic count were estimated by automatic blood cell counter apparatus.

**Clinical sings and macroscopical examination**

Psycological clinical signs were observed in all treated animals. Autopsies were performed on treated and non treated animals

**Statistical analysis**

The obtained data were statistically analysed using analysis of variance (ANOVA) according to **Gelber et al.,(1985).**

# RESULTS

# Results

In the present study, the effect of Triclabendazole (TCBZ) at dose rate of (200 mg/kg b.w.) and (400 mg/kg b.w.) was studied on hepatic, renal functions, blood picture, as well as histological studies for some organs.

## I- Biochemical findings

1- Effect of TCBZ on serum aspartate aminotransferase activity (AST)

TCBZ (200 mg/kg b.w.), induced a significant increase (117.202 U/I) in AST activity compared to level in control group (88.202 U/I). The significant increase appeared from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (126.284 U/I) in AST activity than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are shown in table (1) and fig. (1)

Table (1): Effect of oral administration of triclabendazole (TCBZ); 200 and 400 mg/kg b.w. doses on AST activity in rats treated twice a week from the begining till the end of experiment.

| Main-group | AST activity (U/I) group mean | | | | | | Main-group mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 88.160 m | 88.234 m | 88.202 m | 88.200 m | 88.220 m | 88.198 m | 88.202 C |
| 200 mg/kg b.w. | 96.288 l | 102.902 k | 113.604 h | 119.736 g | 128.974 e | 141.706 b | 117.202 B |
| 400 mg/kg b.w. | 103.964 j | 111.456 i | 122.264 f | 131.154 d | 138.042 c | 150.826 a | 126.284 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range .

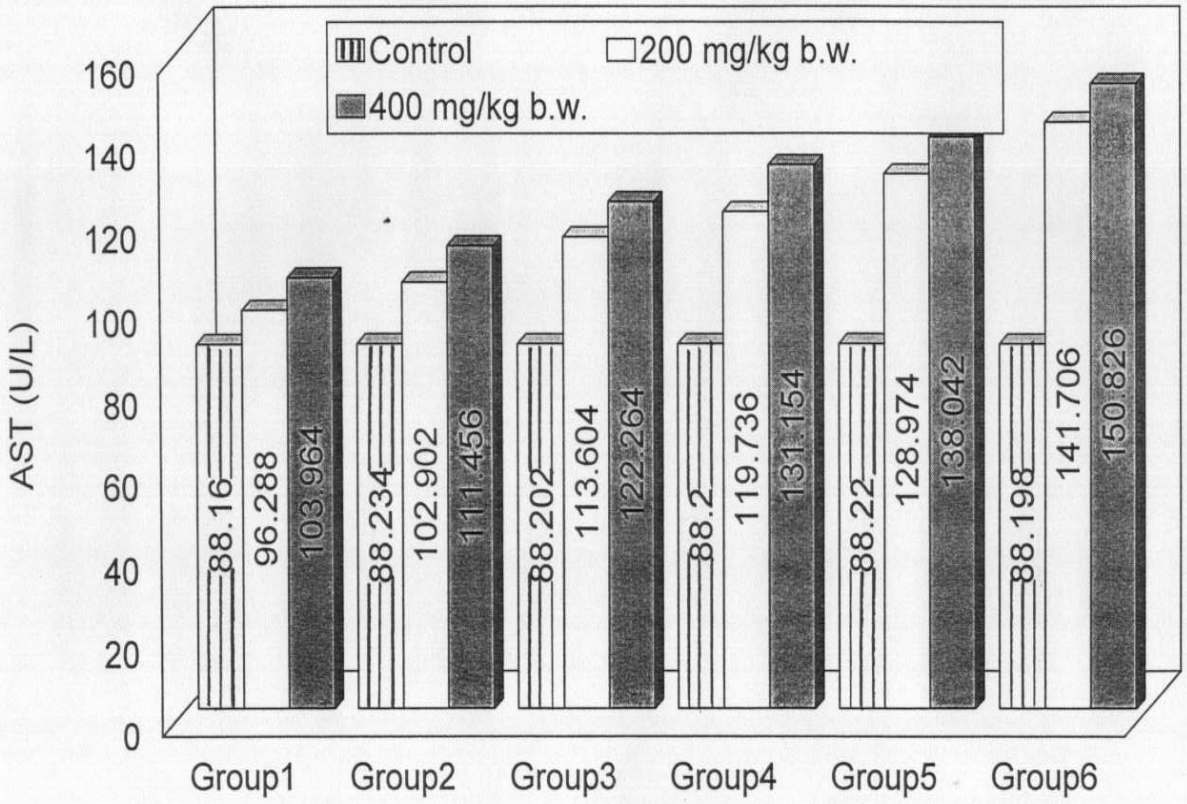



Fig.(1): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on AST activity in rats treated twice weekly from the begining till the end of experiment.

2- Effect of TCBZ on serum alanine aminotransferase ( ALT) activity.

TCBZ (200 mg/kg b.w.), induced a significant increase ( 59.007 U/I ) in ALT activity compared to control level (30.136 U/I). This effect appeared more significantly from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (65.600 U/I) in ALT activity than that in rats treated with 200 mg/kg b.w. compared to control group.

These findings are shown in table (2) and fig. (2)

Table (2): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on ALT activity in rats treated twice a week from the begining till the end of experiment .

| *Main-group* | ALT activity (U/l) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 30.132 m | 30.134 m | 30.138 m | 30.136 m | 30.140 m | 30.136 m | 30.136 C |
| 200 mg/kg b.w. | 35.510 l | 43.610 j | 54.010 h | 62.206 f | 73.414 d | 85.294 b | 59.007 B |
| 400 mg/kg b.w. | 38.964 k | 49.318 i | 60.784 g | 69.818 e | 80.626 c | 94.092 a | 65.600 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

Fig.(2): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on ALT activity in rats treated twice weekly from the begining till the end of experiment.

3- Effect of TCBZ on serum gamma glutamyl aminotransferase ( GGT ) activity.

TCBZ (200 mg/kg b.w.), induced a significant increase (9.348 U/I) in GGT activity compared to control level (2.987 U/I). This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase ( 13.558 U/I ) in GGT activity than that in rats treated with 200 mg/kg b.w. compared to control group.

These findings are shown in table (3) and fig. (3)

Table (3) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on GGT activity in rats treated twice a week from the beginning till the end of experiment .

| *Main-group* | GGT activity (U/I) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| **Control** | 2.958 m | 3.002 m | 2.994 m | 2.982 m | 3.000 m | 2.988 m | 2.987 C |
| **200 mg/kg b.w.** | 4.956 l | 7.320 j | 8.910 i | 10.068 g | 11.636 f | 13.200 d | 9.348 B |
| **400 mg/kg b.w.** | 6.980 k | 9.474 h | 12.028 e | 15.084 c | 17.646 b | 20.134 a | 13.558 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

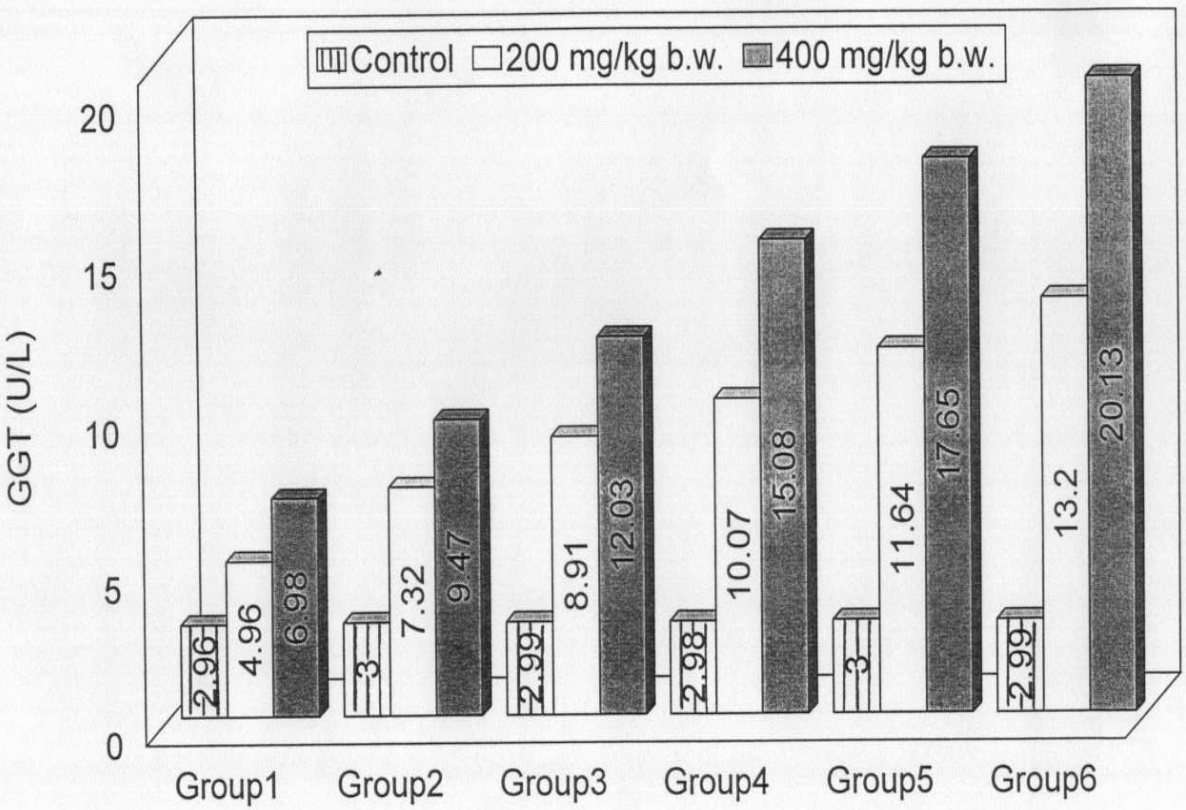



Fig.(3): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on GGT activity in rats treated twice weekly from the begining till the end of experiment.

4- Effect of TCBZ on serum alkaline phosphatase ( AP ) activity

TCBZ (200 mg/kg b.w.), induced a significant increase (119.832 U/I) in serum AP activity compared to control level. This effect appeared more significant from the first week till the end of experiment .

TCBZ (400mg/kg b.w.), induced more significant increase (125.398 U/I) in AP activity than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are recorded in table (4) and fig. (4)

Table (4) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on AP activity in rats treated twice a week from the begining till the end of experiment.

| *Main-group* | AP activity (U/I) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| **Control** | 94.576 m | 94.582 m | 94.600 m | 94.582 m | 94.594 m | 94.590 m | 94.587 C |
| **200 mg/kg b.w.** | 99.146 l | 108.280 j | 116.458 h | 123.800 f | 131.216 d | 140.090 b | 119.832 B |
| **400 mg/kg b.w.** | 103.166 k | 114.498 i | 122.682 g | 129.338 e | 136.906 c | 145.798 a | 125.398 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

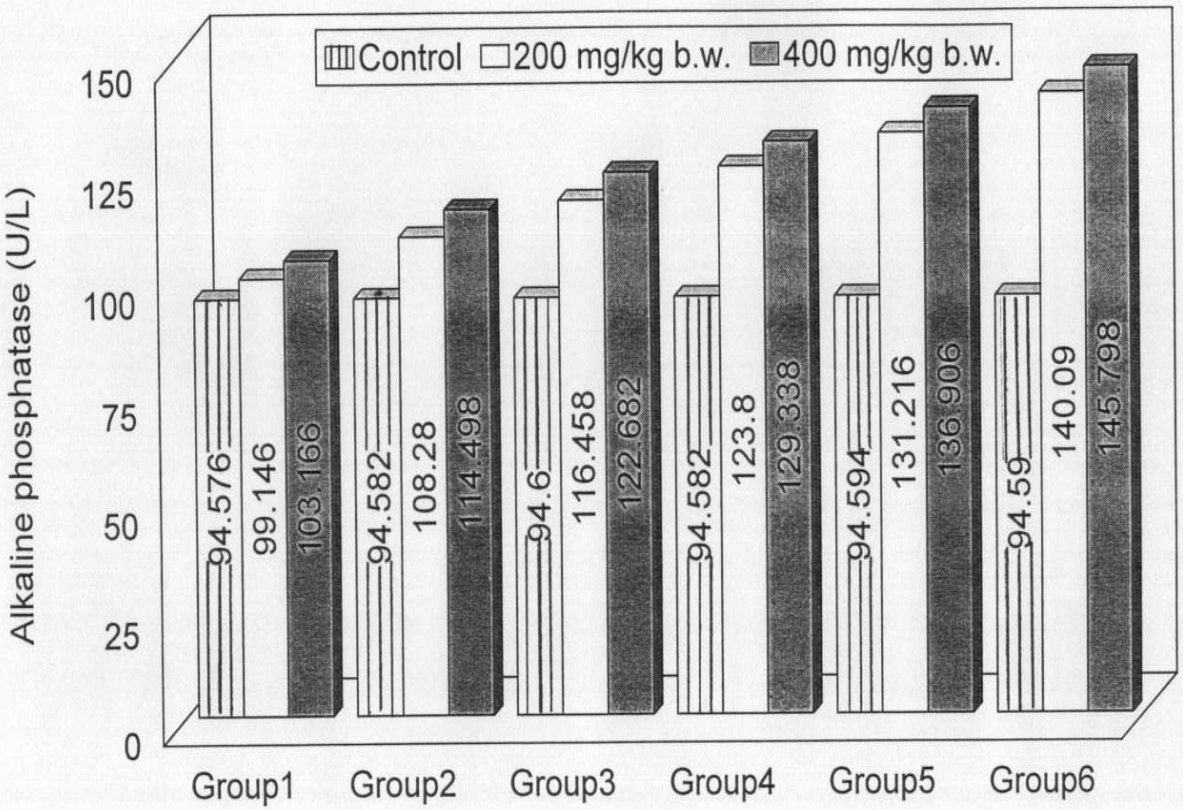



Fig.(4): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on AP activity in rats treated twice weekly from the begining till the end of experiment.

5- Effect of TCBZ on serum total proteins level

TCBZ (200 mg/kg b.w.), induced a significant increase (10.371 g/dl) in serum total proteins compared to the level in control group ( 8.477 g/dl). This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), more significant increase (12.854 g/dl) in serum total proteins than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are recorded in table (5) and fig. (5)

Table (5) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum total proteins level in rats treated twice a week from the begining till the end of experiment .

| *Main-group* | **Total proteins (g/dl)** ---------- *group mean* | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| **Control** | 8.482 m | 8.474 m | 8.474 m | 8.480 m | 8.476 m | 8.476 m | 8.477 C |
| **200 mg/kg b.w.** | 8.766 l | 9.404 k | 9.982 i | 10.764 h | 11.342 g | 11.968 e | 10.371 B |
| **400 mg/kg b.w.** | 9.964 j | 11.438 f | 12.656 d | 13.696 c | 14.244 b | 15.124 a | 12.854 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

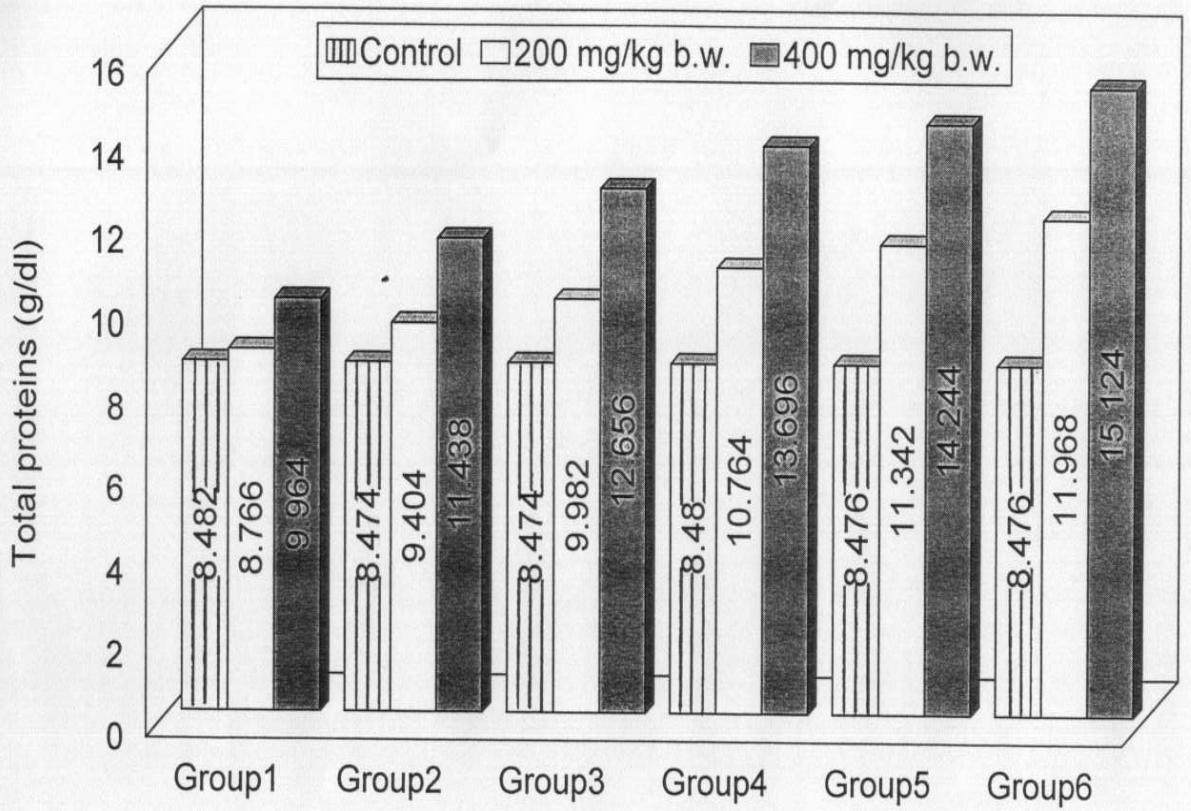



Fig.(5): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum total proteins level in rats treated twice weekly from the begining till the end of experiment.

6- Effect of TCBZ on serum albumin level

TCBZ (200 mg/kg b.w.), induced a significant increase (4.050 g/dl) in serum albumin level compared to control level (3.885 g/dl) . This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (4.561 g/dl) in serum albumin level than that in rats treated with 200 mg/kg b.w. compared to control group.

These findings are recorded in table (9) and fig. (6)

Table (6) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum albumin level in rats treated twice a week from the begining till the end of experiment .

| Main-group | serum albumin (g/dl) group mean | | | | | | Main-group mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 3.896 kl | 3.884 l | 3.874 l | 3.892 kl | 3.882 l | 3.884 l | 3.885 C |
| 200 mg/kg b.w. | 3.912 k | 3.980 j | 3.988 ij | 4.008 i | 4.180 h | 4.234 g | 4.050 B |
| 400 mg/kg b.w. | 4.286 f | 4.384 e | 4.484 d | 4.592 c | 4.754 b | 4.866 a | 4.561 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

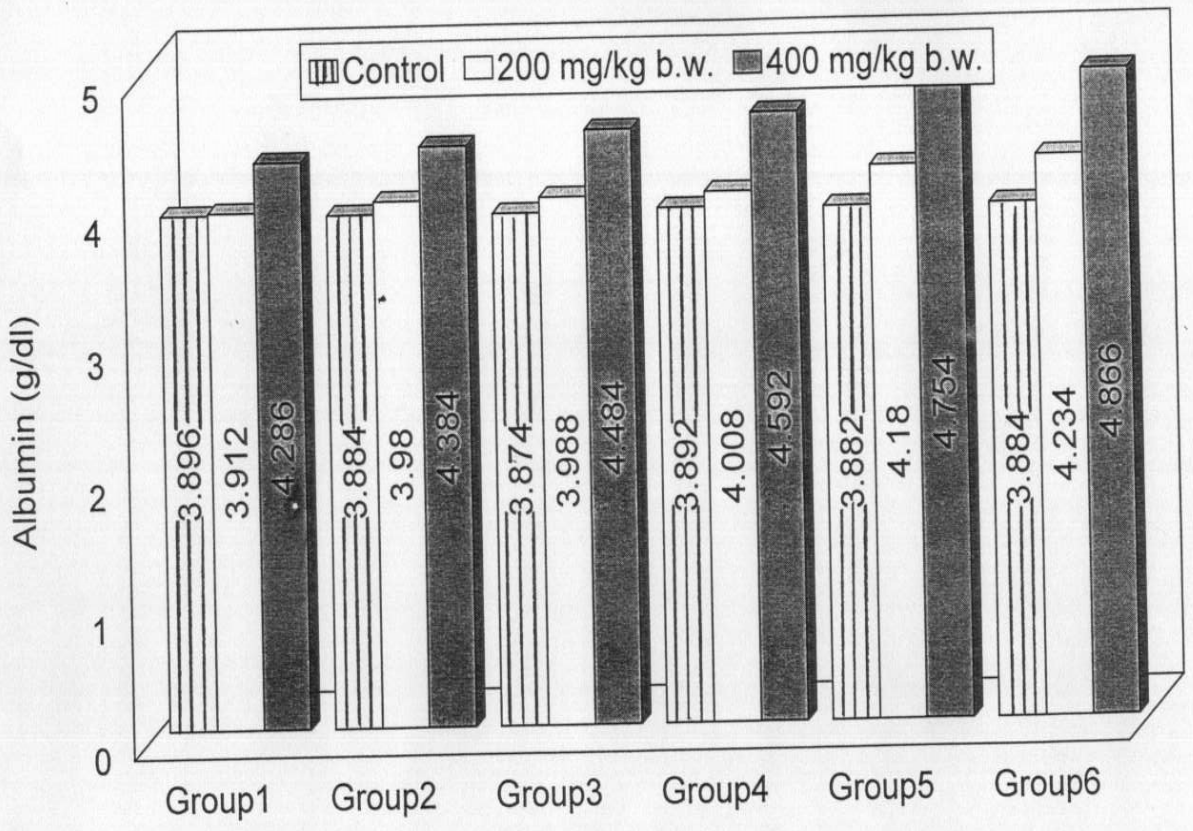



Fig.(6): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum albumin level in rats treated twice weekly from the begining till the end of experiment.

7- Effect of TCBZ on serum globulins level

TCBZ (200 mg/kg b.w.), induced a significant increase (6.321 g/dl) in serum globulins level compared to control level (4.593 g/dl). This effect appeared more significantly from the first week till the end of experiment .

TCBZ (1400 mg/kg b.w.), induced more significant increase (8.292 g/dl) in serum globulins level than that in rats treated with 200 mg/kg b.w. compared to control group.

These findings are recorded in table (7) and fig. (7)

Table (7) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum globulins level in rats treated twice a week from the begining till the end of experiment.

| *Main-group* | Serum globulins (g/dl) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| **Control** | 4.586 m | 4.590 m | 4.600 m | 4.590 m | 4.596 m | 4.598 m | 4.593 C |
| **200 mg/kg b.w.** | 4.854 l | 5.424 k | 5.994 i | 6.756 h | 7.162 f | 7.734 e | 6.321 B |
| **400 mg/kg b.w.** | 5.678 j | 7.054 g | 8.168 d | 9.104 c | 9.490 b | 10.258 a | 8.292 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

Fig.(7): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum globulins level in rats treated twice weekly from the begining till the end of experiment.

8- Effect of TCBZ on total bilirubin level .

TCBZ (200 mg/kg b.w.), induced a significant increase (1.029 mg/dl) in total bilirubin level compared to control level (0.468 mg/dl). This effect appeared more significantly from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (1.205 mg/dl) in total bilirubin level than that in rats treated with 200 mg/kg b.w. compared to control group.

These findings are tabulated in table (8) and fig. (8)

Table (8) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on total bilirubin level in rats treated twice a week from the begining till the end of experiment.

| *Main-group* | **Total bilirubin (mg/dl)** group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| **Control** | 0.466 k | 0.454 k | 0.454 k | 0.498 k | 0.458 k | 0.478 k | 0.468 C |
| **200 mg/kg b.w.** | 0.590 j | 0.786 h | 0.884 g | 1.012 f | 1.306 d | 1.594 b | 1.029 B |
| **400 mg/kg b.w.** | 0.736 i | 0.862 g | 0.988 f | 1.262 e | 1.498 c | 1.886 a | 1.205 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

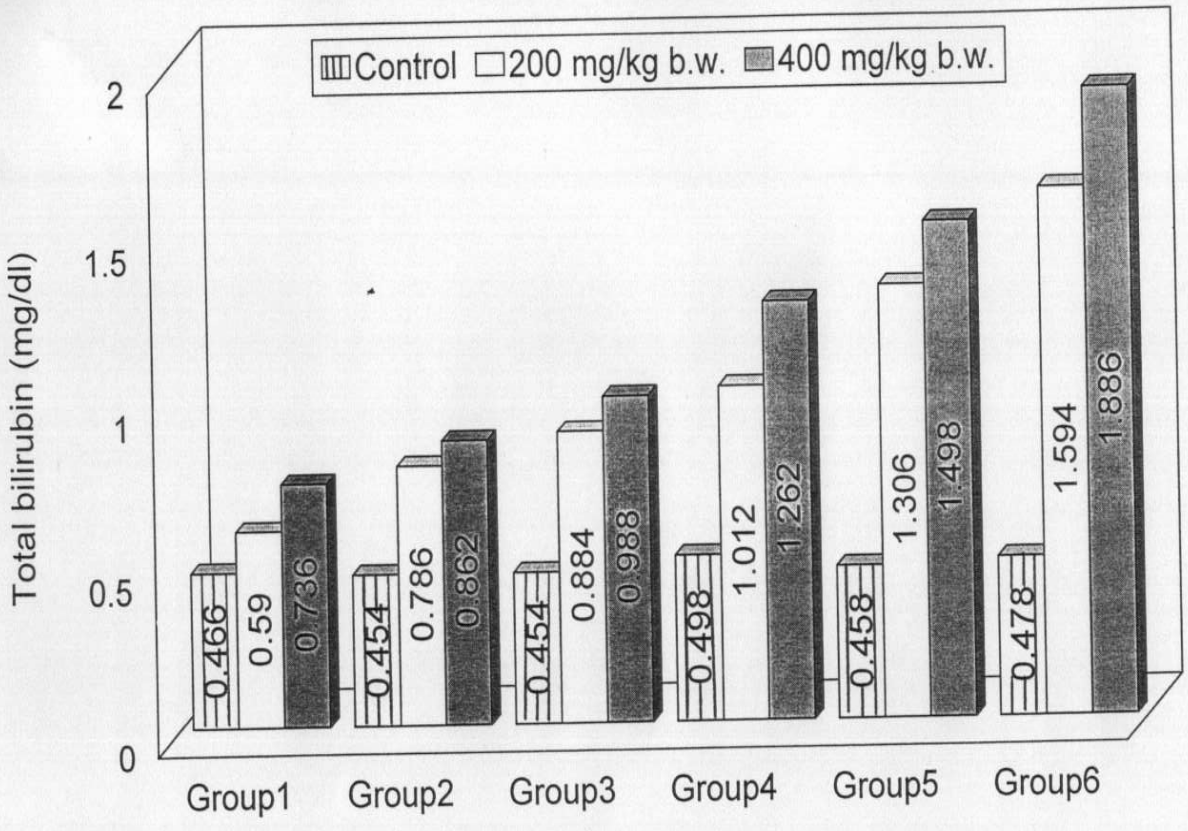



Fig.(8): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on total bilirubin level in rats treated twice weekly from the begining till the end of experiment.

9- Effect of TCBZ on direct bilirubin level

TCBZ (200 mg/kg b.w.), induced a significant increase (0.247 mg/dl) in direct bilirubin level compared to control level ( .110 mg/dl). This effect appeared more significantly from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (0.293 mg/dl) in direct bilirubin level than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are tabulated in table (9) and fig. (9)

Table (9) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on direct bilirubin level in rats treated twice a week from the begining till the end of experiment.

| *Main-group* | Direct bilirubin (mg/dl) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 0.112 i | 0.110 i | 0.108 i | 0.110 i | 0.110 i | 0.110 i | 0.110 C |
| 200 mg/kg b.w. | 0.140 h | 0.190 g | 0.212 f | 0.244 e | 0.312 d | 0.382 b | 0.247 B |
| 400 mg/kg b.w. | 0.178 g | 0.208 f | 0.256 e | 0.304 d | 0.360 c | 0.452 a | 0.293 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

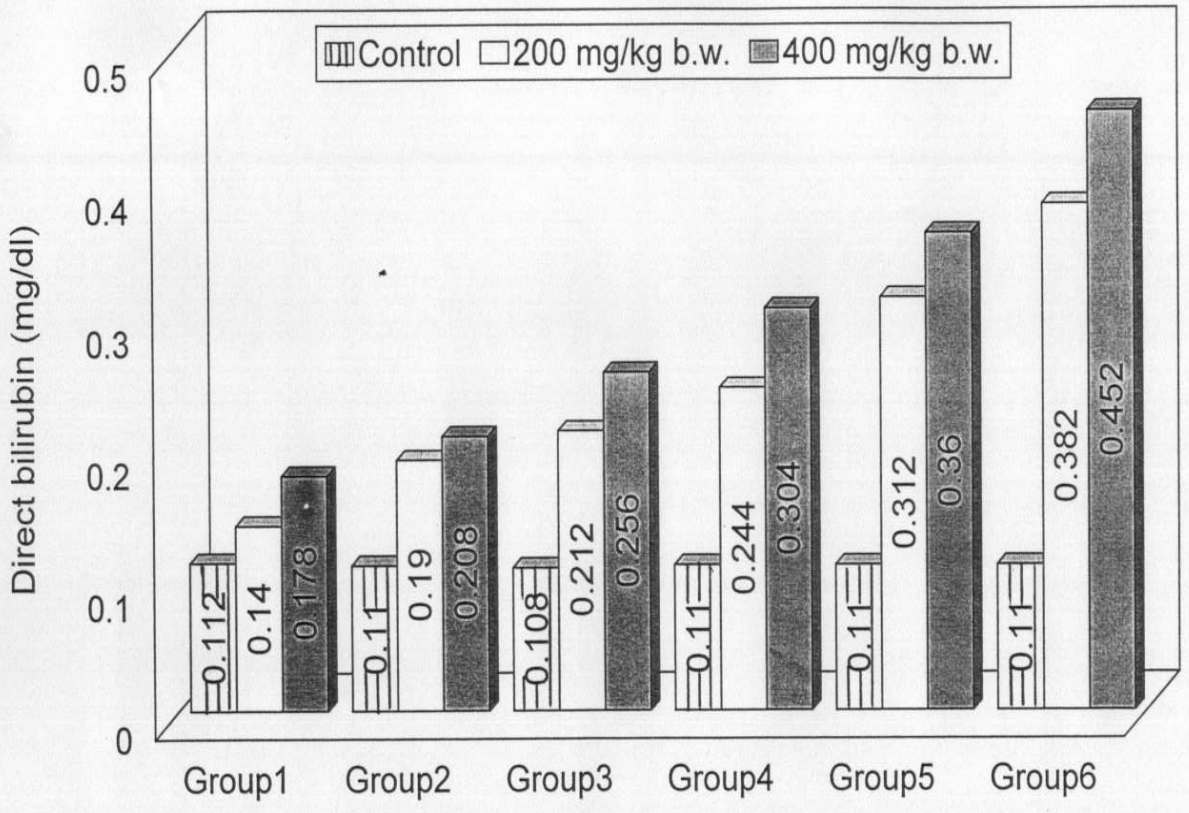



Fig.(9): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on direct bilirubin level in rats treated twice weekly from the begining till the end of experiment.

10- Effect of TCBZ on blood glucose level

TCBZ (200 mg/kg b.w.), induced a significant decrease ( 47.534 mg/dl ) in blood glucose level compared to level in control group (62.291 mg/dl). The significant increase appeared from the first week till the end of experiment

TCBZ (400 mg/kg b.w.), induced more significant decrease (45.234 mg/dl) in blood glucose level than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are tabulated in table (10) and fig. (10)

Table (10) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood glucose level level in rats treated twice a week from the begining till the end of experiment

| *Main-group* | **Glucose level (mg/dl)** | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | *group mean* | | | | | | |
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| **Control** | 62.292 a | 62.282 a | 62.296 a | 62.290 a | 62.294 a | 62.294 a | 62.291 A |
| **200 mg/kg b.w.** | 55.394 b | 52.194 d | 49.496 f | 45.104 h | 42.982 j | 40.032 l | 47.534 b |
| **400 mg/kg b.w.** | 52.982 c | 50.334 e | 46.718 g | 43.270 i | 40.280 k | 37.856 m | 45.234 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

Fig.(10): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood glucose level level in rats treated twice weekly from the begining till the end of experiment.

11- Effect of TCBZ on serum cholestrol level

TCBZ (200 mg/kg b.w.), induced a significant increase (99.477 mg/dl) in serum cholestrol level compared to control level (82.489 mg/dl). This effect appeared more significantly from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (102.904 mg/dl) in serum cholestrol level than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are tabulated in table (11) and fig. (11)

Table (11) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum cholestrol level in rats treated twice a week from the begining till the end of experiment

| Main-group | Serum cholestrol level (mg/dl) group mean | | | | | | Main-group mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 82.510 m | 82.472 m | 82.486 m | 82.494 m | 82.482 m | 82.490 m | 82.489 C |
| 200 mg/kg b.w. | 86.246 l | 89.484 j | 94.890 h | 102.786 f | 109.304 d | 114.150 b | 99.477 B |
| 400 mg/kg b.w. | 88.954 k | 93.306 i | 97.094 g | 105.384 e | 113.202 c | 119.486 a | 102.904 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test.

Fig.(11): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on serum cholestrol level in rats treated twice weekly from the begining till the end of experiment.

12- Effect of TCBZ on blood urea level

TCBZ (200 mg/kg b.w.), induced a significant increase (28.062 mg/dl) in blood urea level compared to control level ( 27.162 mg/dl). The significant increase appeared from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (28.543 mg/dl) in blood urea level than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are shown in table (12 ) and fig. (12)

Table (12) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood urea level in rats treated twice a week from the begining till the end of experiment

| *Main-group* | **Blood urea level (mg / dl)** group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| **Control** | 27.150 h | 27.190 h | 27.138 h | 27.172 h | 27.168 h | 27.156 h | 27.162 C |
| **200 mg/kg b.w.** | 28.008 g | 28.034 fg | 28.048 fg | 28.068 ef | 28.112 e | 28.100 e | 28.062 B |
| **400 mg/kg b.w.** | 28.480 d | 28.512 c d | 28.542 b c | 28.558 a b c | 28.572 ab | 28.596 a | 28.543 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

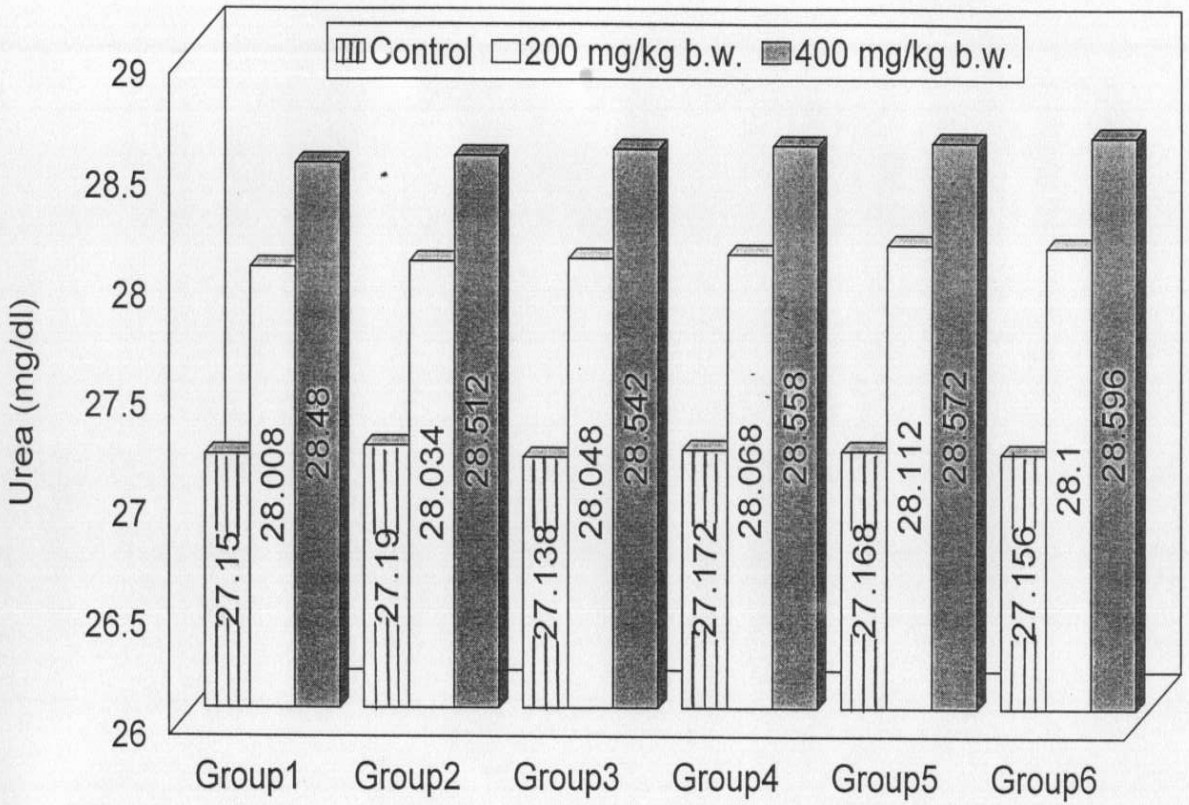



Fig.(12):: Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood urea level in rats treated twice weekly from the begining till the end of experiment.

13- Effect of TCBZ on blood uric acid level

TCBZ (200 mg/kg b.w.), induced a significant increase (7.304 mg/dl) in blood uric acid level compared to control level ( 4.128 mg/dl). This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (7.831 mg/dl) in blood uric acid level than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are shown in table (13) and fig. (13)

Table (13) :Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood uric acid level in rats treated twice a week from the begining till the end of experiment

| *Main-group* | **Blood uric acid level (mg/dl)** group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| **Control** | 4.112 m | 4.134 m | 4.132 m | 4.128 m | 4.136 m | 4.128 m | 4.128 C |
| **200 mg/kg b.w.** | 5.156 l | 5.952 j | 7.144 h | 7.792 f | 8.496 d | 9.286 b | 7.304 B |
| **400 mg/kg b.w.** | 5.866 k | 6.614 i | 7.674 g | 8.088 e | 8.968 c | 9.776 a | 7.831 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

Fig.(13): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood uric acid level in rats treated twice weekly from the begining till the end of experiment.

14- Effect of TCBZ on blood creatinin level

TCBZ (200 mg/kg b.w.), induced a significant increase (1.364 mg/dl) in blood creatinin level compared to control level (1.164 mg/dl). The significant increase appeared from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant increase (1.652 mg/dl) in blood creatinin level than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are shown in table (14) and fig. (14)

Table (14) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood creatinin level in rats treated twice a week from the begining till the end of experiment.

| *Main-group* | **Blood creatinin level (mg / dl)** ---------- *group mean* ---------- | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| **Control** | 1.160 k | 1.166 k | 1.178 k | 1.160 k | 1.152 k | 1.170 k | 1.164 C |
| **200 mg/kg b.w.** | 1.208 j | 1.272 i | 1.340 h | 1.424 g | 1.448 f g | 1.490 e | 1.364 B |
| **400 mg/kg b.w.** | 1.452 f | 1.500 e | 1.642 d | 1.670 c | 1.756 b | 1.894 a | 1.652 A |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

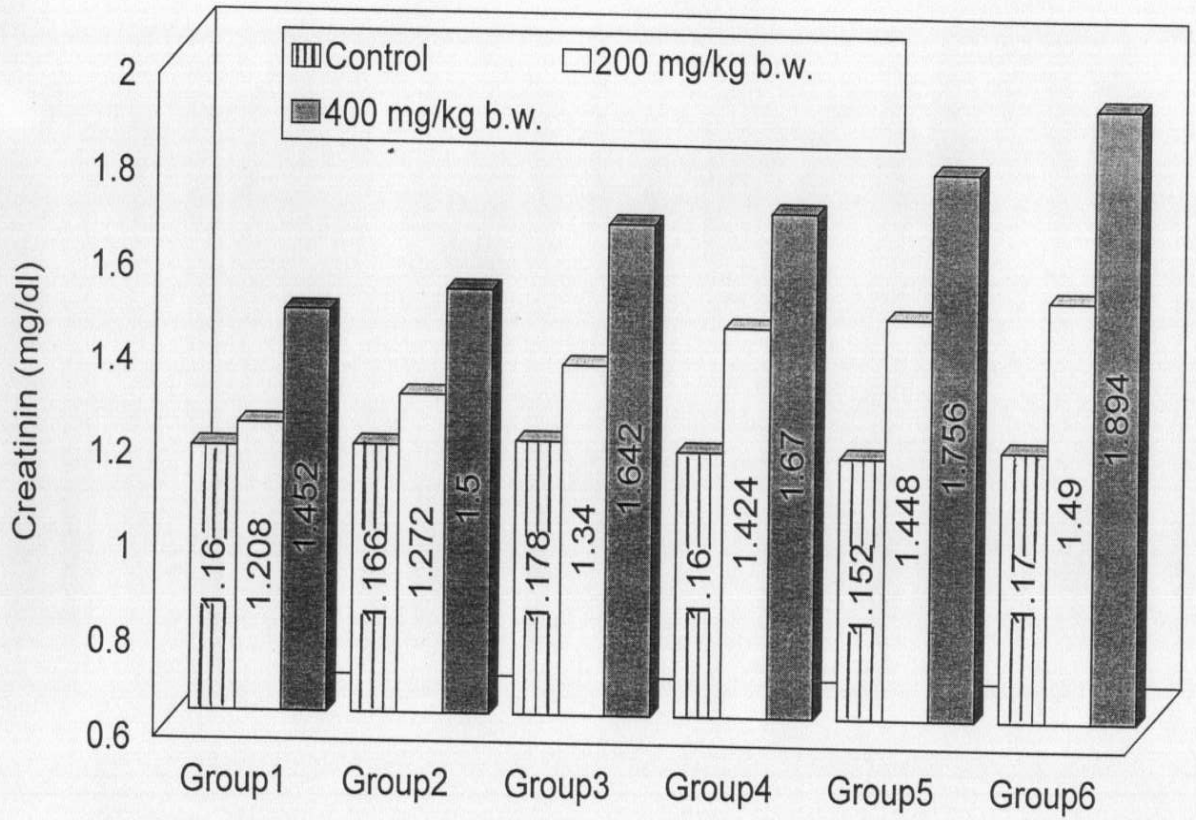



Fig.(14):: Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on blood creatinin level in rats treated twice weekly from the begining till the end of experiment.

## II- Haematological findings

1- Effect of TCBZ on total white blood cells ( W.B.Cs ) count .

TCBZ (200 mg/kg b.w.), induced a significant decrease (3.594 × $10^3$/$mm^3$) in total W.B.Cs count compared to control level (5.872 × $10^3$/$mm^3$). This effect appeared more significant from thefirst week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease (3.132 × $10^3$/$mm^3$) in total W.B.Cs count than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are shown in table (15) and fig. (15)

Table (15) : Effect of oral administration of TCBZ; 200 and 400 mg/kg doses on total W.B.Cs count in rats treated twice a weekl from the begining till the end of experiment

| *Main-group* | **Total W.B.Cs count (×$10^3$/$mm^3$)** *group mean* 1 | 2 | 3 | 4 | 5 | 6 | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| **Control** | 5.834 a | 5.822 a | 5.824 a | 5.834 a | 5.828 a | 5.822 a | 5.827 A |
| **200 mg/kg b.w.** | 5.184 b | 4.400 d | 3.796 f | 3.192 g | 2.674 h | 2.316 i | 3.594 B |
| **400 mg/kg b.w.** | 4.928 c | 3.868 e | 3.208 g | 2.686 h | 2.126 j | 1.974 k | 3.132 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

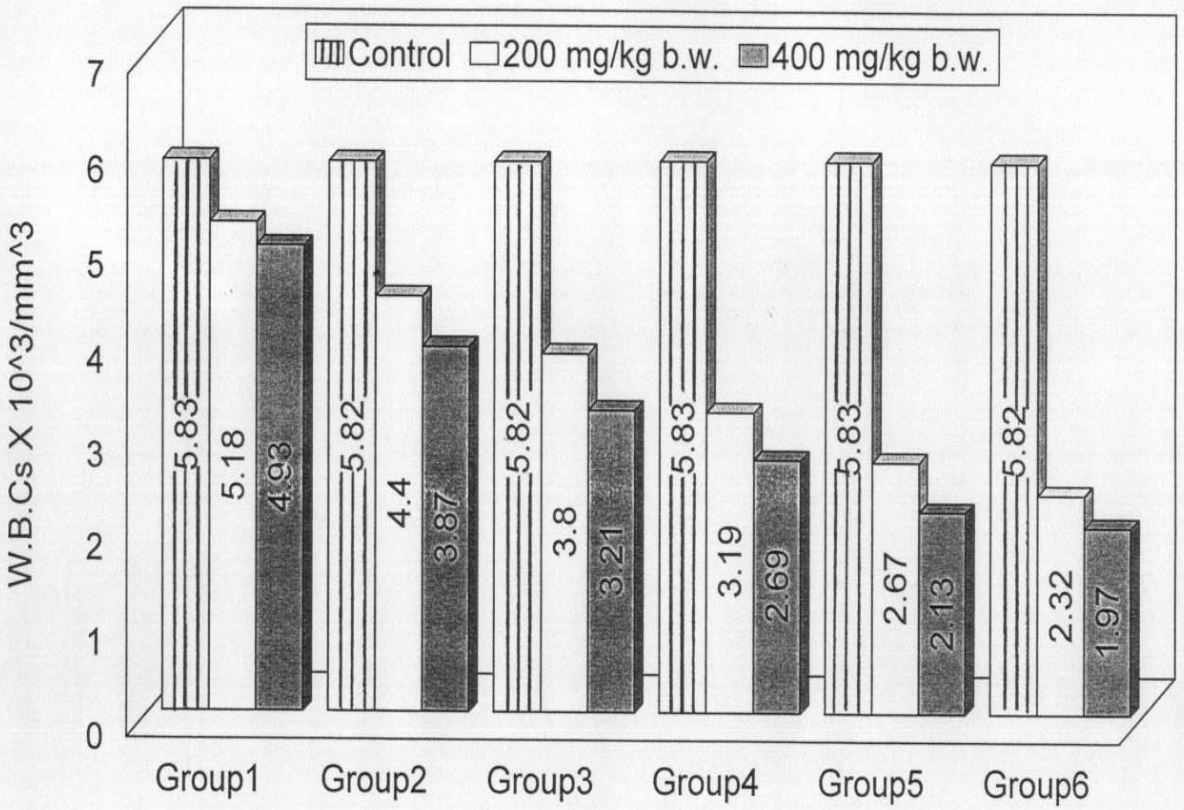



Fig.(15): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on total W.B.Cs count in rats treated twice weekly from the begining till the end of experiment.

2- Effect of TCBZ on lymphocytic count .

TCBZ (200 mg/kg b.w.), induced a significant decrease (3.080 × $10^3$/$mm^3$) in lymphocytic count compared to control level (5.162 × $10^3$/$mm^3$). This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease (2.660 × $10^3$/$mm^3$) in lymphocytic count than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are shown in table (16) and fig. (16)

Table (16) :Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on lymphocytic count in rats treated twice a week from the begining till the end of experiment

| Main-group | Lymphocytic count (×$10^3$/$mm^3$) group mean | | | | | | Main-group mean |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 5.164 a | 5.164 a | 5.160 a | 5.164 a | 5.156 a | 5.164 a | 5.162 A |
| 200 mg/kg b.w. | 4.562 b | 3.822 d | 3.264 f | 2.702 h | 2.224 j | 1.904 k | 3.080 B |
| 400 mg/kg b.w. | 4.326 c | 3.318 e | 2.730 g | 2.242 i | 1.740 l | 1.602 m | 2.660 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

Fig.(16): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on lymphocytic count in rats treated twice weekly from the begining till the end of experiment.

3- Effect of TCBZ on monocytic count

TCBZ (200 mg/kg b.w.), induced a significant decrease ($0.096 \times 10^3/mm^3$) in monocytic count compared to the level in control group ($0.200 \times 10^3/mm^3$). This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease ($0.075 \times 10^3/mm^3$) in monocytic count than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are recorded in table (17) and fig. (17)

Table (17) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on monocytic count in rats treated twice a week from the begining till the end of experiment

| *Main-group* | Monocytic count ($\times 10^3/mm^3$) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 0.200 a | 0.200 a | 0.200 a | 0.200 a | 0.200 a | 0.200 a | 0.200 A |
| 200 mg/kg b.w. | 0.170 b | 0.136 c | 0.100 d | 0.080 e | 0.050 f | 0.040 fg | 0.096 B |
| 400 mg/kg b.w. | 0.160 b | 0.110 d | 0.070 e | 0.050 f | 0.030 g | 0.030 g | 0.075 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

Fig.(17): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on monocytic count in rats treated twice weekly from the begining till the end of experiment.

4- Effect of TCBZ on granulocytic count

TCBZ (200 mg/kg b.w.), induced a significant decrease (0.419 × $10^3$/$mm^3$) in granulocytic count compared to control level (0.470 × $10^3$/$mm^3$).This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease (0.398 × $10^3$/$mm^3$) in granulocytic count than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are recorded in table (18) and fig. (18)

Table (18) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on granulocytic count in rats treated twice a week from the begining till the end of experiment

| *Main-group* | Granulocytic count (×$10^3$/$mm^3$) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 0.470 a | 0.472 a | 0.470 a | 0.470 a | 0.470 a | 0.470 a | 0.470 A |
| 200 mg/kg b.w. | 0.452 b | 0.446 c | 0.432 f | 0.412 g | 0.400 i | 0.372 k | 0.419 B |
| 400 mg/kg b.w. | 0.444 d | 0.438 e | 0.408 h | 0.394 j | 0.354 l | 0.348 m | 0.398 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

Fig.(18): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on granulocytic count in rats treated twice weekly from the begining till the end of experiment.

5- Effect of TCBZ on red blood cells ( R.B.Cs ) count

TCBZ (200 mg/kg b.w.), induced a significant decrease (8.096 × $10^6$/$mm^3$) in R.B.Cs count compared to control level (9.347 × $10^6$/$mm^3$). The significant increase appeared from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease (7.735 × 10^3/mm^3) in R.B.Cs count than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are recorded in table (19 ) and fig. (19)

Table (19) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on R.B.Cs count in rats treated twice a week from the begining till the end of experiment

| *Main-group* | **R.B.Cs count (×$10^6$/$mm^3$)** group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | |
| **Control** | 9.326 a | 9.384 a | 9.384 a | 9.364 a | 9.364 a | 9.388 a | 9.347 A |
| **200 mg/kg b.w.** | 8.976 b | 8.634 d | 8.322 e | 7.944 f | 7.538 g | 7.164 i | 8.096 B |
| **400 mg/kg b.w.** | 8.764 c | 8.320 e | 7.972 f | 7.496 h | 7.052 j | 6.804 k | 7.735 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

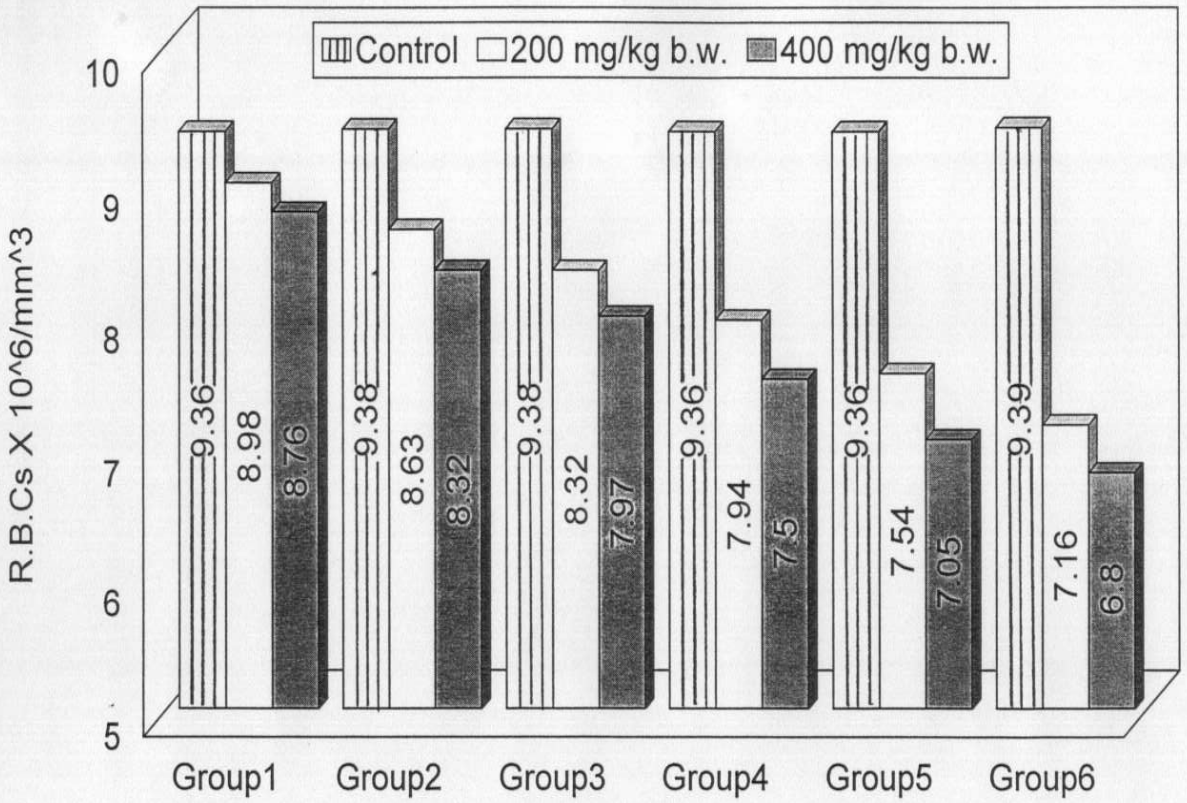



Fig.(19): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on R.B.Cs count in rats treated twice weekly from the begining till the end of experiment.

6- Effect of TCBZ on blood haemoglobin ( Hb ) %

TCBZ (200 mg/kg b.w.), induced a significant decrease (13.920 gm%) in Hb % compared to control level (17.005 gm%). This effect appeared more significant from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease (13.223 gm%) in Hb% than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are tabulated in table (20) and fig. (20)

Table (20): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on haemoglobin (Hb) % in rats treated twice a week from the begining till the end of experiment

| *Main-group* | Hb (gm %) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| Control | 17.006 a | 17.034 a | 17.006 a | 17.010 a | 16.990 a | 16.982 a | 17.005 A |
| 200 mg/kg b.w. | 16.120 b | 14.982 d | 14.146 e | 13.332 g | 12.674 h | 12.266 j | 13.920 B |
| 400 mg/kg b.w. | 15.798 c | 13.806 f | 13.342 g | 12.400 i | 12.100 k | 11.892 l | 13.223 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

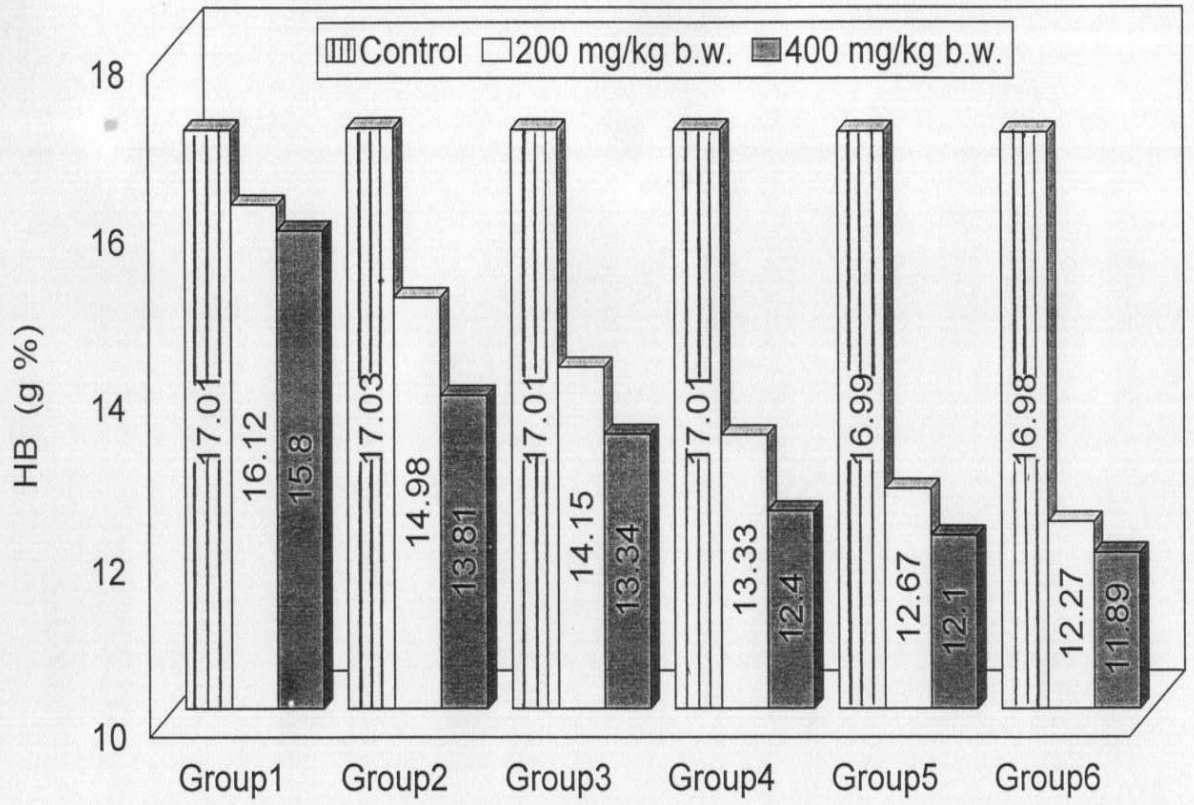



Fig.(20): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on Hb % in rats treated twice weekly from the begining till the end of experiment.

7- Effect of TCBZ on packed cell volume ( P.C.V )

TCBZ (200 mg/kg b.w.), induced a significant decrease (43.809 %) in P.C.V.compared to control level (46.818 %). The significant increase appeared from the first week till the end of experiment .

TCBZ (400 mg/kg b.w.), induced more significant decrease (43.396 %) in P.C.V. than that in rats treated with 200 mg/kg b.w. compared to control group. These findings are tabulated in table (21) and fig. (21)

Table (21) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on P.C.V. in rats treated twice a week from the begining till the end of experiment

| *Main-group* | P.C.V. (%) group mean | | | | | | *Main-group mean* |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| **Control** | 46.840 a | 46.812 a | 46.812 a | 46.816 a | 46.826 a | 46.802 a | 46.818 A |
| **200 mg/kg b.w.** | 45.028 b | 44.596 d | 44.164 e | 43.636 g | 43.194 h | 42.234 j | 43.809 B |
| **400 mg/kg b.w.** | 44.904 c | 44.214 e | 43.874 f | 43.216 h | 42.880 i | 41.290 k | 43.396 C |

Mean of each factor designated by the same latter are not significantly different at 5 % level using Duncan,s Multiple range test

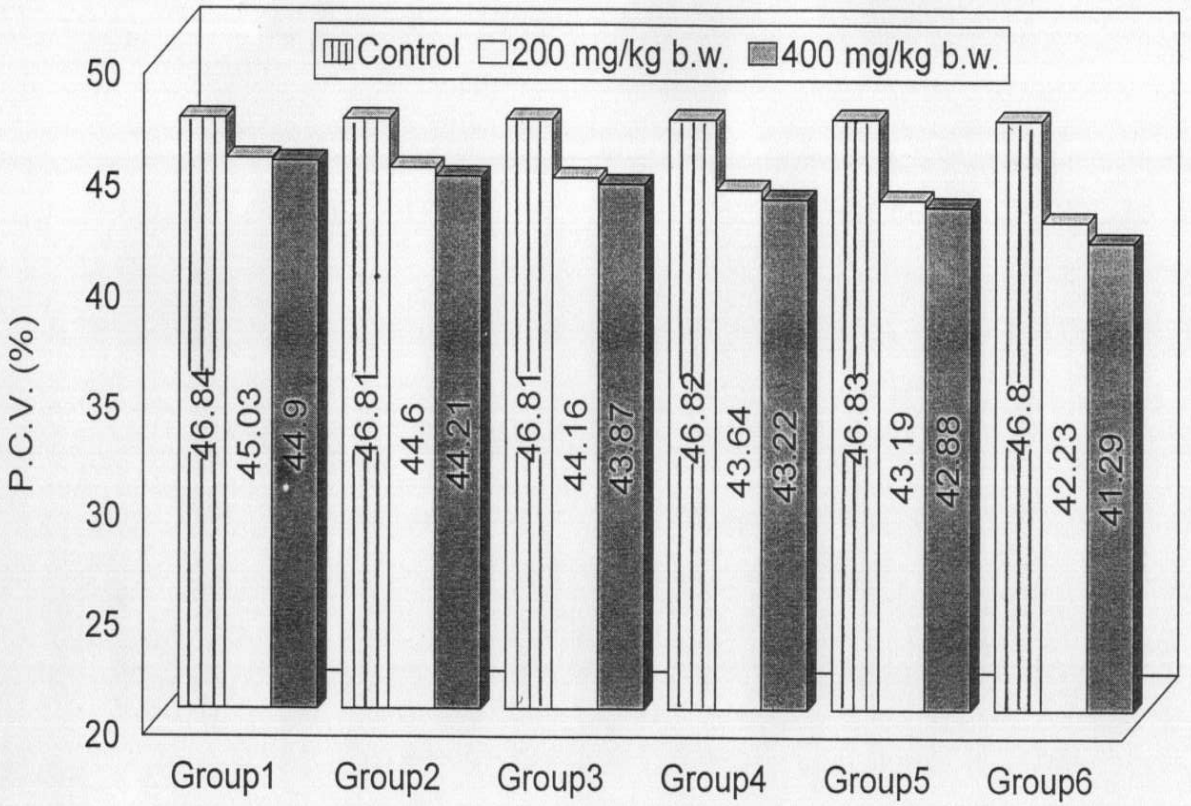



Fig.(21): Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on P.C.V. in rats treated twice weekly from the begining till the end of experiment.

8- Effect of TCBZ on mean corpascular volume ( MCV ), mean corpascular haemoglobin ( MCH ) and mean corpascular haemoglobin concentration ( MCHC ).

TCBZ produced macrocytic hypochromic anaemia as it induced a significant increase in MCV and significant decrease in MCH and MCHC . These findings are showen in table (22)

Table (22) : Effect of oral administration of TCBZ; 200 and 400 mg/kg b.w. doses on MCH, MCH, MCHC in rats treated twice weekly along the period of experiment .

| Main group | MCV | MCH | MCHC |
|---|---|---|---|
| **Control** | 49.3 | 18.19 | 36.3 |
| **200 mg/kg b.w.** | 53.2 | 17.21 | 29.7 |
| **400 mg/kg b.w.** | 55.6 | 17.15 | 29.96 |

**III- Clinical sings**

The clinical sings appeared in the first week after the second dose of the drug were in the form of sedation, depression, loss of weight, weakness, sheivering, ruffled fur, tachypenia and tachycardia . No mortalities occured . These sings were more sever in rats treated with 400 mg/kg b.w. than those treated with 200 mg/kg b.w.

**IV- Pathological findings**

The lesions were nearly similar among 200 mg/kg b.w. and 400 mg/kg b.w. treated rats. The severity of the lesions increased after 6 weeks of administration and were more sever in 400 mg/kg b.w. than 200 mg/kg b.w. treated rats.

1- Gross picture

In sacrificed rats treated with TCBZ yellowish enlarged liver with rounded edges, congested heart and kidney, enlarged spleen were observed . No gross lesions were observed in muscles .

2- Histopathological findings

The hepatic parenchyma showed clowdy swelling, vacuolar and hydropic degenerations one week post administration in rats treated with 200 & 400 mg/kg b.w. of TCBZ. Centrolobular vacuolation of hepatocytes and coagulative necrosis were the common lesions among rats treated with 200 mg/kg b.w. for 6 weeks (fig.22).

The portal areas were dilated and infiltrated with lymphocytes. The portal blood vessels were congested and the bile ducts showed proliferation particularly 5 weeks post administration in rats treated with 400 mg/kg b.w. (fig.23). Extensive replacement of hepatocytes with haemorrhage were noticed at 6 weeks post administration with the tested drug at dose level of 400 mg/kg b.w. (fig.24).

The spleen showed slight depletion of lymphocytes from the white pulp and congestion of red pulp particularly 6 weeks post administration in rats treated with 200 and 400 mg/kg b.w. (fig.25,26).

The myocardium of rats treated with TCBZ at dose level of 200 mg/kg b.w. showed congestion and haemorrhage 6 weeks post administration (fig.27). Mean while rats exposed to 400 mg/kg b.w. for 6 weeks showed extensive haemorrhage among the cardiac muscles .The latter showed hyaline degeneration (fig.28).

The kidneys showed dilatated renal tubules in rats treated with200 mg TCBZ /kg b.w. (fig.29). Cloudy swelling of some renal tubules, congestion of the renal blood vessels with mononuclear cell infiltration could be seen 6 weeks post administration of 400 mg/kg b.w. (fig.30).

The muscles showed no histopathological changes along the entire period of experiment.

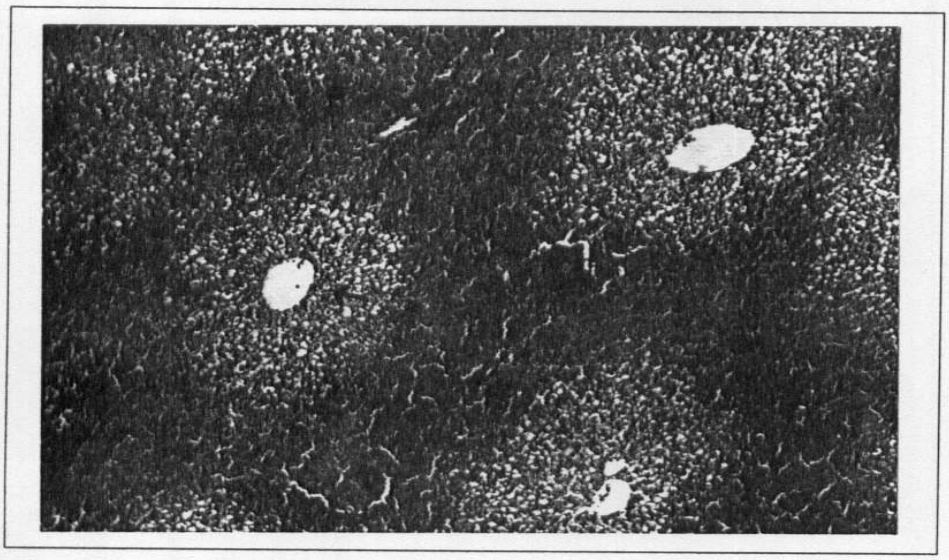


**Fig.(22)** Liver section from rat administered TCBZ orally (200 mg/kg b.w.) twice weekly for 6 weeks showing centrolobular vacuolation of hepatocytes and coagulative necrosis of adjucent cells (H&E, X120) .

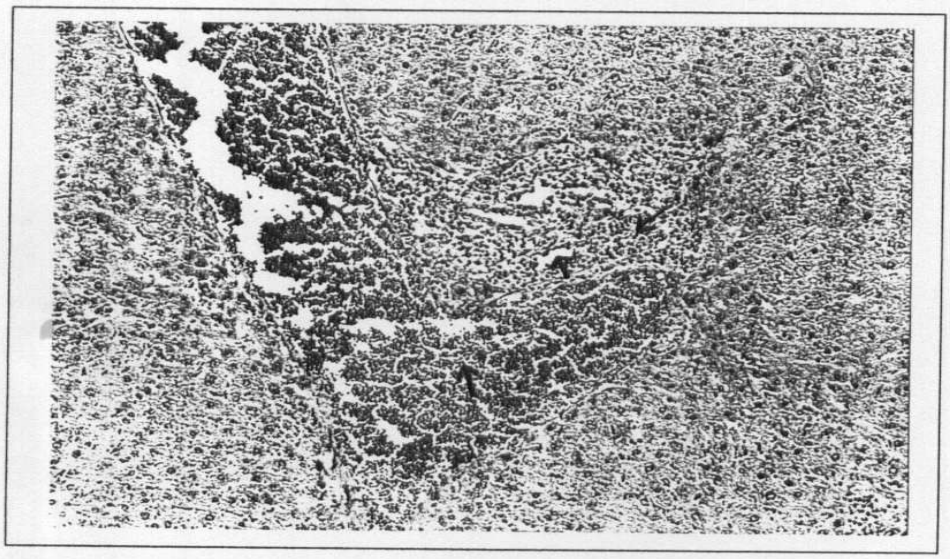


**Fig.(23)** Liver section from rat administered TCBZ orally (400 mg/kg b.w.) twice weekly for 5 weeks showing congestion of portal blood vessels, proliferated bile ducts and proliferation of portal triads with mononuclear cells (H&E, X300) .

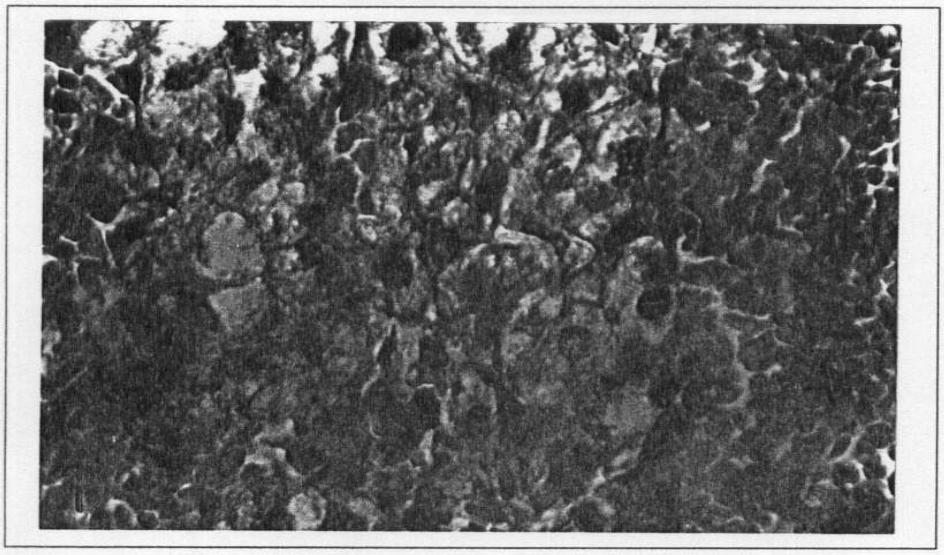


**Fig.(24)** Liver section from rat administered TCBZ orally (400 mg/kg b.w.) twice weekly for 6 weeks showing extensive replacement of hepatocytes with haemorrhage (H&E, X1200) .

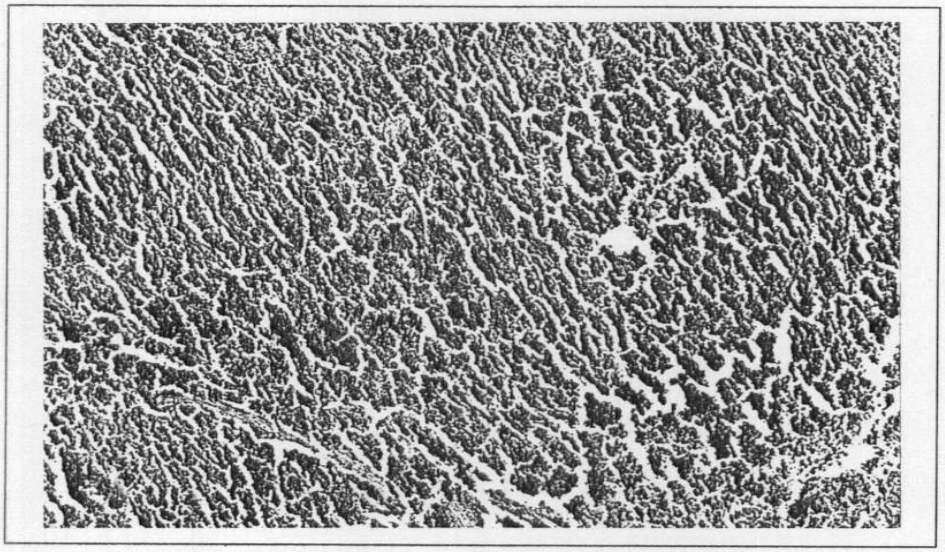


**Fig.(25)** Spleen section from rat administered TCBZ orally (200 mg/kg b.w.) twice weekly for 6 weeks showing congestion of the red pulp and depletion of lymphocytes from the white pulp (H&E, X300) .

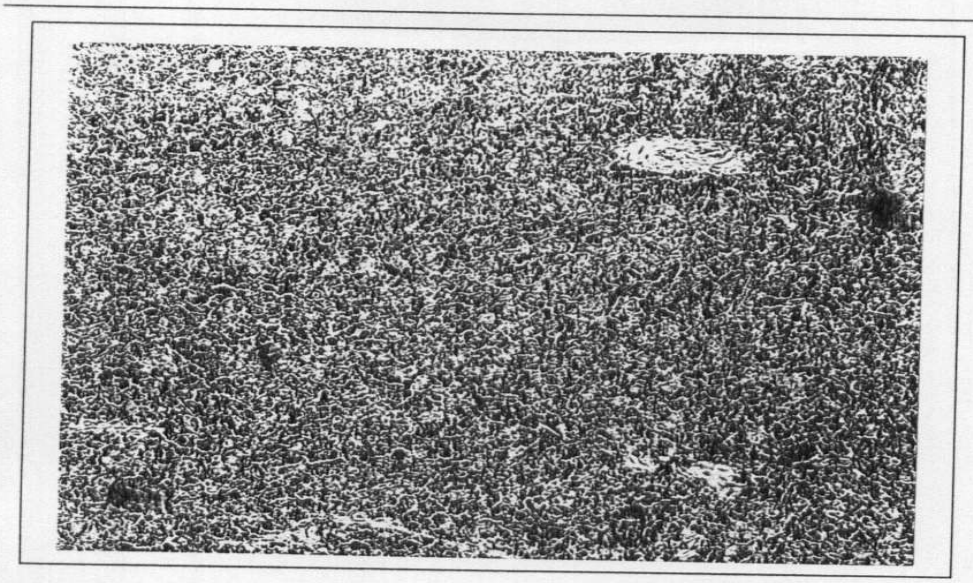


**Fig.(26)** Spleen section from rat administered TCBZ orally (400 mg/kg b.w.) twice weekly for 6 weeks showing congestion of the red pulp and depletion of lymphocytes from the white pulp (H&E, X300).

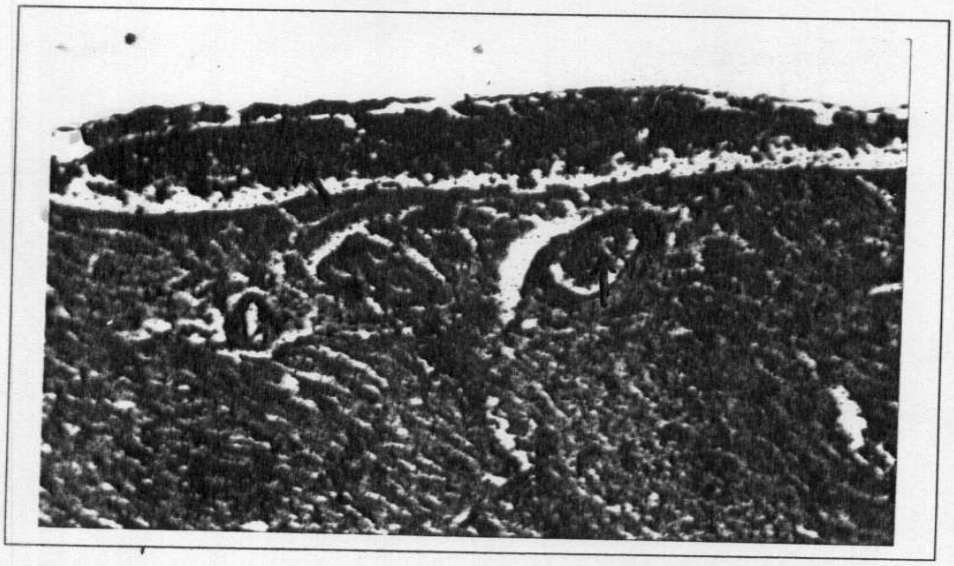


**Fig.(27)** Heart section from rat administered TCBZ orally (200 mg/kg b.w.) twice weekly for 6 weeks showing congestion and haemorrhage (H&E, X300)

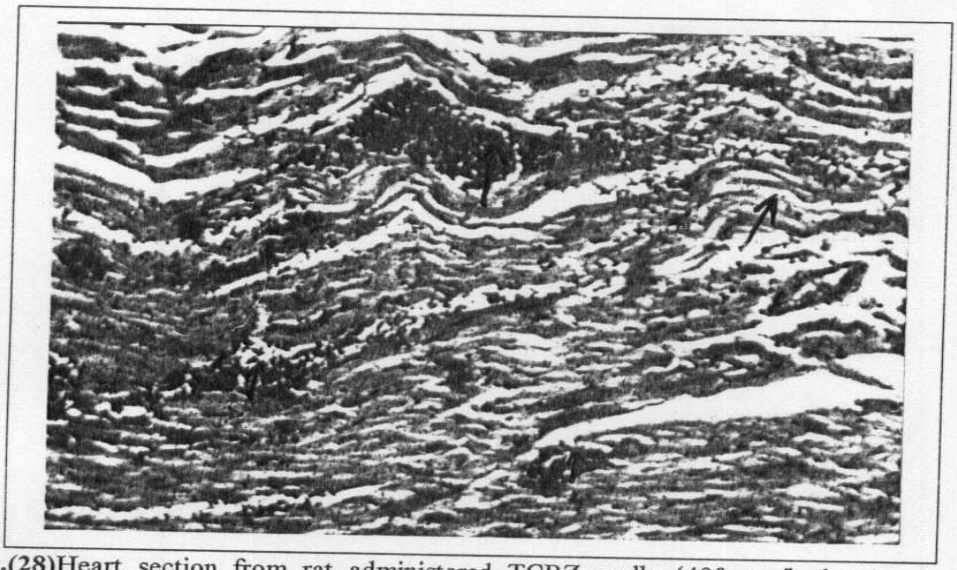


**Fig.(28)**Heart section from rat administered TCBZ orally (400 mg/kg b.w.) twice weekly for 6 weeks showing extensive haemorrhage among the degenerated myocardium (H&E, X300) .

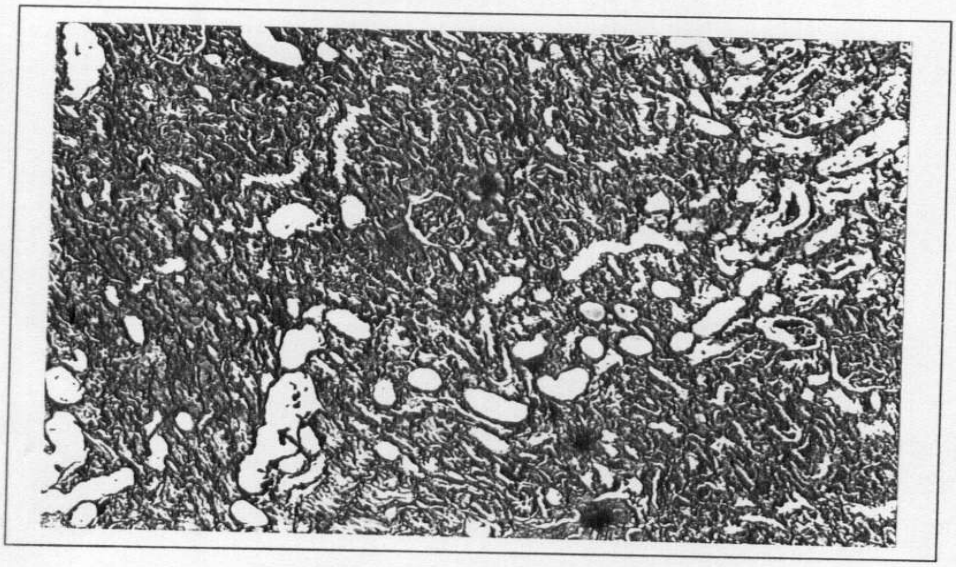


**Fig.(29)** Kidney section from rat administered TCBZ orally (200 mg/kg b.w.) twice weekly for 6 weeks showing cystic dilatation of some renal tubules (H&E, X300)

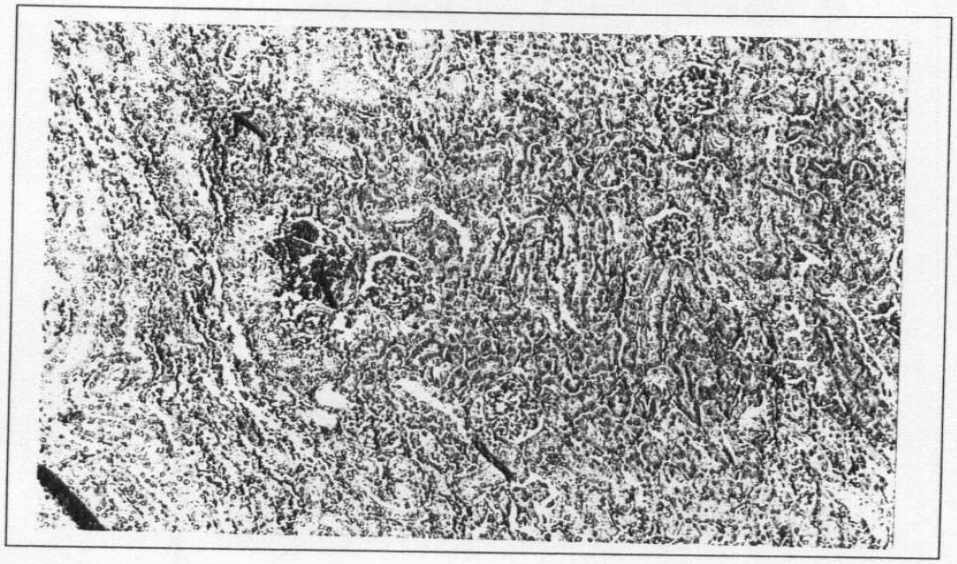


**Fig.(30)** Kidney section from rat administered TCBZ orally (400 mg/kg b.w.) twice weekly for 6 weeks showing congestion of renal blood vessels beside mononuclear cell infiltration (H&E, X300) .

# DISCUSSION

# Discussion

Triclabendazole (TCBZ, Fasinex®) is a derivative of benzimidazoles anthelmintics. This drug is active against early immature and mature fasciola species in sheep and cattle **(Boray,1981)** . The present study investigate the possible toxic effects of TCBZ at 200 mg/kg b.w. and 400 mg/kg b.w. doses on liver and kidney functions, blood picture and histology of some organs. Measurments were made at repeated doses to follow up the induced toxic effects of the drug .

Different investigators reported that hepatic and renal changes were usually induced following administration of benzimidazole drugs **(Dolle,1972 ;Mori,1976;and Patton,1976).** The extent of damage ranged from transient minor hepatic and renal changes to sever lesions or even chronic hepatitis and nephritis with subsequent variation in their enzyme patterns.

**Biochemical analysis**

Liver function examination

The determination of hepatoenzymatic activity performed in this study included the estimation of enzymes which are released from liver cells, biliary system under the condition of hepatic cellular damage.

Transaminases are including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) **(Michael et al., 1992).** ALT and AST are found in most tissues but in unequal proportions, ALT occurs exclusively in the liver, but only in the cytoplasm of parenchymal cells, in

cells, in contrast to AST which is equally distributed between cytoplasm and mitochondria, the dissimillar location of the two transferases provide valuble diagnostic information in various type of liver diseases **(Schmidt and Schmidt,1977 and Moursi et al.,1979).** GGT, in the liver is located in the canalculi of the hepatic cells and particularly in the epithelial cells lining the biliary ductules. Within the hepatic parenchyma, GGT exists to a large extents in the smooth endoplasmic reticulum **(Michael et al.,1992).** Thus GGT is valuble serum marker for disorders of hepatobiliary system resulting in cholestasis **(Braun et al.,1983)** .

The present study revealed that there were significant increase in levels of ALT, AST and GGT . This findings could be attributed to the damage of the hepatic cells by the direct effect of the drug and/or its metabolites resulting in escape of enzymes to plasma . Because these enzymes are found principly in the liver as mentioned before or they are often regarded as being more specific for detecting liver damage, the possible mechanism involved in their elevation may be due to hepatocellular damage **(Rouiller,1964)** or due to increase synthesis or decrease catabolism of them **(Dinman et al.,1963).** However, **Coles,(1986)** also reported that the level of these enzymes is increased following liver damage.

The obtained results are explained by those reported by **(Alvinrie and Galtier,1986)** . They mentioned that the liver is the target organ where the metabolism of TCBZ takes place . These results are supported by those recorded by **(Hunter et al.,1982 and Robinson,1985),** they mentioned that plasma enzymes were elevated at 1000 p.p.m. concentration of the drug. Also recently **(Hamed,1993)** reported in his study that therapeutic dose of TCBZ cause

significant increase in serum ALT and AST levels till 4 weeks post treatment and return to normal levels 60 days post treatment

The present study revealed that there was a significant increase in alkaline phosphatase (AP) level . Simillar results were obtained by **(Hunter et al.,1982 and Robinson,1985)**.

In the liver AP is located on both sinusoidal and bile canalicular membranes **(Michael et al.,1992)**. The increase in serum AP activity in hepatobiliary disorders is due enhanced RNA translation ( chromosomal abnormality ) **(Seetharam et al.,1986)**. This chromosomal abnormality will result in over production of hepatic AP isoenzymes activity in cholestasis **(Kaplan and Righetti,1970)**.

The present study demonstrated that there was a significant increase in serum total proteins including globulins and albumin in rats treated with TCBZ in both tested doses . The TCBZ significantly increases the globulins .This result may be due to increase the synthesis of gamma globulins and this may indicate some error in the protein metabolism **(Varley et al.,1976)**. In addition **(Hamed,1993)** recorded that the therapeutic dose of TCBZ cause significant increase in total proteins and globulins. However, **Coles,(1986)** mentioned that hyperalbuminemia is rarely seen except in the presence of dehydration and this was approved by clinical sings appeared on treated animals in this study, including dehydration, reduced urine volume and low water intake due to depression . Further more all these sings are reported in rats befor by

**(Sarasin,1982a.b)** in acute toxicity study.

The presented work showed that there were significant increase in serum total and direct bilirubin levels . **Coles,(1986)** explained that hepatocellular damage resulting in elevation of serum levels of total and direct bilirubin . Accordingly, this finding could be attributed to the damage of hepatic cells by the direct effect of the drug and/or its metabolites, resulting in decrease the removal of bilirubin by hepatocellular transport .

The significant decrease in the blood glucose level obtained in the present study is explained by **(Coles,1986)** . He mentioned that hypoglycemia may result from general hepatic dysfunction as consequence of circulatory deficiency and cirrhosis, this results in decrease of hepatic gluconeogensis.

The present study revealed significant increase in free cholestrol level and similar results were obtained by **(Hunter et al.,1982 and Robinson,1985)** in rats fed diets containing 1000 p.p.m. of TCBZ.

**Micheal et al.,(1992)** said that esterification and metabolism of cholestrol into bile acids occur in liver . Increased cholestrol may be due to failure of excretion and possibly also due to over production in liver **(Bayers et al.,1951)**. Also **(Kaneko,1989)** found that during hepatic insufficiency, less esterfied cholestrol is formed and transported to plasma because of low lecithin cholestrol acyl transferase (LCAT) activity in the liver. LCAT catalyzes the formation of esters from cholestrol and fatty acyl Co.A.

In the light of previous notions, it could be concluded that significant increase in serum ALT, AST, GGT, AP, total, direct bilirubin, total proteins, cholestrol and the significant decrease in blood glucose, may be due to the hepatocellular damage reported in our histopathological findings which revealed congestion of portal blood vessels, centrolobular vacuolation of hepatocytes, infiltration of mononuclear cells in portal triads and extensive replacement of hepatocytes with haemorrhage.

2- Kidney function examination

The present work shown that there were significant increase in serum levels of urea and creatinine in rats treated with TCBZ (200, 400 mg/kg b.w.)

**(Coles,1986)** recorded that serum urea and creatinine levels are increased iatrogenically following administration of drugs that increase protein catabolism or drugs that decrease protein anabolism.

In the current study, uric acid was significantly increased in rats treated with 200 and 400 mg TCBZ /kg b.w. **Ganang,1995** discussed the significant increase in uric acid level in the blood. He reported that uric acid is an end product of nucleoprotein metabolism, is excreted by the kidney and any increases in its blood level may be due to renal dysfunction.

In the glow of the previous notion, one could view the significant increase

in serum levels of urea, creatinine and uric acid were results from renal damage reported in our histopathological findings which revealed cystic dilatation of some renal tubules, congestion of renal blood vessels and mononuclear cell infiltration .

**Haematological examination**

The influence of this drug on haematological parameters have been little reviewed. The present study revealed marked anaemia (macrocytic hypochromic type) represented by reduction of erythrocytes, haemoglobin and packed cell volume . This was reflected in increased level of MCV and decreased levels of MCH, MCHC. The macrocytic hypochromic anaemia represents a toxic effect on the haemopiotic system **(Coles,1986)** . Simillar results were obtained by **(Hunter et al.,1982 and Robinson,1985)**

On the other hand, the accumulation of metabolic waste products (Ureamia) that was found in this work may had induced short life span and caused depression in the production of erythrocytes by haemopiotic organs **(Jones and Hunt,1983)** .

With white cells there is marked leucopenia especially in lymphocytes, in addition to monocytopenia and granulocytopenia . These findings were in aggreement with those obtained by **Hunter et al.,(1982) and Robinson,(1985)** .

The obtained results were explained by **Coles,(1986)**, as he mentioned that

bone marrow destruction is manifested by decrease in all cell types formed in bone marrow, and the animal often become anaemic in addition to have leucopenia. Also he added that from conditions that may produce leucopenia is chemical agants including drugs . Lymphocytopenia was confirmed histopathologically by the depletion of lymphopiotic tissues in the spleen .

**Histopathological examination**

In the present study, it has been showen that 200 and 400 mg/kg b.w. doses of TCBZ did clearly histopathological changes in parenchymatous organs in albino rats but there were not any changes in muscles.

The lesions in the treated rats were almost similar in most groups, however they were sever in group received 400 mg/kg b.w. than group received 200 mg/kg b.w.. In addition, the lesions were more sever in groups givin larger number of doses.

The hepatic lesions were represented by cloudy swelling, vacuolar and hydropic degeneration. Such findings could be attributed to an interference with energy transfer mechanism in the living tissues as a result of toxic action of this drug on the cell. **Kelly,(1985)** reported that hydropic degeneration was a common retrogressive change in hepatocytes due to intoxication.

Our results revealed co-agulative necrosis of hepatocytes and proliferated bile ducts which could be due to direct toxic effect of the used drug particularly in the higher dose and for long period (6 weeks), as the liver microsomal

sulphoxidation is a common metabolic pathway for TCBZ, **(Souhaili-El-Ameri et al., 1987; Short et al., 1988 and Lanusse et al., 1993a).**

The presence of numerous lymphocytes in the hepatic, renal parenchyma could be attributed to the possible immunologic role played by this drug or to the toxic effect of the drug as the lymphocytes are the main inflammatory cells in case of toxicosis, **(Jones et al., 1997)**.

The depletion of lymphocytes from the white pulp of spleen was more extensive which indicated that this drug have immunosuppresive activity accrding to **Tizard,(1992).**

The kidneys showed congestion of renal blood vessels and cystic dilatation of some renal tubules. Such findings are in accordance with **Smith et al.,(1972) and Atallah et al.,(1997)** who reported that benzimidazoles could lead to these renal changes.

The cardiac lesion represented by haemorrhage and hyaline degeneration of cardiac muscles which could be due to the toxic effect of the used drug as **Jones and Hunt,(1983)** mentioned that these lesions are indicative to intoxication.

It could be concluded that TCBZ at dose rates of 200 and 400 mg/kg b.w. has adverse effects on liver, kidney functions and on blood picture beside the histology of examined organs. It is recomended to aviod the use of the drug at these dose levels.

# SUMMARY

# Summary

Tricalbendazole (TCBZ) is one of the recent benzimidazoles active against immature and mature *F. hepatica* and *F. gigantica*. The drug is commonly used in veterinary medicine.

The present work was designed to study the effect of TCBZ at high doses on the biochemical parameters including liver and kidney function tests, blood picture and histology of some body organs (liver, spleen, kidney, heart, lung, muscles and brain).

In this work, 90 albino rats are divided into 3 main groups, each of 30 rats. The frist group was kept as a control, second group was given 1/40 $LD_{50}$ (200 mg/kg b.w.) dose, third one was given 1/20 $LD_{50}$ (400 mg/kg b.w.) dose. Each group was divided into 6 subgroups, each of 5 rats which was administered the drug orally twice weekly using stomach tube.

Each week one subgroup from each main group was sacrificied.

**The obtained results are summarized as follow:**

1- Effect of TCBZ (200 and 400 mg/kg b.w. doses twice weekly) on liver and kidney function tests.

Blood samples were obtained weekly from control and treated subgroups intended to be sacrificed and serum was prepared to measure the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (AP), total protein, albumin, globulins, total bilirubin, direct bilirubin, glucose, cholestrol, urea, uric acid and creatinine.

The study revealed that rats treated with 200 mg/kg b.w. twice weekly of drug showed a significant increase in the activity of all previous parameters except glucose which showed a significant decrease.

These results were more significant at the dose rate 400 mg/kg b.w. twice weekly. The biochemical abnormalities during this work may be due to liver damage or insufficiency, in addition to kidney dysfunction .

2- Effect of TCBZ (200 and 400 mg/kg b.w. doses twice weekly) on blood picture.

Blood samples were obtained weekly from control and treated subgroups intended to be sacrificed on anticoagulant to measure haematological parameters including white blood cell (W.B.Cs) count, differential leucocytic (lymphocyte, monocyte and granulocyte) count, red blood cell (R.B.Cs) count, haemoglobin (Hb) level and the packed cell volume (P.C.V.).

The correlation between the three haematological parameters, R.B.Cs, Hb and P.C.V. were clearly demonstrated in the blood and showed a significant lowering levels with marked macrocytic hypochromic anaemia which indicated by lower MCH and MCHC, higher MCV than control level.

A marked drop in W.B.Cs count especially in lymphocyte, in addition to monocyte and granulocyte count were observed. All previous haematological results were more significant at the dose rate 400 mg/kg b.w. twice weekly than at 200 mg/kg b.w. twice weekly.

The haematological abnormalities which were seen during the investigation may be due to the effect of drug on blood forming organs or depression of erythropiotic activity.

3- Effect of TCBZ (200 mg/kg b.w.twice weekly) on histlogical picture of some organs.

Organ samples as mentioned befor were obtained and perserved in formaline 10% and presented for histopathological examination.The predominent pathological lesions were observed especially in subgroups recieved larger number of doses and were more sever in 1/20 $LD_{50}$ group than 1/40 $LD_{50}$ one.

1- Liver lesions displaying coagulative necrosis after 6 weeks from giving 200 mg/kg b.w. twice weekly.

2- Spleen of animals treated with showed congestion of red pulp and depletion of lymphocytes from white pulp.

3- Heart showed congestion and haemorrhage.

4- Lung lesions displaying medial hypertrophy and hyperplasia of small muscular arteriols.

5- Kidney, the lesion mainly was cystic dilatation of some renal tubules.

6- Finally, brain showed encephalomalacia .

4- Effect of TCBZ (400 mg/kg b.w.twice weekly) on histlogical picture of some organs.

1- Liver showed extensive replacement of hepatocytes with haemorrhage after 6 weeks and showed congestion of portal blood vessels beside proliferated bile ducts after 5 weeks.

2- Spleen displaying the same lesions of the frist dose .

3- Heart showed degenrated myocardium .

4- Lung showed bronchiolectasia .

5- Kidney displaying congestion of renal blood vessels .

6- Brain showed haemrrhage of meninges after 4 weeks and encephalomalacia after 6 weeks .

Further more, there were no gross or pathological lesions appeared in the muscles.

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# ARABIC SUMMARY

# الملخص العربي


التراي كلابندازول هو أحد البنزيميدازول ذات الفاعلية ضد الأطوار اليافعة و الغير يافعة للديدان الكبدية و هو شائع الاستخدام في الطب البيطري .

تم في هذا البحث دراسة تأثير الجرعات العالية من التراي كلابندازول علي القياسات الخاصة بإنزيمات الدم و مركباته العضوية و تشمل وظائف الكبد و الكلي ، صورة الدم و علي التركيب النسيجي لبعض الأعضاء ( الكبد ، الطحال ، الكلي ، القلب ، الرئة ، العضلات و المخ).

و قد أجريت الدراسة علي ٩٠ جرذا حيث قسمت إلي ثلاث مجموعات رئيسية كلا منها ٣٠ جرذا :

١-المجموعة الرئيسية الأولي : جرعت عن طريق الفم بمحلول ملحي و استخدمت كمجموعة ضابطة للتجربة.

٢- المجموعة الرئيسية الثانية : جرعت عن طريق الفم بعقار التراي كلابندازول بجرعة ٢٠٠ مجم/كجم من وزن الجسم مرتين أسبوعيا أي ١/٤٠ من الجرعة النصف مميتة

٣- المجموعة الرئيسية الثالثة : جرعت بعقار التراي كلابندازول بجرعة ٤٠٠ مجم / كجم من وزن الجسم مرتين آسيويا أي ١/٢٠ من الجرعة النصف مميتة

- و قد قسمت كل مجموعة من المجموعات الرئيسية السابقة إلي ٦ مجموعات فرعية متساوية كلا منها تحتوي علي ٥ جرذان ، حيث تم ذبح مجموعة فرعية من كل مجموعة رئيسية كل أسبوع وذلك لاخذ ا لعينات من الدم و الاعضاء.

و قد أسفرت التجربة عن النتائج التالية :

**١-تأثير التراي كلابندازول ( ٢٠٠ و ٤٠٠ مجم/كجم من وزن الجسم الحي مرتين أسبوعيا عن طريق الفم ) علي وظائف الكبد و الكلي :**

قد تم أخذ عينات من الدم عن طريق العين أسبوعيا من المجموعات الفرعية المزمع ذبحها و فصل مصل الدم لقياس إنزيمات الدم و مركباته العضوية التي تشمل نشاط إنزيم ( الانين امينوترانسفيريز ، الأسبارتات أمينوترانسفيريز ، الفوسفاتيز القاعدى ، البر وتينات الكلية ، الالبيومين، الجلوبيولين ، البيليوربين الكلي و المباشر ، الجلوكوز ، الكوليسترول ، اليوريا ، حمض البوليك و الكرياتينين .

أشارت النتائج أن الجرذان التى اعطيت ٢٠٠مجم/كجم من وزن الجسم الحي مرتين عن طريق الفم أسبوعيا قد أظهرت زيادة معنوية في نشاط كل القياسات السابقة ماعدا الجلوكوز الذي أظهر نقص معنوي في مستواه بالدم.

وقد كانت هذه النتائج اكثر معنوية في حالة الجرذان التى اعطيت ٤٠٠مجم/كجم من وزن الجسم الحي مرتين عن طريق الفم أسبوعيا و أيضا أكثر معنوية في المجموعات الفرعية التي جرعت عدد اكبر من الجرعات.

هذه الانحرافات في قياسات إنزيمات الدم و مركباته العضوية ممكن أن تعزي إلي تلف الكبد أو عدم كفاءته بالإضافة إلي اختلال وظائف الكلي

**٢-تأثير التراي كلابندازول (٢٠٠ و ٤٠٠ مجم/كجم من وزن الجسم الحي مرتين أسبوعيا ) علي صورة الدم**

قد تم أخذ عينات الدم أسبوعيا عن طريق العين من المجموعات الفرعية المزمع ذبحها و ذلك لقياس القياسات الخاصة بصورة الدم و التى تشمل عدد كرات الدم البيضاء و العد

المتابين لهذه الخلايا ( عدد خلايا الليمفوسيت ، المونوسيت ، الجرانيولوسيت) ، عدد كرات الدم الحمراء ، نسبة الهيموجلوبين و نسبة الهيماتوكريت.

الارتباط بين عدد كرات الدم الحمراء ، نسبة الهيموجلوبين ونسبة الهيماتوكريت ظهر بوضوح في الدم و أظهر نقص معنوي بالمقارنة بالمجموعة الضابطة في هذه القياسات مع ميكروسيتيك هيبوكروميك أنيميا.

كان هناك أيضا نقص معنوي في عدد خلايا الدم البيضاء و خاصة الخلايا الليمفاوية بالإضافة إلي النقص المعنوي في عدد خلايا المونوسيت و الجرانيولوسيت.

كل هذه النتائج الخاصة بقياسات الدم كانت أكثر معنوية في حالة الجرعة ٤٠٠مجم عن حالة الجرعة ٢٠٠مجم /كجم من وزن الجسم الحي مرتين أسبوعيا و أيضا كانت أكثر معنوية في المجموعات الفرعية التي جرعت عدد أكبر من الجرعات . قد تعزي هذه النتائج إلي تأثير الدواء علي الأعضاء المكونة للدم أو نتيجة تثبيط النشاط التخليقي للدم.

**٣-تأثير التراي كلابندازول (٢٠٠مجم/كجم من وزن الجسم الحي مرتين أسبوعيا) علي الصورة النسيجية لبعض الأعضاء:**

- أخذت عينات من بعض الأعضاء الداخلية كما ذكر من قبل و حفظت في الفورمالين ١٠٪ لدراسة التغيرات المرضية و النسيجية التي طرأت عليها.

و قد ظهرت التغيرات المرضية و كانت أكثر وضوحا في المجموعات الفرعية التي جرعت عدد أكثر من الجرعات و أيضا كانت هذه التغيرات أكثر حدة في حالة الجرعة ٤٠٠مجم عن ٢٠٠مجم/كجم من وزن الجسم الحي مرتين أسبوعيا.

و قد أظهرت النتائج ما يلي:

-تنكرز في خلايا الكبد ، و نقص شديد في الخلايا الليمفاوية بالطحال ، إحتقان و نزيف بالقلب ، إحتقان في الأوعية الرئوية بعد ثلاثة أسابيع و تكثر و تضخم نسيجي للطبقة

العضلية الخاصة بالشعيرات الدموية بالرئة بعد ٦ أسابيع ، تمدد حويصلي في الأنابيب البولية بالكلي و لين المخ

**٤- تأثير التراي كلابندازول (٤٠٠مجم/كجم من وزن الجسم الحي مرتين أسبوعيا) على الصورة النسيجية لبعض الأعضاء:**

و قد أظهرت النتائج ما يلي:

إحتقان بالأوعية البابية بجانب تشعب القنوات المرارية بعد خمس أسابيع و إحلال الخلايا الكبدية بالأنزفة بعد ٦ أسابيع ، كما ظهرت نفس النتائج الخاصة بالطحال في الجرعة الأولي ، ظهر أيضا تحلل عضلات القلب ، توسع الشعب الهوائية بالرئة ، إحتقان الأوعية البولية الكلي و نزيف السجايا بالمخ بعد أربعة أسابيع بالإضافة إلي لين المخ بعد ستة أسابيع

بسم الله الرحمن الرحيم

وعلمك ما لم تكن تعلم

# و كان فضل الله عليك عظيما

صدق الله العظيم

سورة النساء : آية ١١٣

جامعة طنطا –فرع كفر الشيخ
كلية الطب البيطرى

# قرار لجنة الحكم والمناقشة

**قررت لجنة الحكم والمناقشة بجلستها المنعقدة يوم السبت الموافق / /٢٠٠٠ ترشيح السيد ط.ب./ غادة محمود جمعة للحصول على درجة ماجستير في العلوم الطبية البيطرية تخصص الطب الشرعي والسموم .**

***أعضاء اللجنة:-**

**أ.د./إبراهيم فتوح حسن** — أستاذ الفسيولوجيا والكيمياء الحيوية ووكيل الكلية لشئون الطلاب بكلية الطب البيطرى بكفر الشيخ جامعة طنطا

**أ.د./القلش مصطفى القلش** — أستاذ الطب الشرعي والسموم بكلية الطب البيطرى – جامعة الزقازيق

**أ.د./ فتحي رضوان علي** — أستاذ ورئيس قسم الطب الشرعي والسموم بكلية الطب البيطري جامعة المنصورة والمشرف على الرسالة

**أ.د./ مجدي إبراهيم عبد العزيز** — أستاذ ورئيس قسم الفارماكولوجي والطب الشرعي والسموم بكلية الطب البيطري بكفر الشيخ جامعة طنطا والمشرف على الرسالة