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EFFECT OF EXTRACTED CRUDE VENOM FROM JELLY FISH EUTONINA INDICANS ON LIVER FUNCTION IN MALE MICE

(With 2 Tables and 6 Figures)

By

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تأثير السم المفصول من قنديل البحر أيتونينا انديكانز على وظائف الكبد فى ذكور الفئران

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تناول هذا البحث تأثير السم المفصول من قنديل البحر ايتونينا انديكانز على وظائف الكبد وقد استخدم فى هذا البحث ٢٠ فأر قسمت الى مجموعتين كل مجموعه ١٠ فئران. المجموعه الأولى: استخدمت كضوابط للتجربه والمجموعه الثانيه حقنت فى التجويف البريتونى بجرعه نصف مميته من هذا السم الخام (٧٨ر ١ مليجرام / ٢٠ جرام من وزن الجسم) وقد اثبتت النتائج بعد ٢٤ ساعه من الحقن ان النسب المئويه لجزئيات الف وبيتا وجاما جلوبيولينات والالبيومين لم تتغير معنوياً بالنسبه للحيوانات الغير محقونه. وربما يرجع هذا الى أن السم الخام لا يسبب اى خلل وظيفى للكبد. كما أثبتت النتائج زيادة مستوى كل من البروتين الكلى والجليكوجين وقد اعزيت هذه الزياده الى تأثير البراديكينين الموجود فى السم الخام بطريقه مباشره على نشاط الخليه أو بطريقه غير مباشره عن طريق تأثيره على البروستاجلاندين. أما بالنسبه لمستويات الدهون وحمض الثيوباربتيوريك وأنزيمى GOT , GPT وانزيمى الفوسفاتيز القاعدى والحامضى فقد أوضحت النتائج انها لم تتغير أيضاً تغيراً معنوياً بالنسبه للحيوانات الغير محقونه وهذا ربما يرجع الى أن هذا السم الخام لم يؤثر على وظائف الكبد الحيوية والفسيولوجيه.

SUMMARY

A sublethal dose of crude venom extracted from the Jelly fish *Eutonina indicans* was injected intraperitoneallv to mice (1.78 mg/20 gm b.w.). The percentages of blood serum (α_1, α_2, β, ν,) globulins and albumin were determined. Also, the levels of liver proteins, glycogen, total lipid, TBARS, transaminases (S. GOT & S. GPT) alkaline and acid phosphatases were determined. After 24 hours of treatment, the crude venom induced

insignificant ($P > 0.05$) changes of α_1, α_2, β, ν, globulins and albumiin percentages, on the other hand, this extracted crude venom induced a significant ($P < 0.001$) increase in the liver total proteins and glycogen levels, while the levels of lipid, TBARS, transaminases (S. GOT & S. GPT) alkaline and acid phosphatases activities showed in-significant ($P > 0.05$) changes. This extracted crude venom was performed on Nicolet apparatus cod 710 FT-IR spectrometer which predict that the structure of the venom may be aliphatic compound. The biological effect of this extracted venom may be attributed to the direct effect of crude venom bradykinin or direct effect through the activation of prostaglandin synthesis.

Keywords: Venom- Jelly fish- liver function-Mice

INTRODUCTION

The toxins of the coelenterates have been detected to include bradykinin and related polypeptides which have a kinin-like action (*BURNETT and CALTON, 1974*). *LAMBERT et al. (1986)* reported that bradykinin stimulates DNA synthesis in IMR-90 human fibroblasts. Also, *YU and SADEE (1986)* found that the phosphatidylinositol (PI) can be stimulated in rat neuroblastoma glioma hybrid cells (NG 108-5) and human neuroblastoma cells (SK-N-SH) by bradykinin; according to *OSUGI, et al. (1987)*, the addition of bradykinin to the neuroblastoma glioma hybrid (NG 108-5) cells increases the intracellular calcium concentration (*PORTILLA and MORRISON, 1986)* and the formation of inositol mono, bis and triphosphate.

The relationship between bradykinin and prostaglandins synthesis was studied by *COHEN et al. (1970)*, who reported that bradykinin stimulates the synthesis of PGS. *WHORTON et al. (1982)* recorded that bradykinin stimulates prostaglandin production in endothelial cells. Also, *MENCONI et al. (1984)* reported that the cells in culture could be stimualted to produce PGI_2 by both angiotensin and bradykinin at very low concentrations. *HESSINGER (1986)* found that the venom of *Physalia physalis* caused prostaglandin-induced vasodilation in vivo and in vitro. In addition, prostaglandin synthesis in cultured fibroblasts and isolated smooth muscle was incresed. Therefore, the present work was designed to study the effect of a crude venom extracted from the Jelly fish *Eutonina indicans* on the liver function.

MATERIAL and METHODS

The study was performed on twenty healthy male mice (30 gm, 8 weeks) perchased from the animal house of Assiut University. The animals were fed

on standard diet for two weeks before administration of the jelly fish extracted crude venom. Experimental animals were dlvided into two groups, (10 animals each). The first group used as a control, while the second group was injected intraperitoneally with 1.78 mg/20 b.w. of the crude venom extracted from the Jelly fish *Eutonina indicans*. The jelly fish was collected and isolated by H.M. Aboul-Dahab from the Red Sea. This was determined on the base of the LD50 of the crude venom *(YASSIEN, 1995)*. Blood samples from injected and control animals were collected after 24 hours of the treatment. The blood was collected by heart puncture and the serum separated was used for electrophoresis (SEAC2001) on cellulose acetate strips at pH 8.6 using phosphate buffer and stained with ponceau S for quantitative analysis of electrophoretically separated protein fractions using a densitometer (code 53841210, SEAC) according to the method described by *JEPPSON et al. (1979)*. The tissue of Liver was dissected directly out of the sacrified animals, a part was taken for histochemical technique according to the method of *DRURY and WALLINGTON (1980)*, while the other part of tissue was weighed and homogenized in 10 volumes (W/V) saline solution using glass-glass hand homogenizer. The homogenates samples were kept at -20°C until using. The liver total proteins level was determined according to the method described by *HENRY (1964)*, liver glycogen phosphorylase activities were estimated by the method of Leathwood and *RYMAN (1971)*, liver total lipids was determined by the colorimetric method of *CHRISTOPHER et al., (1970)* and thirobarbituric acid reactive substances (TBARS) were assayed in accordance to the technique described by *FRAGA et al., (1990)* Also, the obtained homogenate liver samples were used to determine glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transminase (GOT) activities according to the colorimetric method of *REITMAN and FRANKEL (1957)*. Alkaline phosphatase and acid phosphatase activities were determined by colorimetric method of *KING (1965)*. The venom was performed on Ft-IR spectrometer cod 710 to predict the groups of compounds contained in it.

RESULTS

The effect of the crude venom, extracted from the Jelly fish *Eutonina indicans*, on liver function of experimental animals was studied.

1- *Electrophoretic pattern of* blood *serum proteins:*

The blood serum protin electrophoretic patterns are shown in Table (1) and Figs. 1, 2 & 3. The percentages of globulins (α_1, α_2, β, ν) and albumin fractions in both control and extracted crude venom treated animals revealed insignificant change ($P > 0.05$) in the above mentined treated group in comparison to respective control ones.

2- Liver *Total roteins Levels:*

It is clear from the date presented in Table (2) that the mean level of total proteins of mice treated with crude venom was increased significantly ($P< 0.001$) + 46% over the corresponding mean level of the control mice.

3- Liver *Glycogen Levels:*

The mean level of liver glycogen of mice treated with extracted crude venom was increased significantly ($P< 0.001$) relative to the control being + 62% (Table 2 and Figs. 4& 5).

Fig. (6.) means the chart of this compond on FT-IR spectrometer. This extracted crude venom may be aliphatic group because it contains ethyl group (-0-) at 1122.5, Carbonyl group 0=C anodic at 1642.9 and hydroxyl group or-NH at 3650.

4- Liver *Total Lipid Levels:*

Analysis of the liver total lipids leveis of both treated and control animals revealed that there was insignificant change ($P< 0.05$) of total lipid levels of treated animals (Table 2).

5- Liver *TBARS Level:*

Table (2) shows that there was insignificant change ($P>0.05$) of TBARS level between the treated and control mice.

6- Liver *Transaminases (S.GOT & S. GPT):*

It is clear from the data presented in Table (2) that the transaminases activities of both treated and control animals showed insignificant change ($P> 0.05$) of treated animals transaminases activities.

7- Liver *Alkaline and acid phosphatase activities:*

The results presented in Table (2) show that the mean activity of both alkaline and acid phosphatases of both treated and control animals showed insignificant change ($P> 0.05$).

Fig (6) means the chart of this compound on FT-IR spectrometer. This extracted crude venom most probably of aliphatic nature group because it is contain ethyl group (-0-) at 1122.5, Carbonyl group 0=C anodic at 1642.9 and hydroxyl group or-NH at 3650.

DISCUSSION

The present study was carried out to detect a probable effect of extracted crude venom from the Jely fish *Eutonina indicans* on the iver function.

The resuts revealed that, the perecentages of (α_1, α_2 , β, ν), gobulins and albumin fractions did not significantly changed ($P>0.05$) in the treated and control animals which indicates that the extracted crude venom has no effect on the liver function. The present results are in agreement with

those obtained by *HARFENIST and MURRARY (1991)* who reported that most of the pllasma proteins are synthesized in liver and the levels of certain proteins in plasma increase during acute inflammatory states or secondary to certain types of tissue damage.

The levels of hepatic total proteins and Glycogen of the treated animals were significantly ($P<0.001$) increased. These elevated levels may be due to the presence of bradykinin in the venom which stimulates and enhances protein and glycogen synthesis. Burnett and *CALTON (1977)*, reported that the coelenterata toxins contain bradkinin related polypeptides which have a kinin like action. *SHIMIZU (1984)* and FREDRIK and XIN-HUA (1991) studied the effect of bradykinin on protein synthesis and glucose production. They reported that bradykinin and the relative bioactive polypeptide stimulate protein, RNA, DNA synthesis and glucose production. The increase of total proteins and glycogen levels after treatment with extracted crude venom may be resulted from an indirect stimulation through the activation of prostaglandin synthesis which in turn promoted the observed effect on liver. This suggestion is supported by COHEN *et al. (1970)*. Who reported that bradykinin stimulates the synthesis of PGS. Also, WHORTON *et al. (1982)* indicated that bradykinin stimulates prostaglandin production in endothelial cells.

The levels of liver total lipids showed that there was no significant change between the treated and the control animals. It can be suggested that the extracted crude venom has no effect on the lipid metabolism in the liver. These results were different from that of FAHIM and ZAHRAN (1986) who found that the level of lipid was significantly decreased after treatment of rats with the crude and fractions from *Naja nigricollis.*

The sublethal dose of extracted crude venom had no significant effect on the level of TBARS. This means that the crude venom did not enhance lipid peroxidation. This result is in support with the reportes that thiobarbituric acid reactive substances (TBARS) is a marker for lipid peroxidation *(FRANKEL, 1984).*

Also, the activities of glutamic pyruvic transaminase (GPT, glutamic oxaloacetic transaminase (GOT), alkaline and acid phosphatase showed that there was no significant change between the treated and the control animals. This unchanged effect could be considered indicative that the extracted crude venom has no effect on the activities of these enzymes. According to *PETER and ANTHONY (1982)* the activities of GOT, GPT, acid and alkaline phosphatases are considered as indicators of liver function. Moreover, *VICTOR (1991)* reported that serum levels of transaminases, acid and alkaline phosphatases are elevated in some disease states. This means that this extracted crude venom did not cause any affection on liver.

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Table 1: Effect of crude venom (1.78 mg/20 gm body weight) extracted from the jelly fish *Eutonina indicans* on serum albumin and globulin fractions in male mice.

Albumin and serum globulin fractions	Treated	Control	Ratio of albumin and globulins to total proteins in treated mice	Ratio of albumin & globulins to total proteins in control mice
Albumin	4.39±1.51*	3.86±1.30	0.0010	0.0013
α_1	23.03±0.42*	22.24±1.61	0.0053	0.0075
α_2	23.9±2.75*	24.38±4.30	0.0055	0.0082
β	25.69±3.64*	26.58±4.10	0.0059	0.0089
υ	22.82±1.72*	22.61±2.28	0.0052	0.0076

Values are expressed as mean of 10 mice ± S.E.

* = Nonsignificant P> 0.05 using student's test.

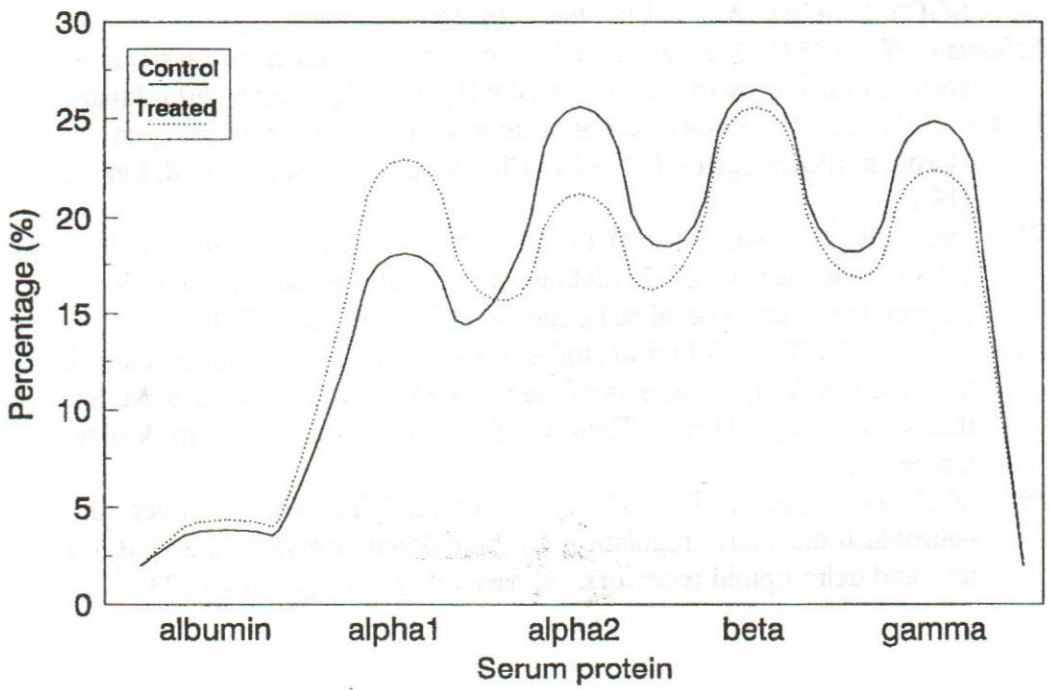


Fig. 3: The electrophoretic scan of the pattern of serum proteins obtained from both control (a) and treated (b) animals.

Table 2: Effect of crude venom (1.78 mg/20 gm b.w.) exracted from the Jelly fish *Eutonina indicans* on the levels of total proteins, glycogen, total lipids (mg/g. tissue) thiobarbituric acid reactive substances; TBARs (m mol/MAD eq./g.. tissue), glutamic-oxaloacetic transaminase; GOT glutamic-pyruvic transaminase; GPT (µmp;e/g. tissue), alkaline phosphatase and acid phosphatase (µmole/g. tissue) in the liver homogenate of mice.

Parameters	Treated	Control
Total proteins % of change	4.336±0.125** + 46	2.95±0.146
Glycogen % of change	0.684±0.037** + 62	0.420±0.0183
Total lipids % of change	1.863±0.125* - 0.55	1.875±0.021
TBARs, % of change	0.201±0.0048* + 1.5	0.198±0.0023
GOT activity level % of change	12.077±0.183* - 2.5	12.39±0.314
GPT activity level % of change	13.673±0.344* + 1.1	13.529±0.324
Alkaline phosphatase activity level % of change	2.481±0.161* - 3.1	2.654±0.5
Acid phosphatase activity level % of change	3.283±0.204* - 2.9	3.382±0.208

Each value represents the mean ± S.F. using students test:

** = Highly significant ($P<0.001$).

* = Non significant ($P>0.05$).

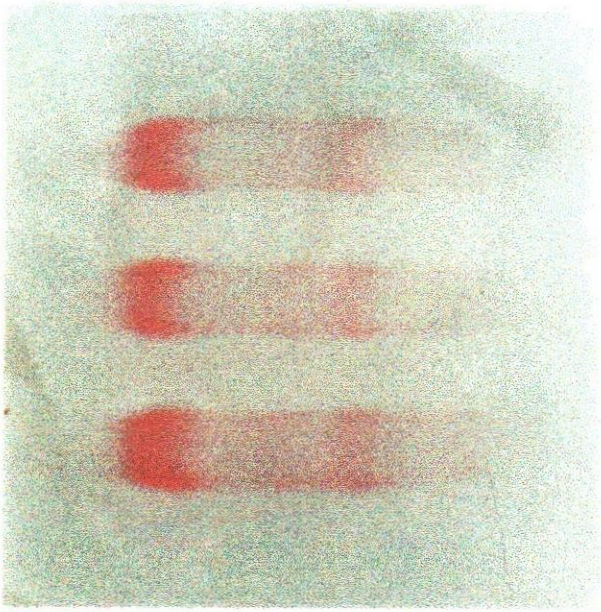


Fig. 1: (A) Control

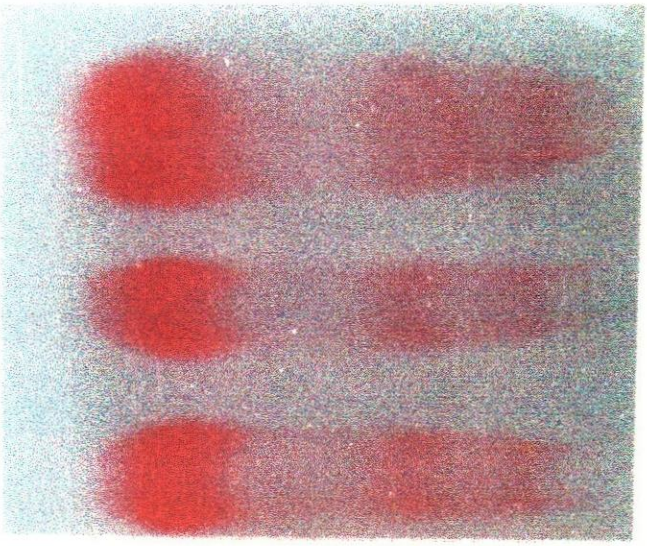


Fig. 2: (B) Treated after 24h. Electrophoretic patterns of serum protein (A) control and (B) treated mice [albumin, α_1, α_2 , β, ν].

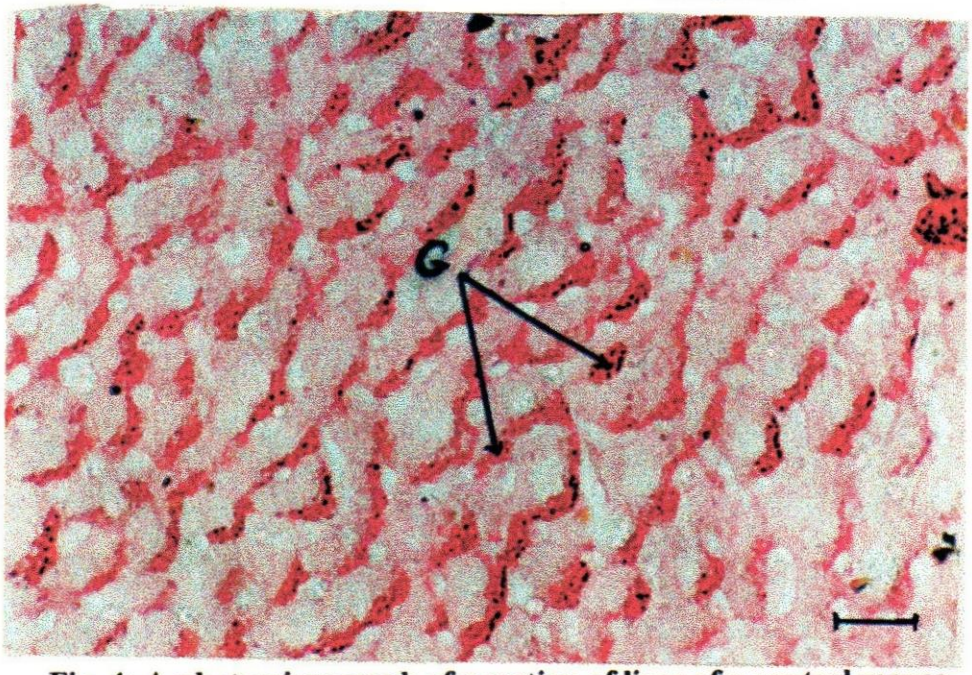


Fig. 4: A photomicrograph of a section of liver of a control mouse. It shows a glycogen within the hepatocytes.
G = Glycogen. PAS. Scale bar = 0.01 mm.

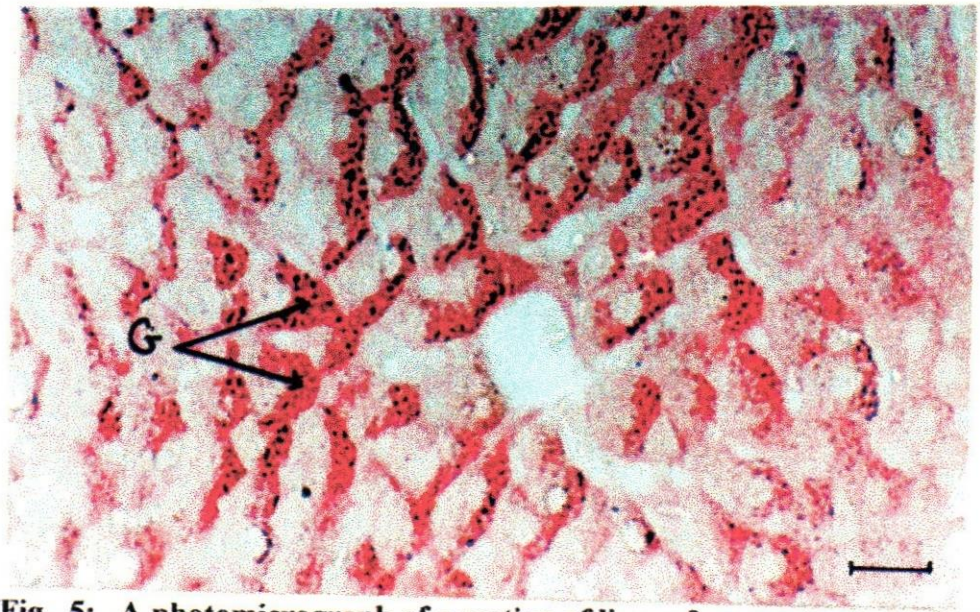
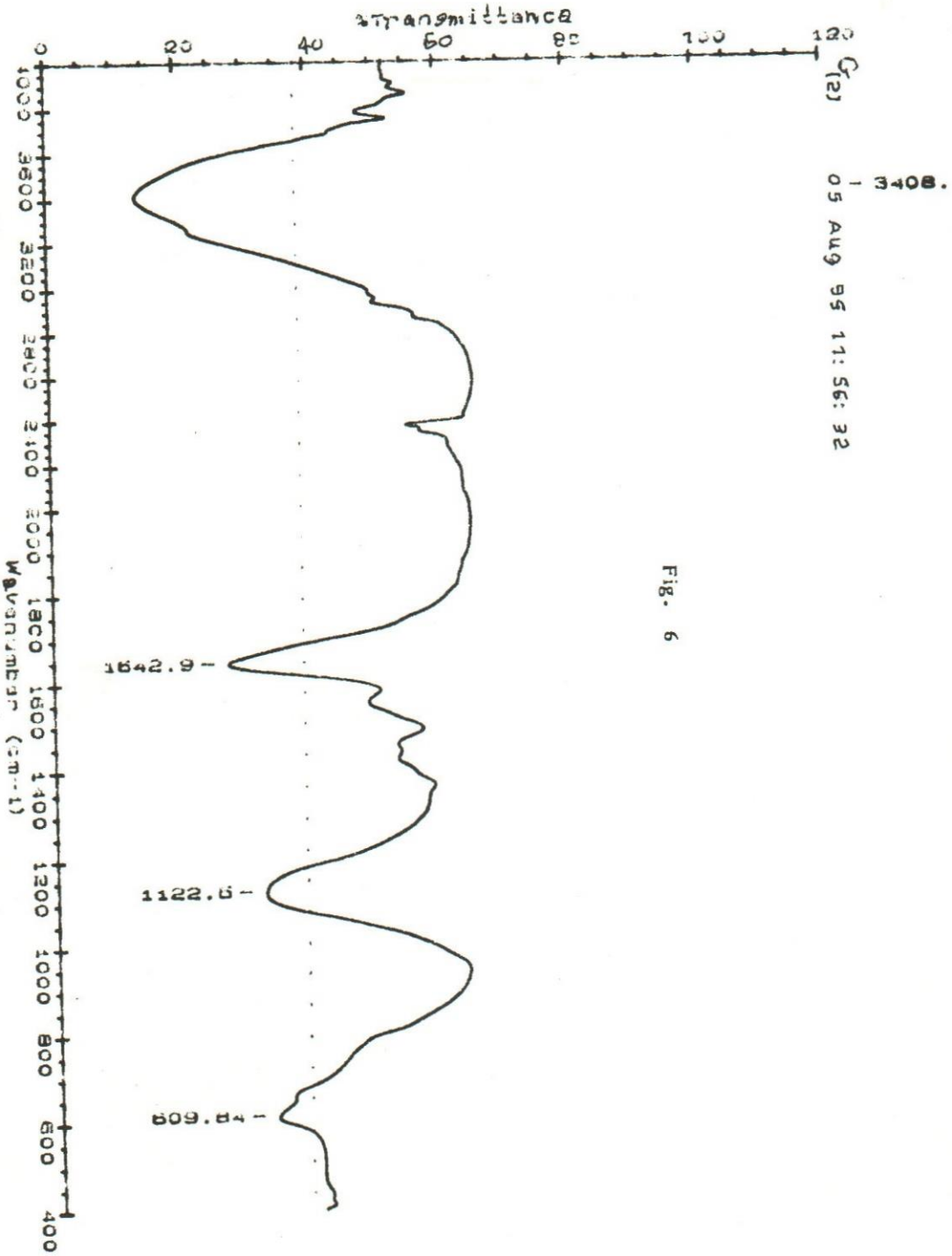


Fig. 5: A photomicrograph of a section of liver of mouse treated with a sublethal dose of extrated crude venom significant increase of glycogen quantities.
G = Glycogen PAS. Scale bar = 0.01 mm.


%Transmittance
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40
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1200
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400
Wavenumber (cm-1)
1642.9
1122.6
609.84
3408.
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Fig. 6