

Evaluation The Toxic Effects Of Herbicide Stomp 50% EC (Pendimethalin) On Health Of *Oreochromis niloticus*

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ABSTRACT

Two hundred and ten *Oreochromis niloticus* with an average weight 50 ± 0.4 g. were used to evaluate the toxic effects of herbicide Stomp (Pendimethalin). The results revealed that, the 96 hrs LC_{50} of Stomp 50% EC was 3.55 mg/L. The characteristic findings of exposed *Oreochromis niloticus* to acute toxicity were bloody dark coloration on operculum and anterior part of isthmus, erected pectoral fins, opened mouth and pale gills with excessive mucous secretion on gills and skin. The mortality rate of *Oreochromis niloticus* exposed to $1/10$ and $1/20$ 96 hrs LC_{50} of Stomp for four weeks was 30% and 16.7% respectively. The exposed fish didn't respond to escape reflex and showed congested gills with congested and enlarged liver. A significant increase of Creatinine, AST and Cortisol and a significant decrease of Immunoglobulin M (IgM) were recorded. Histopathological investigations of liver, kidney and gills confirmed the aforementioned findings.

INTRODUCTION

Pesticides, including herbicides, insecticides and fungicides, are used extensively to improve crop yields and as a result, they accumulate in the environment (1).

Dinitroanilines constitute an important group of herbicides being used on a wide variety of crops for the control of annual grasses and broadleaf weeds (2). Pollution of crops and ground water by dinitroaniline herbicides is being recognized as one of the serious environmental problems (3).

The herbicide Stomp belongs to the dinitroanilines compounds. Its half-decay in the surface waters is 61 days. This herbicide is widely used in the plant protection (4).

Pendimethalin is highly toxic to fish and aquatic organisms. Drift and runoff from treated areas may be hazardous to fish and aquatic organisms in neighboring areas. In addition, the Slackwater, darter and certain freshwater mussels are endangered species at risk from the use of pendimethalin on cotton (5).

There is no available literature on the effects of Stomp on warm water fishes. Only little data was obtained on rainbow trout and channel cat fish. Therefore, the objective of

this study was to determine the acute 96 hrs LC_{50} and investigate the effects of exposure to $1/10$ and $1/20$ 96 hrs LC_{50} for four weeks on health of *Oreochromis niloticus*.

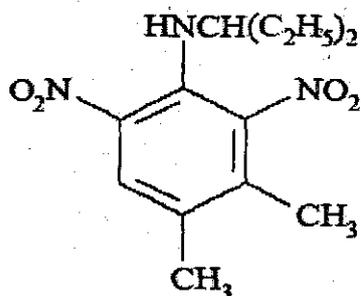
MATERIAL AND METHODS

Fish

A total number of 210 *Oreochromis niloticus* with an average weight 50 ± 0.4 g., were used. Fish were obtained from Abbassa Fish Hatchery, Sharkia Province. Fish were apparently healthy and free from any skin lesions and external parasites. They were maintained in glass aquaria (each, 80 x 40 x 30 cm capacity) having 60 liters of dechlorinated tap water. Each aquarium provided with aerator, thermostatically controlled heater and thermometer. Fish were acclimatized to laboratory environment for two weeks.

Stomp 50% EC

Stomp 50% EC (BASF PLC) is an orange-yellow liquid emulsive herbicide of the dinitroaniline type, whose active ingredient is pendimethalin [N-(1-ethylpropyl)-2,6-dinitro-3,4-xylylidine]. Empirical Formula: $C_{13}H_{19}N_3O_4$ (5).



Chemical formula of Pendimethalin (6).

Determination of 96 hrs LC₅₀ of Stomp in *Oreochromis niloticus*

A total number of 120 *Oreochromis niloticus* were divided into 12 equal groups (each 10 fish) kept in glass aquaria. All groups of tested fish, except control group, were exposed to different concentrations of Stomp (Table 1).

The experimental fish were observed daily. The clinical signs, behavioral response, post mortem lesions and the mortality rate were recorded (7). The 96 hours LC₅₀ was determined (8).

Effects of ¹/₁₀ and ¹/₂₀ 96 hrs LC₅₀ of Stomp on health of *Oreochromis niloticus*

A total number of 90 *Oreochromis niloticus* were divided into 3 equal groups,

each with two replicates (15 fish replicate⁻¹). The fish of the first group were kept as control, while fish of the second and third groups were exposed to ¹/₁₀ and ¹/₂₀ 96 hrs LC₅₀ respectively. The experiment was conducted for four weeks.

The experimental fish were fed on a basal diet contained 35.4 % crude protein. The amount of food (on dry matter basis) per day was 3 % of fish body weight. The fish were fed 3 times daily.

Blood samples for biochemical analysis were collected from the caudal blood vessels (7), left for one hour at room temperature for clot formation and centrifuged at 3000 r.p.m. for 15 minutes for serum separation. Creatinine (9), aspartate aminotransferase (AST) (10), cortisol level (11) and immunoglobulin M (12) were determined.

Histopathological examination of liver, kidney and gills of tested *O. niloticus* was carried out (13).

Statistical analysis using one-way ANOVA Statistical Analysis System (14) was performed to obtain the significant difference at P < 0.05 on various parameters between tested groups.

Table 1. Preliminary trials for determination of zero and hundred % mortalities in *Oreochromis niloticus* exposed to different concentrations of Stomp.

Group (n=10)	Concentration of Stomp (mg/L)	Mortality during 96 hours.				Total MR*	Mortality %
		1 st day	2 nd day	3 rd day	4 th day		
1	control	0	0	0	0	0	0
2	1	0	0	0	0	0	0
3	1.5	0	0	0	0	0	0
4	2	1	1	0	0	2	20
5	2.5	1	1	0	0	2	20
6	3	2	0	1	0	3	30
7	3.5	2	1	0	1	4	40
8	4	3	1	1	0	5	50
9	4.5	4	2	1	1	8	80
10	5	7	1	1	1	10	100
11	5.5	8	1	1	0	10	100
12	6	10	0	0	0	10	100

*MR = Mortality.

RESULTS AND DISCUSSION

Acute toxicity (96 hrs LC₅₀) of Stomp on *Oreochromis niloticus*

The results demonstrated in Table 2 revealed that, the 96 hrs LC₅₀ of Stomp in *Oreochromis niloticus* was 3.55 mg/L. Previous study (15) demonstrated that, 96-hours LC₅₀ for pendimethalin in rainbow trout was 138 ug/L and 420 ug/L in channel catfish. These differences may be attributed to the difference in concentration of Stomp active principle used, difference of fish species, environmental conditions as well as individual variation.

The exposed *Oreochromis niloticus* showed abnormal swimming movement, sluggish, didn't respond to escape reflex, the respiration rate was gradually slow, bloody dark coloration on operculum and anterior part of isthmus, the fish lying down on one side with erected pectoral fins followed by convulsion till die with opened mouth (Fig.1. A, B, C). Pale gills with excessive mucous secretion. These clinical signs may be attributed to the inhibition of acetylcholine esterase (CHE) enzyme by this group of herbicides, specially at neuromuscular junction which leads to continuous muscular contraction until complete exhaustion is attained (16). Previous study (17) indicated that, there was an increase in methemoglobin in rats administered 3,5-Dinitro-4-chloro-alpha,alpha,alpha-trifluorotoluene; one of the dinitroaniline herbicides. The bloody dark coloration may be attributed to hemorrhage which presumably due to damage resulting from spasms. Stomp herbicide act as uncoupler of oxidative phosphorylation in rats hepatic mitochondria which consequently enhances the respiration and diminishes membrane potential (18). Pendimethalin causes central nervous system depression in mice and rats (19).

Effects of ¹/₁₀ and ¹/₂₀ 96 hrs LC₅₀ of Stomp on health of *Oreochromis niloticus*

The results demonstrated in Table 3 revealed that, the mortality rate of fish exposed to ¹/₁₀ (0.355 mg/L) and ¹/₂₀ (0.177 mg/L) 96

hrs LC₅₀ of Stomp for four weeks were 30% and 16.7% respectively. The exposed fish appeared sluggish and didn't respond to escape reflex, dark coloration of the skin and covered with thick layer of mucous, congested gills with congested and enlarged liver (Fig.1. D). Enlarged liver of rats exposed to Stomp was previously recorded (19).

The results in Table 4 revealed that, there was a significant increase of creatinine, AST activity and cortisol while there was a significant decrease of IgM of exposed *O. niloticus*. The increased AST may be attributed to in spite of pendimethalin herbicide has quite rapid excretion from the rat body (up to 90% within 24hrs) about 70% of hydrophobic compound goes through enterohepatic pathway (20). Moreover, absorbed pendimethalin is rapidly metabolized in the liver and kidney which explained the significant increase of creatinine (21). The increase of cortisol may be due to the stress of Stomp on exposed *O. niloticus*. The decrease of IgM may be due to inhibition of protein biosyntheses (22). Also in this respect our results coincide with those previously obtained in rats administered one of dinitroaniline herbicide (DNCTT) and showed increased in spleen relative weights and microscopic examination revealed marked congestion with increase density of spleen white pulp which may indicate immunotoxic effect of dinitroaniline herbicides (17). Nearly similar findings were previously recorded with other pesticides in different fish species (23-25).

The previously mentioned results concerning clinical and biochemical findings were confirmed with our histopathological findings which revealed swollen of the hepatic cells with pyknosis or karyolysis of majority of their nuclei and congestion of blood vessels and hepatic sinusoid (Fig.2- B). Hepatopancreatic elements had slightly eosinophilic granules in their cytoplasm, also extravasated erythrocyte could be seen, bile duct epithelium showed proliferation forming newly formed bile ductules with some lymphocytic infiltration. Melanomacrophages could be seen scattered among hepatic tissue.

Moreover, there were hydropic degeneration and congested blood vessels which were common (Fig.2- C). Portal areas showed hyperplastic bile duct epithelium forming finger like projection and slight periductular fibrosis. The results similarly with those previously reported in alachlor (26) and glyphosate (27) herbicide toxicity on Nile tilapia. Kidney illustrated that majority of renal parenchyma was destructed and replaced by edematous fibrous tissue containing melanomacrophages (Fig.2 - E). Interstitial eosinophilic granular cells could be seen with a few fibroblastic proliferation. Some glomeruli had thickened basement membrane and contracted glomerular tufts with dilated glomerular space and degenerated renal tubular epithelia with melanomacrophage contents were common (Fig.2 - F). Hemobiotic elements were proliferated, renal capsule was thickened by fibrotic tissue. Renalotoxic effect

was recorded in alachlor (26), glyphosate (27) exposure of Nile tilapia and different herbicide in two Venezuelan cultured fish (28). Histopathological investigation of gills illustrated dilated capillaries of secondary lamellae and branchial blood vessels together with hyperplastic lamellar epithelium on the tops of gill filaments were seen (Fig.2 - H). Gill filaments appeared edematous or disorganized. Hemorrhages and edema in gill arches could be seen. Besides, branchial blood vessels and capillaries of secondary lamellae were dilated and hyperemic (Fig.2 - I). Gill arch revealed edema, hemorrhages, lymphocytic and esinophilic granular cells infiltration. Muscles of gill rakers were partially hyalinized and epithelium covering showed metaplasia to goblet cells. The results were in the same context with those previously reported (26,27).

Table 2. Actual determination of 96 hours LC₅₀ of Stomp in *Oreochromis niloticus*.

Group	Concentration of Stomp (mg/L)	Number of dead fish after 96 hours	a	b	a X b	Σ (a x b)
1	1.5	0	0	0	0	
2	2	2	0.5	1	0.5	
3	2.5	2	0.5	2	1	
4	3	3	0.5	2.5	1.25	
5	3.5	4	0.5	3.5	1.75	
6	4	5	0.5	4.5	2.25	
7	4.5	8	0.5	6.5	3.25	
8	5	10	0.5	9	4.5	
						14.5

$$96 \text{ Lc}_{50} = \text{highest dose} - \frac{\sum (a x b)}{n}$$

$$= 5 - \frac{14.5}{10} = 3.55 \text{ mg/L}$$

a= Constant factor between two successive doses.
 b= The mean of dead fish in the groups.
 n= Number of fish in each group
 Σ= The sum of (a x b).

Table 3. Effects of different concentrations of Stomp on behavioral response and mortality of exposed *Oreochromis niloticus* at the end of experiment (4weeks).

Group n=30	Concentration of Stomp (mg/L)	Observation (week)								Mortality rate	
		1 st		2 nd		3 rd		4 th			
		ER	MR	ER	MR	ER	MR	ER	MR	No	%
1	Control	+	0	+	0	+	0	+	0	0	0.0
2	$1/10$ 96 hrs LC_{50} (0.355)	-	5	-	2	-	1	-	1	9	30.0
3	$1/20$ 96 hrs LC_{50} (0.177)	-	3	-	1	-	1	-	0	5	16.7

ER= escape reflex.

MR= mortality.

+ = fish respond well to escape reflex.

- = fish not respond to escape reflex.

Table 4. Effects of different concentrations ($1/10$ and $1/20$ 96 hrs LC_{50}) of Stomp on some biochemical parameters of exposed *Oreochromis niloticus* at the end of experiment (4weeks).

Parameters	Group (dose)	1	2	3
		Control	$1/10$ 96 hrs LC_{50} (0.355 mg / L)	$1/20$ 96 hrs LC_{50} (0.177mg / L)
Creatinine (mg / dL)		0.22 ± 0.12^b	0.39 ± 0.02^a	0.38 ± 0.15^a
AST (μ / L)		17 ± 0.21^c	34 ± 0.06^a	29 ± 0.24^b
Cortisol (μ g/dL)		0.80 ± 0.16^b	1.5 ± 0.03^a	1.4 ± 0.07^a
Ig M (g/L)		0.60 ± 0.33^a	0.17 ± 0.01^c	0.19 ± 0.18^b

Means within the same row bearing different superscripts are significant at $p \leq 0.05$.

AST= aspartate aminotransferase

Ig M= Immunoglobulin M

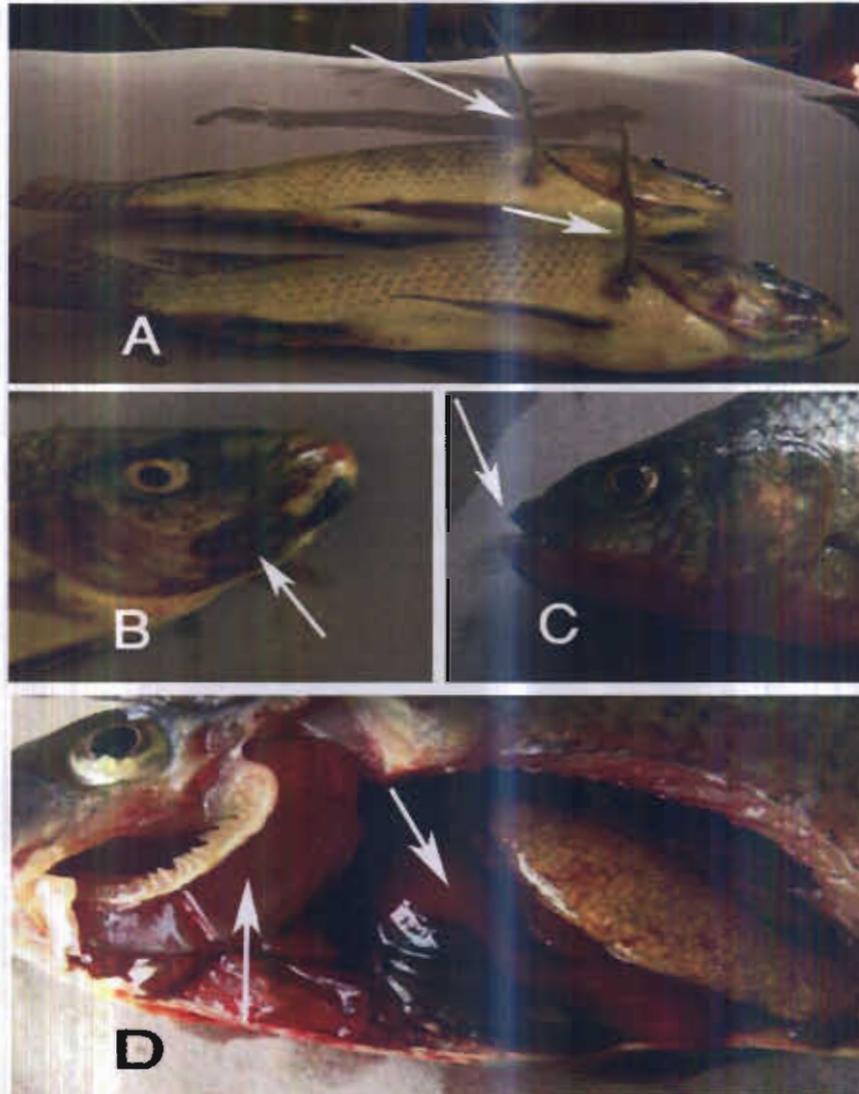


Fig.1. Clinical signs and postmortem finding of *Oreochromis niloticus* exposed to acute toxicity showing erected pectoral fins (A), bloody dark coloration on operculum and anterior part of isthmus (B) and opened mouth (C). Exposure to $1/10$ 96 hrs LC_{50} of Stomp for four weeks showing congested gills with congested and enlarged liver (D).

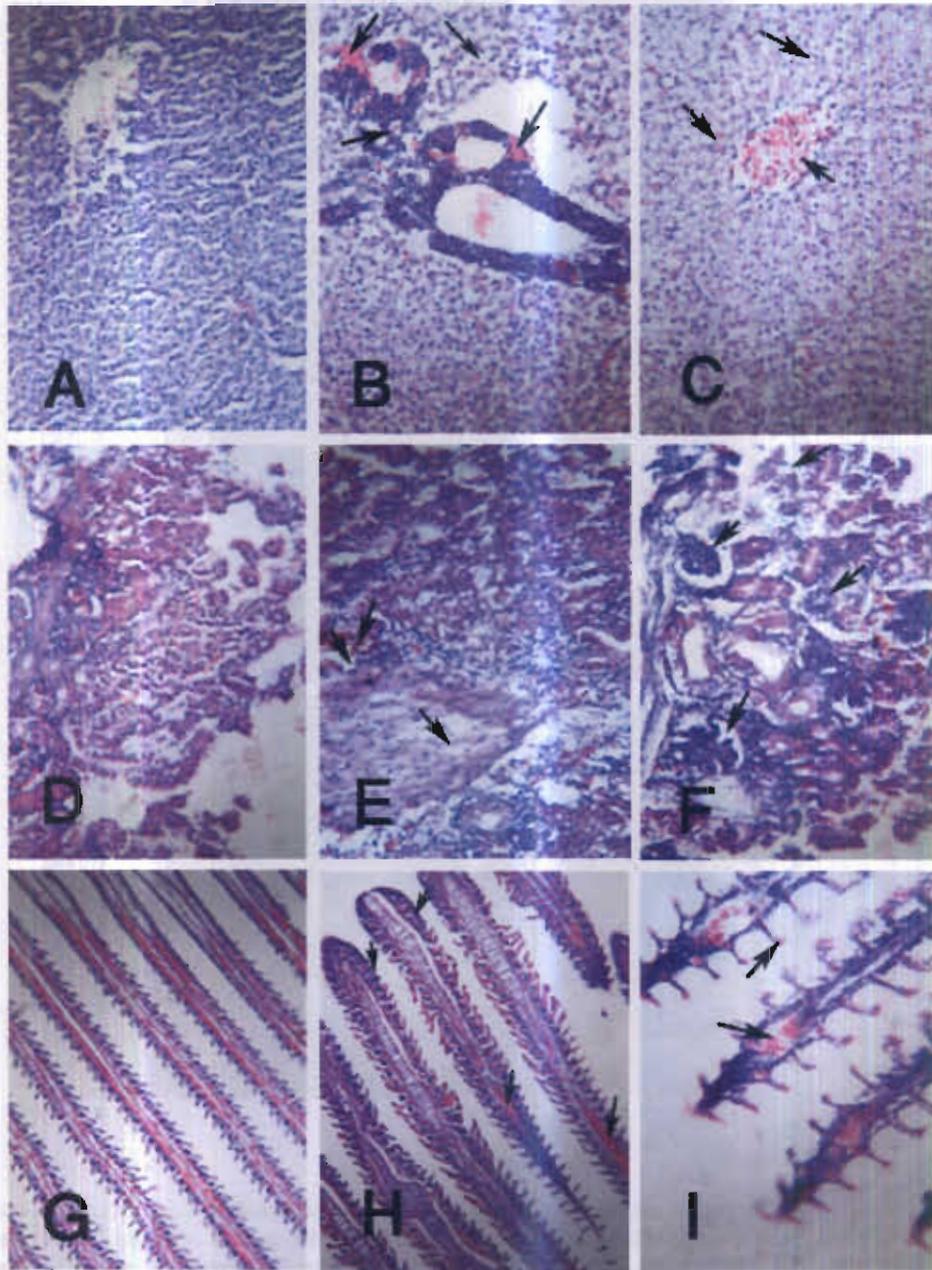


Fig. 2. **A:** Section in liver of control fish showing normal histological structure (H&E X300). **B:** Section in liver of fish (exposed to $1/10$ 96 hr LC_{50} of stomp herbicide) showing necrotic hepatocytes, inactivated or destroyed hepatopancreas and presence of melanomacrophages (H&E X300). **C:** Section in liver of fish (exposed to $1/20$ 96 hr LC_{50} of stomp herbicide) showing hydropic degeneration of hepatic cells and congestion (H&E X300). **D:** Section in kidney of control fish showing normal histological structure (H&E X300). **E:** Section in posterior kidney of fish (exposed to $1/10$ 96 hr LC_{50} of stomp herbicide) showing focal replacement of renal tissue with edematous fibrous tissue and melanomacrophage cells (H&E X300). **F:** Section in posterior kidney of fish (exposed to $1/20$ 96 hr LC_{50} of stomp herbicide) showing lobulated or contracted glomerular tufts and degenerated tubular epithelium (H&E X300). **G:** Section in gills of control fish showing normal histological structure (H&E X120). **H:** Section in gills of fish (exposed to $1/10$ 96 hr LC_{50} of stomp herbicide) showing hyperplasia of capillaries and secondary lamellar epithelium with hyperemia in blood vessels of primary and secondary filaments (H&E X120). **I:** Section in gills of fish (exposed to $1/20$ 96 hr LC_{50} of stomp herbicide) showing hyperemia in branchial blood vessels and capillaries of secondary lamellae (H&E X300).

CONCLUSION

It could be concluded that, the 96 hrs LC₅₀ of herbicide Stomp 50% EC (Pendimethalin) was 3.55 mg/L. The exposed *O. niloticus* to acute toxicity showed respiratory and nervous disorders. Exposure to concentrations ¹/₁₀ and ¹/₂₀ 96 hrs LC₅₀ of Stomp for four weeks had adverse effects on behavioral, some biochemical parameters and histopathological alterations of some organs of tested *O. niloticus*. Therefore we recommend further studies on herbicide Stomp 50% EC (Pendimethalin) on farmed warm water fishes.

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الملخص العربي

تقييم التأثيرات السمية لمبيد الحشائش ستومب EC 50% (بنديميثالين) على صحة البلطي النيلي

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استخدم عدد 210 من البلطى النيلي بمتوسط وزن 50 ± 0.4 جرام لتقييم التأثيرات السمية لمبيد الحشائش ستومب (بنديميثالين) على صحة البلطى النيلي.

أوضحت النتائج أن الجرعة نصف المميّنة لمدة 96 ساعة لمبيد الحشائش ستومب هي 3.55 ملجرام/لتر وكانت الأعراض المميزة للبلطى النيلي المعرض لهذا التركيز هي وجود لون دموى داكن على غطاء الخياشيم ومقدمة أسفل الرأس (البرزخ) وانتصاب الزعنفة الصدرية وفتح الفم وشحوب الخياشيم مع افرازات مخاطية كثيفة على الخياشيم والجلد. وجد أن نسبة النفوق للبلطى النيلي المعرض لمدة أربعة أسابيع لتركيزات $1/10$ و $1/20$ من الجرعة نصف المميّنة لمدة 96 ساعة لمبيد الحشائش ستومب هي 30% و 16.7% على التوالي كما أن الأسماك المعرضة لا تستجيب لانعكاس الهروب مع احتقان الخياشيم واحتقان وتضخم الكبد. وجد أن هناك زيادة معنوية للكرياتينين والأسبرتيت أمينوترانسفيريز والكورتيزول بينما كان هناك نقص معنوى للأميونوجلوبولين بالإضافة الى الفحص الباثولوجى لبعض الأعضاء الداخلية الممثلة فى الكبد والكلية والخياشيم والتي تؤكد النتائج المذكورة أعلاه .