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Possible Protective Role of Ginger Extract on Methomyl Induced Hepatotoxicity in Adult Male Albino Rats

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Abstract- This study aimed to evaluate the possible histological and ultrastructural changes of liver induced by methomyl pesticide exposure and estimation the possible protective effect of ginger extract for hepatic damage in rats. Ginger is used worldwide primarily as a spicy condiment and of the herbal sources of natural protection from contamination and oxidative stress thus play an important role in chemoprevention of liver diseases. Methomyl is one of the most frequently prescribed pesticides, that are used as a pesticide. It is characterized as a highly toxic compound and has been reported to cause multiple organs damage. Fifteen male albino rats were allocated into 5 main groups. (3 rats in each); one served as control and the four remained groups were for different treatments. GC-MS analysis of ginger extract revealed the content of gingerol, quercetin (3.20%), limonene and zingiberene. Treatment of methomyl treated rats with ginger extract maintained serum ALP, AST& ALT levels and reduced the damage effect with protective efficacy against pesticide induced hepatotoxicity, which appears also more effective than its therapeutic application.

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Abstract- This study aimed to evaluate the possible histological and ultrastructural changes of liver induced by methomyl pesticide exposure and estimation the possible protective effect of ginger extract for hepatic damage in rats. Ginger is used worldwide primarily as a spicy condiment and of the herbal sources of natural protection from contamination and oxidative stress thus play an important role in chemoprevention of liver diseases. Methomyl is one of the most frequently prescribed pesticides, that are used as a pesticide. It is characterized as a highly toxic compound and has been reported to cause multiple organs damage. Fifteen male albino rats were allocated into 5 main groups. (3 rats in each); one served as control and the four remained groups were for different treatments. GC-MS analysis of ginger extract revealed the content of gingerol, quercetin (3.20%), limonene and zingiberene. Treatment of methomyl treated rats with ginger extract maintained serum ALP, AST& ALT levels and reduced the damage effect with protective efficacy against pesticide induced hepatotoxicity, which appears also more effective than its therapeutic application. Coadministration of methomyl with ginger extract showed a slight improvement in some hepatocytes that looked normal in the examination but still markedly affected and showing signs of degeneration. Results obtained in this study demonstrated that high doses of methomyl induced histological and ultrastructural changes in the liver and the levels of enzymes were raised. due to oxidative stress and the use of ginger extract had partially improved the toxic effect of methomyl.

Keywords: methomyl, hepatotoxicity and ginger extract.

I. Introduction

Plants have been the major source of drugs for the treatment of various diseases in many ancient systems of medicine in the world. Ginger is an underground rhizomes of plant Zingiber officinale belonging to the family Zingiberaceae which is widely consumed as spice for the flavoring of foods *(Ajith et al., 2007)*. It has been reported that ginger and its extracts possess some pharmacological activities including hypoglycemic, insulinotropic and hypolipidemic in human and in experimental animals *(Kondeit et al., 2005).*

The antioxidants in ginger include gingerols, shogaols, monoterpenes, sequiterpenes, some phenolic derivatives and other phytochemicals which are responsible for their pharmacological activities *(Li et al., 2001).*

Many previous studies investigated the hepatoprotective effects of ginger extract against liver toxicity induced by ethanol, carbon tetrachloride, bromobenzene and acetaminophen with significant decrease in the level of ALT and AST *(Mallikarjuna et al., 2008; El-Sharaky et al., 2009).*

The present work was conducted to study the effect of ginger on the liver tissues& serum profile enzymes.

II. Materials & Methods Experimental Design

Fifteen male albino rats were allocated into 5 main groups. 3 rats in each,

Group 1: served as control were administered 1ml once daily of tween 80, vegetable oil and distilled water.

Group 2: received ginger extract in a dose 1/10 LD_{50} (1250 mg/kg /day orally) *Zaki et al (2022).*

Group 3: animals received methomyl orally in a dose 1/10 LD_{50} (1.25 mg/kg /dayorally) Araki et al (1982).

Groups 4: animals received ginger extract simultaneously with methomyl after two hours in the previous doses.

Group 5: (treatment) received ginger extract for 2 hours after methomyl exposure

The treatments were given for rats for 28 days, then, rats were sacrificed by ether anesthesia and specimens from liver were taken for light and electron microscopic examination. Gas chromatography–mass spectrometry (GC-MS)analysis. The chemical composition of water extract of ginger roots was performed using Trace_ GC Ultra-ISQ mass spectrometer(Thermo-Scientific, Waltham, MA) with a

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direct capillary column TG–5MS (30m_0.25mm_0.25 mm film thickness). The column oven temperature was initially held at 40_C, and then increased by 5_C/min to 280_C. The injector and detector (MS transfer line) temperatures were kept at 250_C. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. Extract derivatization was done using BSTFA/TMCS (80:20, v:v) for 1 h at 70_C, after evaporation to dryness of dichloromethane/methanol mixture. The resulting solution was dried and then dissolved in hexane. The solvent delay was 2 min and diluted samples of 1 ml were injected automatically using Auto sampler AS3000 (Thermo-Scientific, Waltham, MA) coupled with GC in the split less mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source and quadrupole temperatures were set at 200_C and 150_C, respectively. The components were identified by comparison of their retention times (Rt) and mass spectra with those of WILEY 09 (Flavor & Fragrance Natural & Synthetic Compounds) and NIST 11 (National Institute of Standards and Technology, Gaithersburg, MD) Mass Spectral databases.

a) Plant material

The ginger rhizomes were collected from the Faculty of Pharmacy, Heliopolis University for Sustained Development, Medicinal Plant Reserve. It was identified and authenticated by, Medicinal plants branch at the national research center (Latin name: *Zingiber officinale*; plant part: *Rhizome*).

b) Methomyl

Technical grade methomyl (90% active ingredient) was obtained from (Tabouk) Pesticide Company, Egypt.

c) Animals used

Healthy adult male albino rats (120-180 gm) were obtained from laboratory animal breeding unit (Faculty of Medicine, Zagazig University). The rats were kept in metal cages during the whole experimental period under hygienic condition, fed on well balanced ration and provided water ad-libitum, through the experiment. The light system was 12/12 hrs. dark/night cycle. The rats were accommodated to laboratory conditions for two weeks before the experiment.

Aqueous ginger extract was prepared from available ginger (Zingiber officinale) roots. The ginger roots were peeled on crushed ice, and (100 gm) of ginger was cut into small pieces and homogenized in 75 ml of cold, sterile 0.9% NaCl in the presence of crushed ice. The homogenization was carried out in a blender at high-speed bursts for total 15 minutes.

i. *Blood samples collection and preparation of plasma*

The blood was collected by the retina and allowed to clot for 30 min. at room temperature. The clotted blood was then centrifuged at 3500 RCF for 30 min. The serum was separated and stored at -25°C until protein and enzyme analyses were performed.

a. *Determination of alanine amino transferase (ALT) and aspartate amino transferase (AST)*

ALT and AST were determined colorimetrically according to the method of *Reitman, (1957).* The reaction mixture consisted of 1 ml of a mixture of phosphate buffer (pH 7.2), 0.2 mM α-ketoglutaric and 200 mM L-aspartate. Incubate for exactly 30 min., add 1 ml of 0.001 M 2,4-dinitrophenyl hydrazine, wait for at least 30 min., and then 10 ml of 0.4 N NaOH were added. The optical density of the produced brown color is measured after 5 minutes, using a spectrophotometer at 520 nm.

b. *Determination of acid and alkaline phosphatases (ALP)*

The activities of acid and alkaline phosphatases were determined using the method of *Powell and Smith, (1954).*

In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate reacts with 4-aminoantipyrine,and by the addition of potassium ferricyanide, the characteristic brown color is produced. The reaction mixture consisted of 1 ml carbonate buffer (pH 4.5 & 10.4),1ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml sample, and then incubate for exactly 30 min. at 37°C. At the end of incubation period 0.8 ml of 0.5 N NaOH was added to stop the reaction. Then add 1.2 ml of 0.5 N $NaHCO_3$, followed by the addition of 1 ml of 4-aminoantipyrine solution (1 %) and 1 ml potassium ferricyanid (0.5%). The produced color was measured immediately at 510 nm. The enzyme activity is expressed by unit (U),where 1 unit hydrolyze 1.0μ mole of p-nitrophenyl phosphate per minute at 37°C, and pH 10.4.

d) Statistical analysis

The significance differences were determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range tests ($p < 0.05$). Data were subjected to statistical analyses using the software package *Costat® Statistical Software (2005)* a product of Cohort Software, Monterey, California, USA, The values of each measurement of the tested parameters were recorded as mean of five readings i standard error. Statistical analysis was carried out using simple one-way analysis of variance (ANOVA) test, using spss software windows version 17 (SAS, 2001). A probability of $P < 0.05$; and $P< 0.0$ as the level of significance unless stated otherwise. Statistical significant differences among all treatments were carried out by least significant differences (LSD).

III. Results

Chemical composition of GE The GC-MS analysis of GE revealed that it contains phenolics, alkaloids, flavonoids, tannins, anthraquinones,

terpenoid, and steroids. The identified bioactive components of GE are listed in Table 1, with their respective RT and percent composition (area %), where the most important substances are gingerol, quercetin, DL-limonene, ar-curcumene, zingiberene, b-sesquiphellandrene, linalool, pyrazine, and 1,8-terpin hydrate. The major identified compound was the gingerol (7.09%), while from total ion chromatogram (TIC), we note that the flavonoid quercetin has been appeared at multiple RTs with total percent composition of 3.2%.

Table (1): Bioactive components of aqueous extract of ginger roots as determined by using gas chromatography-mass spectroscopy (GC-MS)

Compound name	Molecular formula	Molecular weight	Retention time	Area%
Nerolidol	$C_{15}H_{26}O$	222	29.3	0.05
Lucenin	$C_{27}H_{30}O_{16}$	610	33.75	0.06
Clionasterol	$C_{40}H_{56}$	414	33.55	0.09
Curcumene	$C_{15}H_{22}$	202	28.38	0.09
Cineole	$C_{10}H_{18}O$	154	24.86	0.10
Phenol	$C_9H_{12}O_2$	152	22.88	0.11
Sesquiphellandrene	$C_{15}H_{24}$	204	29.39	0.12
Zingiberene	$C_{15}H_{24}$	204	28.69	0.18
Isochiapin	$C_{19}H_{22}O_6$	348	41.21	0.18
Dihydrostillbene	$C_{16}H_{18}O_4$	275	41.97	0.20
Quercetin	$C_{18}H_{16}O_7$	344	43.03	3.41
Pyrazine	$C_5H_6N_2$	95	8.35	12.71
Hydroxylinalool	$C_{10}H_{18}O_2$	170	23.52	1.15
Terpinhydrate	$C_{10}H_{20}O_2$	171	24.29	1.09
Rosifoliol	$C_{15}H_{24}O$	220	36.34	0.74
Limonene	$C_{10}H_{16}$	140	15.17	0.27
Gingerol	$C_{17}H_{26}O_4$	294	45.17	0.79

Table (10): Effect of tested materials on the liver enzymes esterase of albino rats

	G1 Control	G2 Ginger	G3 Methomyl	G4 Ginger& methomyl	G5 Methomyl & ginger
ALT (U/L)	134.16 ±1.211	124.12 ±5.21	152.15 ±1.379*	142.4 ±1.455**	144.27 ±1.205
AST (U/L)	35.45 ±0.684	40.56* ±5.96	73.32 ±1.215*	43.77 ±0.997**	65.55 ±1.990*
ALP (U/L)	375.16 ±0.921	395.22 ±11.54*	655.65 ±1.655*	511.28 ±1.568*	579.52 ±4.403*

*The values were given as means ± SEM (Standard Error of Mean); n=5, Significant difference levels versus control: *p≤0.05; ** p≤0.01, compared with control.*

The serum enzyme activities in rats fed supplemented with ginger extract; hot and cold for 28 days are given in Table 3. All treated groups showed significant decrease of serum aspartate and alanine aminotransferase, gamma glutamyl transferase and alkaline transferase activities as compared with control group.

The liver enzymes levels were reduced by the same amount after pesticide withdrawal with or without GE treatment, suggesting that GE does not exhibit metal-chelating ability and does not provide hepato protection against methomyl accumulation. Additionally, the markers of liver function remained persistently high, particularly AST, at early exposure periods in rats treated with methomyl, which may be indicative of hepatic damage *(Omobowale et al., 2014)*. GE initially improved transaminases activities (Farag et al., 2010) but failed to restore their activity to normal towards the end of the exposure period. Thus, GE could protect against the early methomyl induced hepatotoxicity. Administration of ginger improved liver function s as it reduced liver enzymatic activities. These results were in accordance with that of *Mallikarjuna et al. (2008)* who showed that administration of ginger ethanolic extract (200 mg/kg) orally from day 15 to day 21 along with country-made Liquor (CML) produced significant lowering of AST, ALT, ALP and tissue lipid peroxide levels. Treatment of ginger significantly decreased serum urea and increased serum creatinine concentration since, ginger contain polyphenols and flavonoids that influence removing certain waste products from plasma. These results agree with *Ajith et al., (2007)* who reported that the

presence of polyphenols and flavonoids in ginger extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea and creatinine levels.

a) Histopathological Work Up

Fixation and tissue processing: The formalin preserved liver, specimen was processed in an automated tissue processor. The processing consisted of an initial 2 step fixation and dehydration. Fixation comprising tissue immersion in 10% buffered formalin for 48 hours, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70% , 90% ,and 100%). The tissue was initially exposed to 70% alcohol for 120 minutes followed by 90% alcohol for 90 minutes and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin, *(Suvarna et al., 2012)* Stained sections were examined for circulatory disturbances, inflammation, degenerations, apoptosis, necrosis, and any other pathological changes in the examined tissues.

b) Histopathologic Finding

i. *Group 1. (Control free)*

Liver: Examined serial sections from liver revealed normal hepatic parenchyma with preserved lobular pattern, portal triades and associated structures (portal vein, hepatic artery, hepatic vein, bilsductiles and lymphatics). Central vein sinusoids and hepatic cords were apparently normal. Hepaticlobules were separated by fine fibrous stromal connective tissue.(Fig.1).

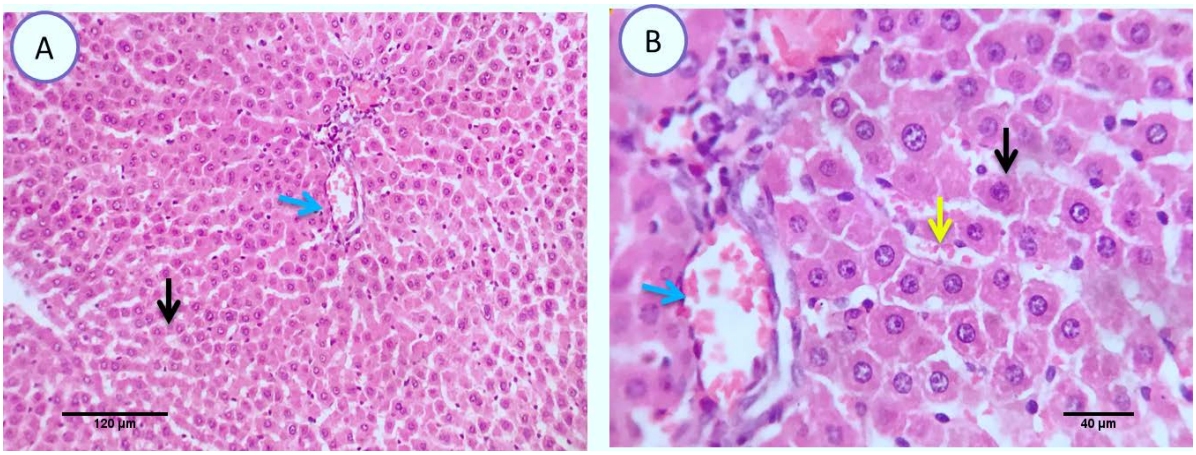

Fig. 1: Photo-micrograph from liver, control group, showing normal hepatic parenchyma with preserved lobular pattern, central veins(blue arrow), Sinusoids and hepatic cords(black arrow) . Scale bars 120, 40 um

ii. *Group 2 Ginger administration*

Liver: Examined serial sections denoted apparently normal hepatic parenchyma, lobular arrangement, portal triads structures, vascular tributaries and biliary tree, however some sections showed vascular dilatation and mild portal round cells aggregation. (Fig.4).

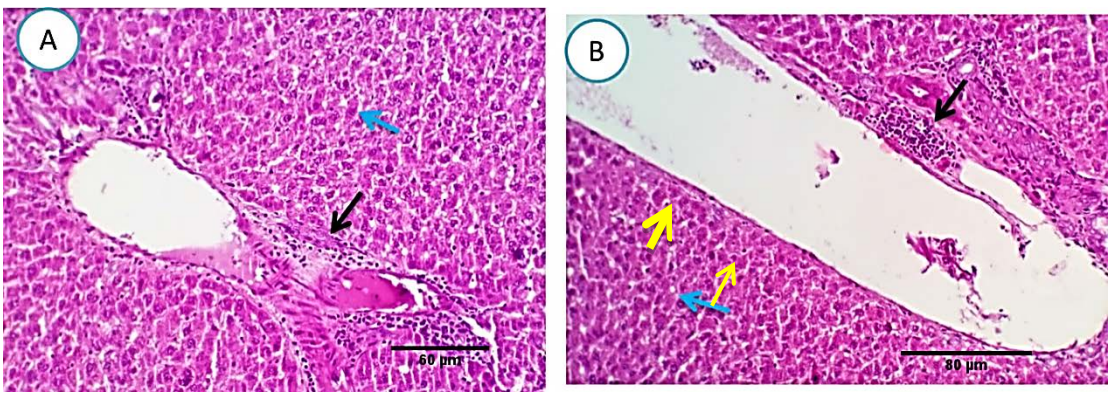

Fig. 2: Photomicrograph of liver (Group .G) showing normal hepatic parenchyma(blue arrows), vascular dilatation(yellow arrows)and mild portal round cells aggregation(black arrows). Scale bars 60, 80 um

Here, as a proof of concept, we used a mouse model to show that orally from ginger extracts resulted in protecting mice against methomyl-induced liver damage.

c) *Group3 (Methomyl pesticide)*

Liver: Examined sections from liver of this group denoted moderate portal biliary proliferation, congestion of portal blood vessels, round cell infiltration, multifocal interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches beside degenerative changes in a few hepatocytes. (Fig.4).

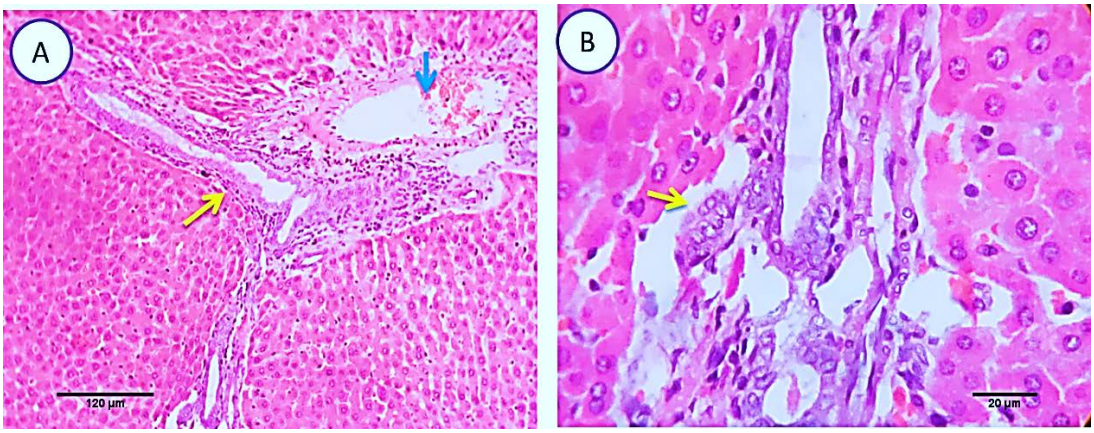

Fig. 3: Photo-micrograph from liver, group (2), showing, portal biliary proliferation (yellow arrow), congestions of portal blood vessels (blue arrow) and round cell infiltration beside degenerative changes in a few hepatocytes. Scale bars 120, 20 um.

d) *Group 4 (Ginger protection followed by methomyl administration)*

Liver: Sections from liver of this group denoted moderate portal biliary proliferation, congestion of portal blood vessels, round cell infiltration, multifocal interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches beside degenerative changes in a few hepatocytes. (Fig.10)

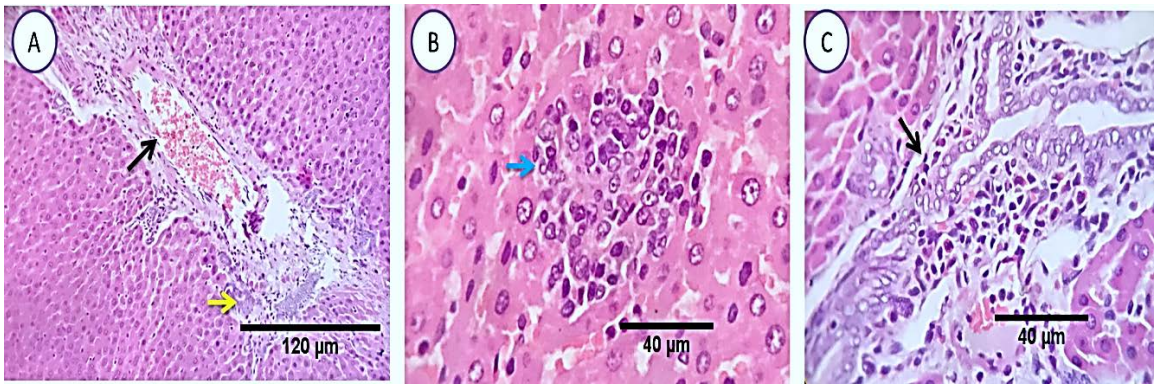

Fig. 4: Photo-micrograph from liver, group (4), showing, portal biliary proliferation (A, yellow arrow, B, black arrow), congestions of portal blood vessels(A, black arrow), round cell infiltration and interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches (B, blue arrow). Scale bars 120, 40, 40 um.

e) *Group 5 (Ginger treatment after methomyl administration)*

Liver: Examined sections from liver of this group denoted moderate portal biliary proliferation, congestion of portal blood vessels, round cell infiltration, multifocal interstitial lymphocytic and macrophages aggregations replacing previous necrotic patches beside degenerative changes in a few hepatocytes.(Fig.7).

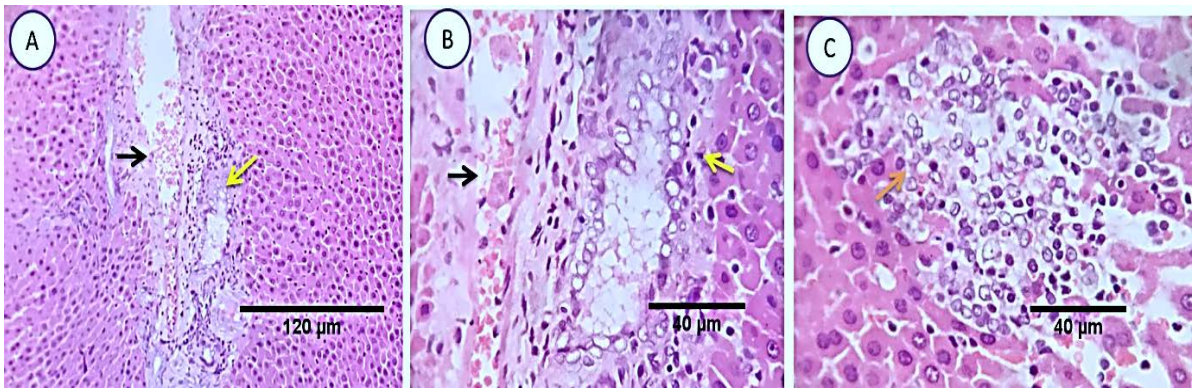

Fig. 5: Photo-micrograph from liver, group (3), showing, portal biliary proliferation (yellow arrow), congestions of portal blood vessels (black arrow) and round cell infiltration and interstitial lymphocytic and macro-phages aggregations replacing previous necrotic patches (orange arrow). Scale bars 120, 40, 40 um.

IV. Conclusion

Ginger extract appear to be highly effective in improving the toxic effects caused by pesticide, and the use of ginger was beneficial in lowering liver enzymes and has hepato protective effect.

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