

POSSIBLE HEPATOTOXIC EFFECTS IN ADULT MALE ALBINO RATS ON COMBINATION OF IVERMECTIN AND PARACETAMOL DRUGS USED IN COVID-19 INFECTION MANAGEMENT PROTOCOL

BY

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ABSTRACT

Introduction: Ivermectin (IVM) and paracetamol (APAP) are among drugs used for treatment of mild and moderate cases of covid-19 infection. **Aim:** this study aimed to evaluate the potential hepatotoxic effects on combination of both Paracetamol and Ivermectin in adult male albino rats. **Methodology:** fifty adult male albino rats were divided into control groups (negative and Positive) and IVM and APAP treated group which included thirty rats and subdivided equally into 3 subgroups (III A, B, C). Each rat received orally 3.7mg/kg/day of Ivermectin concomitantly with 370 mg/kg /day of paracetamol (groups III A, B, C were sacrificed after 7 days, 14 days and 28 days respectively). Serum levels of aminotransferases ALT, AST, glutamate dehydrogenase (GLDH) and Hyaluronan (HA), Histopathological examination of liver and immunohistochemical staining for inducible nitric oxide synthase (iNOS) were performed. **Results:** HA was elevated after 7 days followed by GLDH which increased after 14 days while aminotransferases levels increase only after 28 days of combined IVM and APAP treatment. After 7 days, the histopathological changes in IVM and APAP treated group was restricted to congestion of central vein with some vaculation in the hepatocytic cytoplasm then aggravated with increased duration of treatment to periportal fibrosis, inflammatory infiltration areas and necrotic foci. **Conclusion:** Combination of IVM and APAP induced liver injury, detected 7 days after treatment by HA level and accompanied by histopathological changes which became more severe with increasing duration of treatment. IVM and APAP should be used cautiously to avoid possibility of aggravation of liver injury which is believed to be one of covid-19 complications.

Keywords: Ivermectin, Paracetamol, Hepatotoxic, Covid-19, Hyaluronan.

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INTRODUCTION

The year 2020 was demarcated by COVID-19, the worst pandemic in the past 100 years. COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had a major impact on human health globally; infecting about 75 million people causing 1.6 million deaths worldwide and associated with long-term health sequelae. Covid -19 has disrupted nearly every aspect of life, including work, education, trade, travel, sports, routine healthcare services and social activities. This had a great impact on people physical and mental health (**WHO, 2020a**). According to WHO reports, the estimated confirmed cases of COVID-12 in Egypt from Jan. 2020 to June, 2021 were 276,756 with 15,829 deaths (**WHO, 2021**).

Since the emerging of this pandemic, researchers and doctors have searched for medications and vaccines to treat covid 19. Although there is no specific curative treatment till now, many drugs such as tocilizumab, heparin, remdesivir, chloroquine and ivermectin have been introduced in the treatment protocols. Also, symptomatic treatment for fever and pain relief by paracetamol as a safer drug rather than NSAID has been introduced in almost all treatment protocols (**NIH, 2021**). In Egypt, **Ministry of Health** protocol included ivermectin and paracetamol for treatment of mild cases which required only isolation and treatment at home and in moderate cases which may require hospitalization. (**MOHP, 2020**). Ivermectin is a Food and Drug Administration (FDA) approved antiparasitic drug used for treatment of onchocerciasis, helminthiases, and

scabies where it is well tolerated in such conditions (Yang *et al.*, 2020). On the other hand, it is not FDA approved for treatment of viral infections since pharmacokinetic and pharmacodynamics studies stated that a 100-fold higher doses than those approved for use in humans is needed to get the antiviral efficacy (Chaccour *et al.*, 2020). However, in vitro studies suggested that ivermectin inhibit the replication of SARS-CoV-2 in cell cultures (Caly *et al.*, 2020) and interfere with the attachment of (SARS-CoV-2) spike protein to the human cell membrane (Lehrer and Rheinstein, 2020). Although, insufficient clinical trials or observational studies to confirm the clinical effectiveness of ivermectin for COVID-19 treatment (NIH, 2021), it was proposed in some treatment protocols including that of Egypt where it is used as one of the treatment and prophylactic drugs (Hellwig and Maia, 2021) against covid19 (MOHP, 2020).

Paracetamol is recommended as a safe drug for symptomatic treatment of pain and fever in COVID-19 at a dose of 1 gm/6 hr with a maximum of 4 gm/day since any higher dose can be dangerous if not advised (WHO, 2020 b). However, it can be toxic to the liver especially when used with some other medications or in patients with existing liver problems, malnourished, underweight or old age. Although paracetamol is one of over the counter medications, medical consultation and following packaging instructions are important on use to avoid toxicity (FDA, 2015).

Some studies reported that liver injury associated with administration of therapeutic doses of ivermectin and for short durations (3-7 days) such as in mild and moderate cases of covid-19 is mild and self-limited (Veit *et al.*, 2006 and Hanafy & Abd-Elsalam, 2020). However, no enough studies have been carried out to evaluate risk of toxicity of both ivermectin and paracetamol when used in combination as in some protocols of covid-19 treatment especially that home management of such drugs doesn't guarantee use of therapeutic doses and and/or recommended duration.

In case of suspected drug-induced liver injury (DILI), biomarkers can be used to confirm drug causality, the type of DILI, the severity of liver damage and its prognosis (Meunier and Larrey, 2019). Alanine and aspartate aminotransferases (ALT and AST, respectively) are the most commonly used hepatic injury biomarkers. However, they have low prognostic utility which led to emergence of new and early biomarkers of liver injury (McGill, 2016).

Glutamate dehydrogenase (GLDH) is a protein that is embedded in the matrix of the mitochondria (Schmidt and Schmidt, 1988). It is abundant in the liver (Braakman *et al.*, 1991 and Kasarala & Tillmann, 2016) and has advantage over ALT in terms of liver specificity because extrahepatic sources of GLDH are negligible when compared with those of ALT which can be elevated in cases of cardiac and skeletal injury. (Mastorodemos *et al.*, 2005)

Hyaluronan (HA) is a straight-chain glycosaminoglycan polymer of the extracellular matrix (ECM). The liver is the most important organ involved in the synthesis and degradation of HA. Circulating HA occur very early during periods of rapid cell turnover following liver injury. Serum HA are often used clinically to assess liver function and necro inflammatory injuries in parallel with liver biopsy since increased HA provides a rapid response survival mechanism following acute liver damage and considered a very early markers of toxic hepatic injury (George and Stern, 2004 & Rostami and Parsian, 2013)

Inducible nitric oxide synthase (iNOS) is an enzyme that controls production of nitric oxide. The expression of this enzyme is increased by endotoxins, a variety of inflammatory mediators as cytokines, and disturbances in the cellular environment. This enzyme can be used as an efficient indicator and good monitor of tissue damage. iNOS is involved in pathological inflammation at different organs including the liver (Bachmann *et al.*, 2017)

Clinical studies revealed that liver injury is associated with Covid 19 and is related to its severity (Zhang *et al.*, 2020). So, prevention of

the mechanisms underlying pathogenesis of liver injury are urgently required for COVID-19 patients (Xu *et al.*, 2020). Direct viral cytotoxicity, uncontrolled cytokine storm and/or drug-induced toxicity have been introduced as possible underlying pathogenesis of covid 19 induced hepatic affection (Wu *et al.*, 2020).

The aim of this work was to evaluate the possible hepatotoxic effects in adult male albino rats on combination of both paracetamol and ivermectin drugs which are used in treating mild and moderate cases of covid 19 infection according to Egyptian ministry of health protocol through biochemical assessment of aminotransferases (ALT, AST), Glutamate Dehydrogenase (GLDH), Hyaluronan (HA), histopathological, immunohistochemical and morphometric studies of the liver

MATERIAL AND METHODS

• **Materials:**

I- Chemicals:

Iversine tablets, each containing 6 mg of ivermectin and manufactured by Uni pharma company, Egypt. Paracetamol tablets each containing 500 mg acetaminophen and manufactured by El-Nasr pharmaceutical chemicals Company (ADWIC). Both drugs purchased over the counter from retail pharmacies.

II- Animals:

Fifty adult male albino rats, weighing between 180-200 grams were obtained from the Animal House of the Faculty of Medicine, Zagazig University. All animals were treated in compliance with the Animal Care Guidelines and Ethical Regulations in 'The Guide for the Care and Use of Laboratory Animals (ILAR, 2011). The study procedures were approved by the institutional animal care and use committee, Zagazig University (ZU-IACUC/3/f/177/2021). Two weeks before the experiment, the rats were subjected to passive preimination for acclimatization to their new environment and to assess their wellbeing and exclude the diseases rats. They were fed with a standard chow diet in equal amounts to all animals in each cage and

water was offered in separate clean containers. The animals were kept in separate plastic cages free from any source of chemical pollution under controlled circumstances with an ambient temperature range of 22 ± 2 °C, relative humidity of $50 \pm 5\%$ and a 12 hr light-cycle. Soft wood shavings were used for bedding and changed during the washing of cages every other day to keep animals clean. Over crowding and isolation were avoided. Wearing Protective clothing as gloves, laboratory coat, masks, protective eye wear, using sterile oral gavage needle and avoiding eating or drinking when dealing with the animals were adopted.

• **Experimental design:**

The rats were divided into three groups as follows: Group I (negative control): 10 rats, each rat received water and regular diet to measure the basic parameters of the experimental animals. Group II (Positive control group): 10 rats, each rat received 5 ml of normal saline 0.9% (solvent of paracetamol) once daily by oral gavage. Group III (ivermectin and paracetamol treated group): thirty rats were subdivided equally into 3 groups (group IIIA, BandC). Each rat received ivermectin dissolved in distilled water (Lytvynets *et al.*, 2010) at a dose of 3.7mg/kg/day concomitantly with paracetamol dissolved in 5ml saline (Iyandaand Adeniyi, 2011) at a dose of 370mg/kg /day. Group III A was sacrificed after 7 days, group III B was sacrificed after 14 days and group III C was sacrificed after 28days (Colerangle, 2017).

These doses are equivalent to the human dosing regimen of the Egyptian protocol for management of mild and moderate cases of covid 19 which are 36mg/day of ivermectin and 3000- 4000mg /day of paracetamol. It was calculated using body surface area conversion equation {animal dose (mg/kg) = human equivalent dose (mg/kg) x 6.2}, assuming that an adolescent person weighs 60 kg (Reagan-Shaw *et al.*, 2008). The calculated doses of ivermectin is within the range of 1/10 of oral LD50 in rats(10-50mg/kg) as reported by Campbell *et al.*, (1983) and Lankas *et al.*,

(1989). Also, the calculated dose of paracetamol equals 1/10 of oral LD50 in rats (3.7g/kg as reported by *Boyd and Berezcky (1966)*).

• **Methodology:**

Whole blood samples were collected from retro-orbital plexuses of animals, centrifuged at 3000 x g for 10 minutes at room temperature and then serum samples were collected and kept frozen at -80°C until analysis at the medical biochemistry department, Faculty of medicine, Zagazig university. Immediately after blood collection, animals were sacrificed and the livers were dissected and weighed. Liver tissue samples were frozen in liquid nitrogen and kept in a -80°C for histopathological and immunohistochemical examination.

I. Biochemical study:

Serum levels of aminotransferases ALT, AST were measured using commercially available Elisa kits (Olympus Corp, Hamburg, Germany) by enzyme-colorimetric methods in an Olympus AU600 auto analyzer (*Kutlu et al., 2007*). Serum levels of GLDH were measured by using commercially available kits of Rat Glutamate Dehydrogenase (GLDH) ELISA Kit cat no. MBS3807923 according to manufacturer protocol. Serum levels of Hyaluronan was determined using HA ELISA based micro titer plate procedures according to *Fosang et al., 1990 and Frost & Stern, 1997*.

II. Histopathological study:

The hepatic lobe was preserved in 10% neutral buffered formalin embedded in paraffin, sectioned with 5 µm thickness and then stained with hematoxylin and eosin (H&E) and Mallory's trichrome (*Bancroft and Gamble, 2007*), examined and photographed under light microscope at Anatomy department, Faculty of Medicine, Zagazig University.

III. Immunohistochemical study:

Paraffin-embedded liver sections (5 µm) were applied in the detection of inducible nitric oxide synthase (Inos). At histology department, Faculty of Medicine, Menufeya university; sections were dewaxed in xylene for about 20 minutes then transferred to subsequent

descending grades of ethanol. After that they were immersed in preheated target retrieval solution for 40 minutes and then removed to cool for 20 minutes. The sections were immersed in 3% H2O2 in deionized water for 10 minutes to quench nonspecific endogenous peroxidase activity. For blocking of nonspecific binding sites, the sections were then incubated with 10% normal rabbit serum for 10–15 min for two hours at room temperature. Then the sections were incubated with polyclonal rabbit anti-Inos, which is specific for iNOS enzyme in mouse and rat (M-19/Sc 650, Santa Cruz Biotechnology) at dilution of 1:25 followed by incubation by biotinylated goat-antirabbit IgG for 10–15 min at room temperature. Two drops of Streptavidin labeling reagent were added to the sections for 10 minutes. The detection of antigen antibody reaction was demonstrated by the addition of diaminobenzidine (DAB) chromogen for 10 minutes. After rinsing the sections were counter-stained with hematoxylin. Positive reaction was recognized as Brown-yellow granules in the cytoplasm (*Atik et al., 2008*).

IV- Morphometric study:

The image analyser computer system was used in this work (using the software Fiji Image J program (1.51n, NIH, USA) aiming to measure area percentage % of collagen fibers in the Mallory's trichrome sections, area percentage % of iNOS immune reaction in the iNOS immunohistochemical section and optical density of iNOS immunoreaction. Values for each group were obtained from 5 different non overlapping fields from different slides. The measurements were performed at magnification 400x via using Leica Q500 Image analysis computer System (Leica Q500) at Anatomy department, Faculty of Medicine, Zagazig University.

STATISTICAL ANALYSIS

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Shapiro-Wilk test was used to verify the normality of distribution of variables, ANOVA was used for comparing the four studied groups and followed

by Post Hoc test (Tukey) for pairwise comparison. For all comparison $P < 0.05$ is considered as significant difference.

RESULTS

Comparison between negative control group and positive control group showed non-significant difference regarding the parameters included in the study so, negative control group was used for comparison with different treated groups.

• **Biochemical parameters:**

Regarding ALT and AST, no significant differences were detected when group III (A) or group III (B) were compared with control group ($p < 0.05$). On the other hand a significant increase in group III (c) was found on comparing with control group as regard ALT and AST. A significant increase in the mean values of ALT was found when group III (c) was compared with either group III (A) or

group III (B) with no significant difference was detected between different treated groups regarding AST. Significant increases in the mean values of GLDH in group IIIB and group IIIC were detected on comparison with the mean values of control group and group IIIA ($p < 0.05$). Also, there was a significant increase in mean values of GLDH in group IIIC when compared with group IIIB ($p < 0.001$). On assessment of hyaluronan, there were significant increase in the mean values of hyaluronan level in treated groups (IIIA, B, C,) when compared with control group ($p < 0.05$). Also, there was a significant increase in the mean values of hyaluronan level in group IIIB when compared with group IIIA ($p < 0.05$) with group IIIC showed significant increase when compared with both groups IIIA and B ($p < 0.05$) (table 1).

Table (1): Comparison between the studied groups according to biochemical parameters

Biochemical parameters	Group I (control group) (n=10)	Group III (A) Ivermectine and paracetamol treated group After 7 days (n=10)	Group III (B) Ivermectine and paracetamol treated group After 14 days (n=10)	Group III (C) Ivermectine and paracetamol treated group After 28 days (n=10)	F	p
ALT (IU/L)						
Min. – Max.	51.0 – 64.0	52.0 – 66.0	54.0 – 68.0	56.0 – 73.0	6.66	0.001*
Mean ± SD.	58.30 ± 4.37	58.70 ± 4.67	60.10 ± 4.98	66.90 ± 5.55	4*	
Median (IQR)	59.50 (55.0 – 61.0)	58.50 (55.0 – 63.0)	59.0 (56.0 – 64.0)	68.50 (63.0 – 71.0)		
pcontrol		0.998	0.845	0.002*		
Sig. bet. grps.		$p_1=0.919, p_2=0.003^*, p_3=0.019^*$				
AST (IU/L)						
Min. – Max.	61.0 – 79.0	65.0 – 82.0	68.0 – 83.0	69.0 – 87.0	4.41	0.010*
Mean ± SD.	71.50 ± 5.62	75.20 ± 5.45	77.30 ± 4.92	80.20 ± 5.98	9*	
Median (IQR)	73.50 (68.0 – 75.0)	76.0 (72.0 – 80.0)	79.0 (77.0 – 80.0)	81.50 (75.0 – 86.0)		
pcontrol		0.447	0.105	0.006*		
Sig. bet. grps.		$p_1=0.829, p_2=0.196, p_3=0.645$				
GLDH (IU/L)						
Min. – Max.	5.40 – 7.30	5.80 – 7.50	8.90 – 17.70	15.20 – 23.60	86.9	<0.001*
Mean ± SD.	6.51 ± 0.64	6.89 ± 0.54	11.68 ± 2.82	19.55 ± 2.88	54*	
Median (IQR)	6.45 (6.0 – 7.10)	7.0 (6.50 – 7.30)	10.80 (9.3 – 13.20)	19.85 (16.9 – 21.6)		
Pcontrol		0.976	<0.001*	<0.001*		

Biochemical parameters	Group I (control group) (n=10)	Group III (A) Ivermectine and paracetamol treated group After 7 days (n=10)	Group III (B) Ivermectine and paracetamol treated group After 14 days (n=10)	Group III (C) Ivermectine and paracetamol treated group After 28 days (n=10)	F	p
Sig. bet. grps.		p ₁ <0.001*, p ₂ <0.001*, p ₃ <0.001*				
Hyaluronan (Ug/ml)						
Min. – Max.	275.0 – 622.0	1530.0 – 2890.0	2070.0 – 3840.0	2780.0 – 4730.0	89.3	<0.001*
Mean ± SD.	450.90 ± 132.15	2220.0 ± 441.26	3026.5 ± 586.1	3856.7 ± 625.6	36*	
Median (IQR)	465.0(340.0–574.0)	2230.0(1860.0–2530.0)	3145.0(2530.0–3450.0)	3897.5(3540.0–4430.0)		
Pcontrol		<0.001*	<0.001*	<0.001*		
Sig. bet. grps.		p ₁ =0.004*, p ₂ <0.001*, p ₃ =0.003*				

F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using **Post Hoc Test (Tukey)**

p: p value for comparing between the studied groups

*: Statistically significant at $p \leq 0.05$

p control: p value for comparing between **Control** and each other groups

p₁: p value for comparing between **Group III (A)** and **Group III (B)**

p₂: p value for comparing between **Group III (A)** and **Group III (C)**

p₃: p value for comparing between **Group III (B)** and **Group III (C)**

• **Histopathological parameters:**

I-Light microscopic results:

a- HandE stain results:

The Hand E stained sections from negative and positive control groups exhibited normal characteristic features of hepatic parenchyma where the well-formed hepatic cords were radiating from the thin walled central vein. Small sinusoidal spaces appeared in between the hepatic cords and containing few amount of kupffer cells. The hepatocytes exhibited central located rounded vesicular eosinophilic nuclei with prominent nucleoli and acidophilic cytoplasm. Few hepatocytes were doubly nucleated (**Fig. 1A**). Normal portal area was observed containing portal triads; branch of the portal vein with thin lining endothelium, bile ductules lined by simple cuboidal epithelial and branch of the hepatic artery (**Fig. 1B**). Liver sections in group III A (IVM and APAP treated group for 7 days) showed normal characteristic histological features of hepatic parenchyma except for congestion of central and portal veins, some hepatocytes show vacuolated cytoplasm with dark stained nuclei (arrow) and double nucleated hepatocytes appeared (Fig. 1 C, D). Liver parenchyma sections from group III B (IVM and APAP treated group for 14

days) showed congestion of portal veins. Most of hepatocytes appeared normal except for few amount that had vacuolar cytoplasmic degeneration with dark stained nuclei. Some doubly nucleated hepatocytes were observed. The kupffer cells that lining the blood sinusoid showed a noticeable proliferation. Dilatation and distortion of the congested central vein lining epithelium, localized area of mononuclear cell infiltration in addition to dark stained nuclei of some hepatocytes and increase the number of proliferated kupffer cells were detected (**Fig. 2 A, B**)

The hepatic parenchyma in group C (IVM and APAP treated group for 28 days) showed marked congestion and dilatation of central vein, hepatocytes appeared with pyknotic dark stained nuclei with basophilic cytoplasm. Apparent increase in the number of doubly nucleated hepatocytes cells and proliferation of kupffer cells were observed. Congestion and dilatation of portal vein. Thick periportal fibrosis and mononuclear cellular inflammatory infiltrations with fibrosis surrounding the proliferated bile ductules were also noticed in the portal area. Necrotic foci with vacuolation and loss of the cytoplasmic organelles were also detected (**Fig. 2 C, D, E, F**).

b- Mallory's trichrome stain results:

Sections of rat liver from control group and group IIIA (IVM and APAP treated group for 7days) showed thin layer of blue colored collagen fibers around the central vein, in the portal area and also in the lining of blood sinusoid (**Fig 3. A, B, C, D**). On the other hand group III B (IVM and APAP treated group for 14 days) showed few blue colored collagen fibers around the central and portal veins (PV) and in the lining of the blood sinusoid (**Fig.3 E, F**) which increased in group III C. (IVM and APAP treated group for 28 days) (**Fig3. G, H**)

II-Immunohistochemical results of iNOS

A negative immune reaction for iNOS was detected in control groups and group III A (IVM and APAP treated group for 7 days) (**Fig4 A, B**). In the group III B (IVM and APAP treated group for 14 days) scattered hepatocytes showed positive immune reactions for iNOS. This reaction appeared as

yellowish brown cytoplasmic coloration (Fig.4C). While more positive cells appeared in group III C (IVM and APAP treated group for 14 days) (Fig. 4D).

III- Morphometric results:

Groups III (B) and Group III (C) showed significant increase in area % of collagen when compared with that of both control group and Group III (A) ($P < 0.05$). Also, area % of collagen was significantly increased in Group III (C) when compared with Group III (B) ($p < 0.05$). The mean values of area percentages (area %) and optical density of iNOS showed significant increase ($P < 0.05$) in treated groups IIIB and C when compared with that of both control group and treated group IIIA. Also, a significant increase in the mean value of area % of iNOS in treated group IIIc was detected when compared with treated group IIIB ($p < 0.05$) (Table 2).

Table 2: Comparison between the studied groups according to histopathological findings

Parameters	Group I (control group) (n=10)	Group III (A) Ivermectine and paracetamol treated group After 7 days n=10	Group III (B) Ivermectine and paracetamol treated group after 14 days n=10	Group III (C) Ivermectine and paracetamol treated group after 28 days n=10	F	P
	Mean ± SD					
Area % of collagen fibers	1.91 ± 0.64	1.97 ± 0.65	2.76 ± 0.67 ^a	9.55 ± 1.08 ^{a,b,c}	222.3861	0.000*
Area % of iNOS	0.023 ± 0.001	0.033 ± 0.002	2.579 ± 0.558 ^{a,b}	7.204 ± 1.476 ^{a,b,c}	183.9344	0.000*
Optical density of iNOS	0.093 ± 0.006	0.098 ± 0.007	0.320 ± 0.124 ^{a,b}	0.417 ± 0.040 ^{a,b,c}	61.9315	0.000*

F: F for ANOVA test, Pairwise comparison bet. each 2 groups were done using Post Hoc Test ((Tukey)

*p: p value for comparing between the studied groups * $p < 0.05$*

^a: $p < 0.05$ vs control group, ^b: $p < 0.05$ vs group III A, ^c: $p < 0.05$ vs group III B

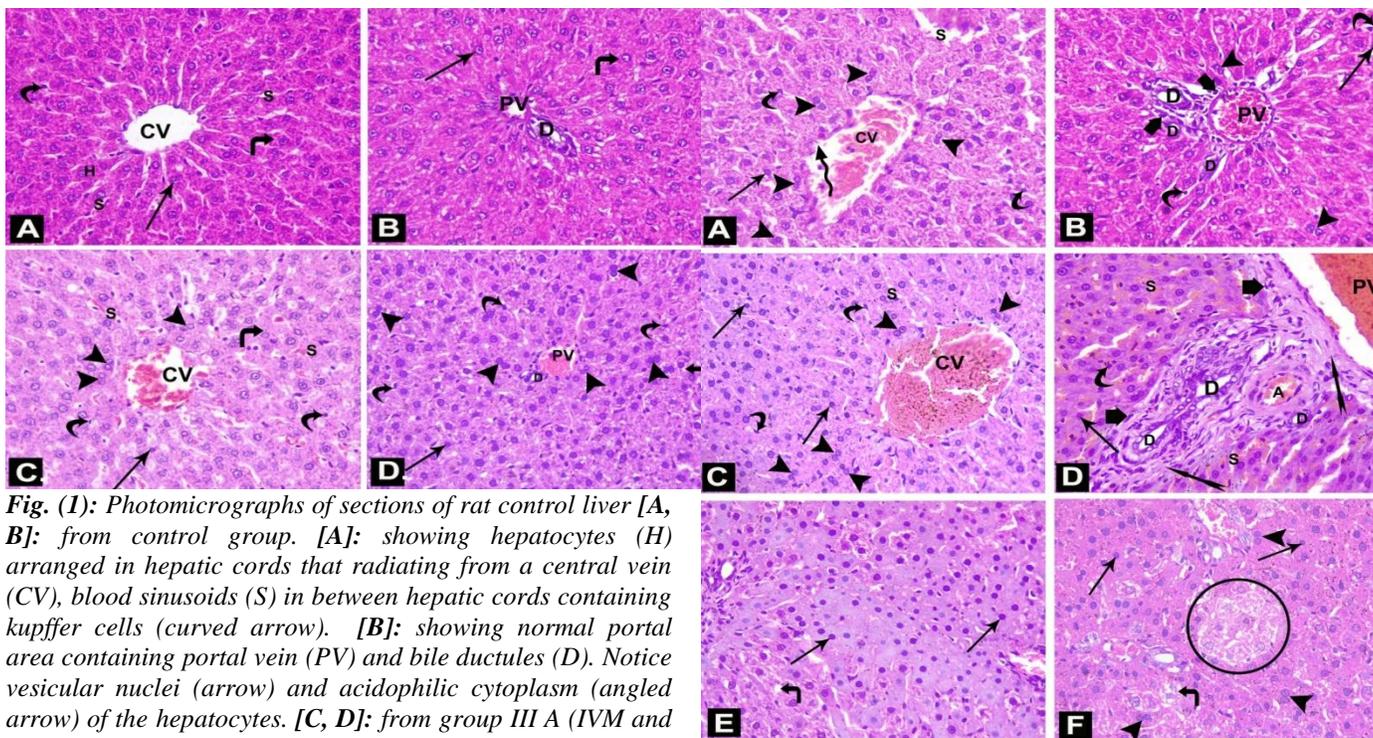


Fig. (1): Photomicrographs of sections of rat control liver [A, B]: from control group. [A]: showing hepatocytes (H) arranged in hepatic cords that radiating from a central vein (CV), blood sinusoids (S) in between hepatic cords containing kupffer cells (curved arrow). [B]: showing normal portal area containing portal vein (PV) and bile ductules (D). Notice vesicular nuclei (arrow) and acidophilic cytoplasm (angled arrow) of the hepatocytes. [C, D]: from group III A (IVM and APAP treated group for 7days) [C]: showing dilated congested central vein (CV), most of normal hepatocytes with acidophilic cytoplasm (angled arrow), dark stained nuclei (arrow). The double nucleated hepatocytes (arrow head) are apparently increased. [D]: hepatic cords containing kupffer cells (curved arrow), congested portal vein (PV), hepatocytes with vacuolated cytoplasm (angled arrow), dark stained nuclei (arrow), double nucleated hepatocytes (arrow head) H&E (X 400).

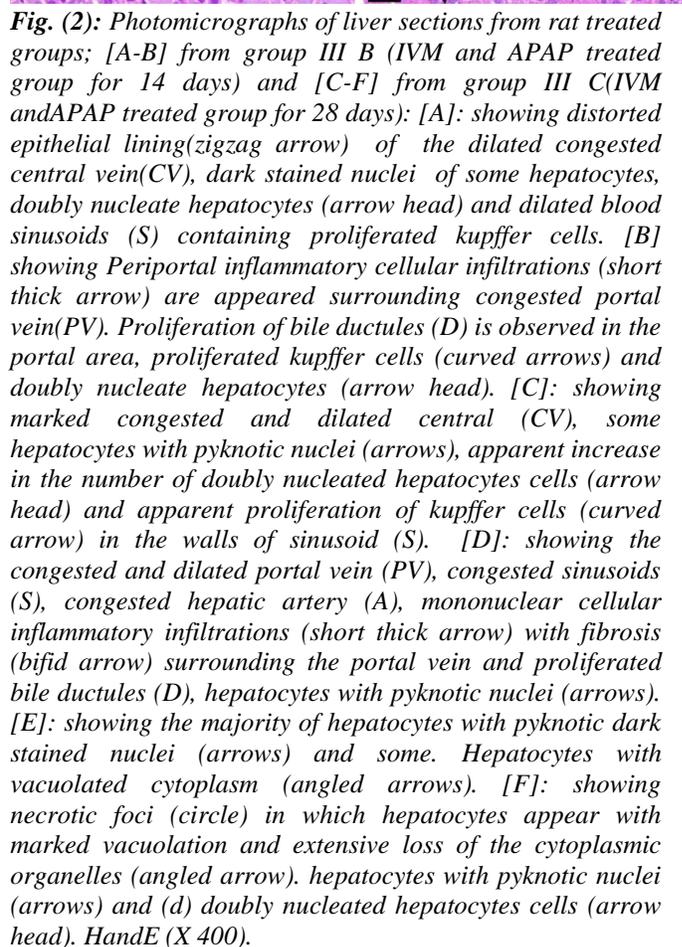


Fig. (2): Photomicrographs of liver sections from rat treated groups; [A-B] from group III B (IVM and APAP treated group for 14 days) and [C-F] from group III C (IVM and APAP treated group for 28 days): [A]: showing distorted epithelial lining (zigzag arrow) of the dilated congested central vein (CV), dark stained nuclei (arrow) of some hepatocytes, doubly nucleate hepatocytes (arrow head) and dilated blood sinusoids (S) containing proliferated kupffer cells. [B] showing Periportal inflammatory cellular infiltrations (short thick arrow) are appeared surrounding congested portal vein (PV). Proliferation of bile ductules (D) is observed in the portal area, proliferated kupffer cells (curved arrows) and doubly nucleate hepatocytes (arrow head). [C]: showing marked congested and dilated central (CV), some hepatocytes with pyknotic nuclei (arrows), apparent increase in the number of doubly nucleated hepatocytes cells (arrow head) and apparent proliferation of kupffer cells (curved arrow) in the walls of sinusoid (S). [D]: showing the congested and dilated portal vein (PV), congested sinusoids (S), congested hepatic artery (A), mononuclear cellular inflammatory infiltrations (short thick arrow) with fibrosis (bifid arrow) surrounding the portal vein and proliferated bile ductules (D), hepatocytes with pyknotic nuclei (arrows). [E]: showing the majority of hepatocytes with pyknotic dark stained nuclei (arrows) and some. Hepatocytes with vacuolated cytoplasm (angled arrows). [F]: showing necrotic foci (circle) in which hepatocytes appear with marked vacuolation and extensive loss of the cytoplasmic organelles (angled arrow), hepatocytes with pyknotic nuclei (arrows) and (d) doubly nucleated hepatocytes cells (arrow head). HandE (X 400).

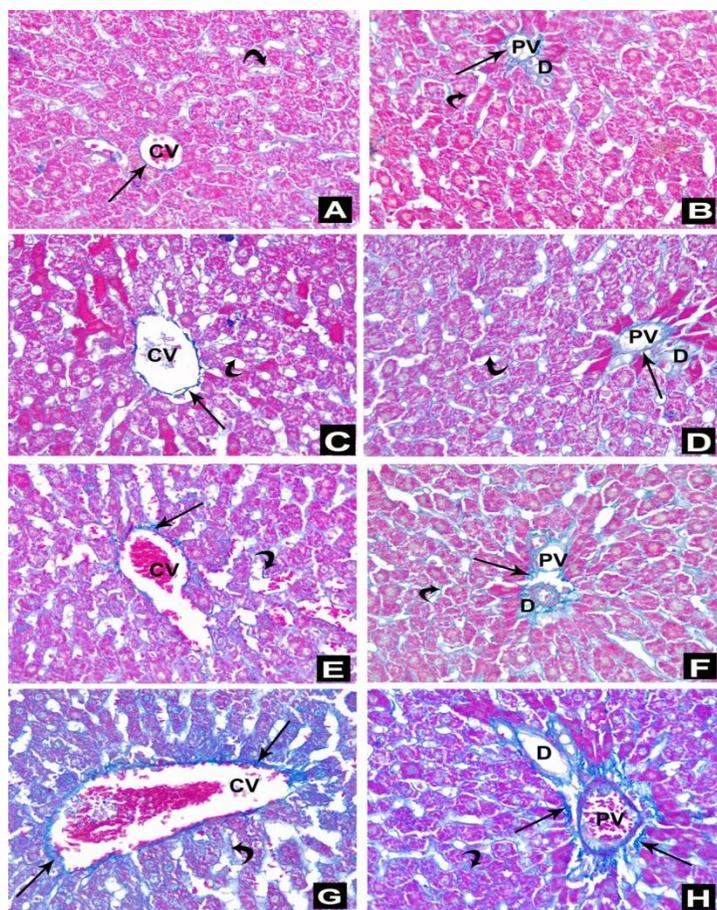


Fig. (3): Photomicrographs of sections of rat liver [A, B]: from control group and [C, D] from group IIIA (IVM and APAP treated group for 7days) showing thin layer of blue colored collagen fibers (arrow) around the central vein (CV), in the portal area (PV) (arrow) and also in the lining of blood sinusoid (curved arrow). [E, F] from group III B (IVM and APAP treated group for 14 days): showing few blue colored collagen fibers (arrow) around the central vein (CV), around portal vein (PV) and in the lining of the blood sinusoid and [G, H] from group III C. (IVM and APAP treated group for 28 days): showing increased blue colored collagen fibers (arrow) around the central vein (CV), in the portal area (PV) (arrow) and also in the lining of blood sinusoid (curved arrow) (Mallory stain X400).

DISCUSSION

Quispe-Cañari, 2021 assessed self-medication practices during COVID-19 and reported prevalence of self-medication up to 88.35 % of general population which included mainly acetaminophen, antibiotics, chloroquine and ivermectin. Pointless prophylaxis, prevention and treatment protocols of COVID-19 pushed people to adopt self-medication and more concerning, self-dosing without prescription. **Stahl et al. (2015)** reported that a dose of 2g paracetamol resulted in a significant decrease of the antioxidants plasma glutathione and free

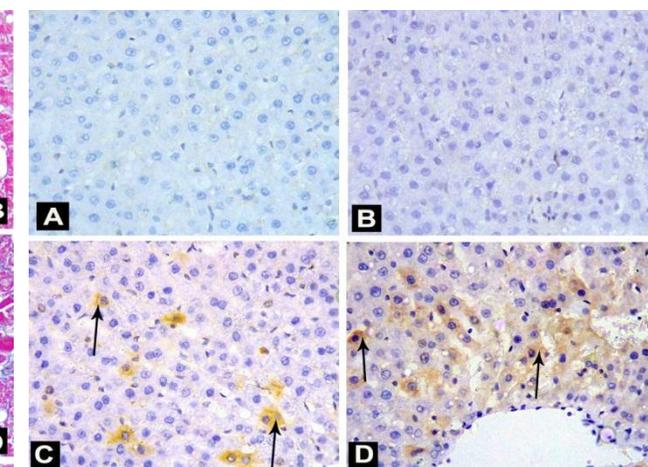


Fig. (4): Photomicrographs of sections of rat liver [A]: from control group and [B]: from group III A (IVM and APAP treated group for 7days) : showing negative cytoplasmic immune reaction for iNOS [C]: from group III B (IVM and APAP treated group for 14 days) showing positive cytoplasmic immune reaction for iNOS in some hepatocyte [D]: from group III C (IVM and APAP treated group for 14 days): showing many hepatocytes with positive cytoplasmic immune reaction for iNOS (Immunoperoxidase technique for iNOS X400).

cysteine for 3 hours. This is of particular importance in cases of COVID-19 infection which itself adds a burden on liver by many suggested mechanisms including direct cytotoxicity from viral infection, hypoxia due to respiratory failure, inflammatory cytokine storm and drug induced liver injury (**Cha et al., 2020 and Aydemir & Ulus, 2020**). Also, liver damage has been observed with long term use of paracetamol even at therapeutic doses in patients with viral infections (**Day et al., 2000**). However, its consumption and sales increased significantly during the COVID-19 pandemic

(*Romano et al., 2020*). In addition, people started taking ivermectin, which is well known for its good margin of safety, on a regular basis. This may increase its toxic effect especially with concomitant drug intake potentiating the risk of harmful health effects (*Molento, 2020*). The aim of this work was to assess the potential hepatotoxic effects on combination of both paracetamol and ivermectin which are used in treating mild and moderate cases of covid 19 infection according to Egyptian ministry of health protocol in adult male albino rats. The animals received both drugs at doses equivalent to human dosing regimen used in covid 19 management protocol after conversion according to surface area ratio. The Evaluation of the possible hepatotoxic effects was studied at different durations (after 7, 14 and 28 days). The biochemical parameters result of this study revealed that hyaluronan level was the earliest biomarker to be increased. The elevation of its level was detected as early as 7 days after treatment with IVM and APAP, while GLDH measures increased after 14 days of treatment. On the other hand, ALT and AST levels didn't increase except after 28 days of combined IVM and APAP treatment.

These results coincide with *George & Stern (2004)* who conducted a study on the hepatotoxic effect of Dimethyl nitrosamine and found that HA is the earliest biomarker of acute liver injury which was elevated within one to two days and reached about 35 folds more than control, with the elevation of conventional liver injury biomarkers (ALT and AST) was delayed to day 7. This may be explained by *Litwiniuk et al. (2016) and Gupta et al. (2019)* stated that HA presents in significant amount in the extracellular matrix in the liver and its synthesis increases in response to inflammation and injury.

As regard GLDH measures, the results of this work are in agreement with *Church et al., 2019* who reported that a multicenter study found that GLDH is the most helpful in identifying DILI. Also, several studies found that GLDH is more effective biomarker of acute hepatic injury than ALT and AST because of its great

increase following hepatocellular injury, prolonged persistence in the blood following injury, high sensitivity for detection of injury (including pre-necrotic injury), high tissue specificity, and lower susceptibility to inhibition or induction (*O'Brien et al., 2002; Khalaf et al., 2017 and Schomaker et al., 2020*).

Also, *Fu et al., 2020* stated that ALT and AST are not elevated in the circulation except after liver injury has occurred and so they cannot be used for identifying potential DILI before substantial liver injury. On the other hand, GLDH is considered an early biomarker of liver injury with advantage over ALT and AST in early recognition of DILI being elevated even without apparent hepatotoxicity. Adding to this it is not affected by age, gender or muscle injury

The histopathological changes detected in IVM and APAP treated group after 7 days was restricted to congestion of central vein with some vacuolation in the hepatocytic cytoplasm. These changes were aggravated with increased duration of treatment and showed congestion and fibrosis of portal vein, dilatation of blood sinusoids, proliferation of kupffer cells, distortion of central vein epithelium, with areas of mononuclear cell infiltration and necrotic foci

The histopathological results of this study coincides with *Elzoghby et al. (2015)* who reported histopathological changes in the liver of rats after injection of ivermectin at therapeutic dose and double therapeutic doses in the form of congestion of central vein, hepatic blood vessels and blood sinusoids in association with vacuolated cytoplasm of hepatocytes. They stated that the severity of these changes varied from mild to severe changes according to the dose. Moreover, *Al-Jassim et al. (2015) and Mahmoud et al. (2017)* who studied the effects of therapeutic and double therapeutic doses of ivermectin on adult male and female rabbits and detected congestion of central vein, portal vein together with sinusoidal dilatation which were more severe with increasing dose and duration. They

also stated that hepatocytes revealed degenerative changes in the form of vacuolar and hydropic degeneration with therapeutic doses to, hyperplasia of epithelial cell lining bile duct with periductal mononuclear leucocytic cellular infiltration, fibrosis and focal necrosis with double therapeutic doses.

Veit et al. (2006) reported a case of a 20-year-old woman who developed severe hepatitis 1 month after a single dose of ivermectin in which liver biopsy revealed intralobular inflammatory infiltrates, confluent necrosis and apoptosis, compatible with drug-induced liver affection. Also, **Leticia et al. (2021)** stated that a clinically apparent liver injury with minimal increase in aminotransferases was reported in a case with single dose therapy of ivermectin. On the other hand, **Dadarkar et al. (2007)** mentioned that cases of liver affection associated with ivermectin use are usually mild and self-limited

Also, our results are parallel with that of **Nesreen et al. (2014)** who stated that the histopathological changes in liver of paracetamol-treated rats included congested central vein, inflammatory changes and activated Von Kupffer cells. In addition, an Experimental study on male albino rats was conducted by **AL-DOAISS, (2019)** revealed that a 30 days administration of 470mg/kg of acetaminophen (which is calculated based on surface area ratio of rats by conversion of the maximum therapeutic daily dose in human) induced hepatic necrosis, blood sinusoidal dilatation and congestion, and severe inflammatory infiltrations which is initiated by the formation of toxic reactive metabolites, N-acetyl benzoquinone imine (NAPQI), which is responsible for hepatotoxicity through the depletion of glutathione.

Woolbright & Jaeschke (2017a) and (2017b) stated that the pathophysiological role of inflammation in acetaminophen-induced liver injury is still conversial. Many studies suggested that inflammation with activation of kupper cells and neutrophils aggravates liver injury induced by paracetamol (**Woolbright and Jaeschke, 2018**). **Kim & Naisbitt (2016)**

and Jaeschke & Naisbitt (2018) explained this by sustained cytokine release from Kupffer cells which also recruits other inflammatory cells such as eosinophils, dendritic cells, T cells resulting in DILI. Moreover, **Oliveira et al. (2006)** stated that the inflammatory process following liver injury can possibly induce fibrogenesis. This goes in parallel with fibrosis findings in this study where after 28 days of treatment , the deposition of collagen fibers around portal vein and bile ducts increased concomitantly with elevation of serum HA which is known to be a non-invasive biomarker to assess the presence of liver fibrosis, and to monitor disease progression (**Neuman et al., 2016**).

The results of this work revealed increased hepatic iNOS protein in treated groups with IV MandAPAP after 14 and 28 days. This is in parallel with that of **Mühl et al. (2011)** who detected increased iNOS in the liver of acetaminophen treated mice. Also, **Pautz et al. (2010) and Burrack & Morrison (2014)** stated that increased expression of iNOS together with increased its biological activity were detected in Kupffer cells and hepatocytes and is related to the pathological inflammation associated with DILI. Biological activity of iNOS with concok, mitant cytoplasmic expression depends on the pathogenesis of drug-induced liver injury (**Burrack and Morrison, 2014**).

Moreover, **Atakisi et al., 2009** found that subcutaneous administration of 1mg/kg of ivermectin in rabbits resulted in increased plasma Nitric oxide level while the level was not significantly altered at doses of 0.5 mg/kg. INOS is responsible for high production of free radical molecule; Nitric oxide (NO) over a short duration. Nitric oxide (NO) is considered not only as a crucial key for efficient innation of host defense, cell-mediated immunity but also in cell injury (**Iwakiri and Kim, 2015**)

The results of this study highlighted the risk of liver injury within short duration following combination of two drugs used in treatment protocol of covid-19. Added to this, studies carried out on COVID-19 reported abnormal liver functions with liver injury in COVID-19

cases (Chen et al., 2020; Wang et al., 2020 and Guan et al., 2020). Also, Histological study of autopsies from patients infected with Covid-19 revealed signs of liver affection ranged from apoptosis, abundant mitosis, mixed inflammatory infiltration in the portal area to severe bile duct injury and centri-lobular necrosis (Fiel et al., 2020 and Bradley et al., 2020). Moreover, Melquist et al., (2020) reported a case of COVID-19 patient presented with acute hepatitis which rapidly progressed to fulminant hepatic failure without any respiratory manifestations.

CONCLUSION

Combination of ivermectin and paracetamol resulted in liver injury which was detected as early as 7 days after treatment by HA level and was accompanied by histopathological changes which became more severe with increasing duration of treatment.

RECOMMENDATIONS

Ivermectin and Paracetamol should be used with precautions and under medical supervision especially on combination to avoid possibility of aggravation of liver injury which is believed to be one of covid-19 complications. More researches are required to assess safety of drugs included in management protocols of covid-19

LIMITATION OF THE STUDY

The study didn't include paracetamol treated group alone and ivermectin treated group alone since the hepatotoxic effects reported may be due to one of them alone, but since the aim of the study to simulate the clinical treatment protocol in which both drugs are used for management of mild and moderate cases of COVID-19 management, the treated group involved the 2 drugs concomitantly

REFERENCES

1. AL-DOAISS, A. A. (2020): Hepatotoxicity-induced by the therapeutic dose of acetaminophen and the ameliorative effect of oral co-administration of selenium / Tribulus terrestris extract in rats. *Int. J. Morphol.*; 38(5):1444-1454.
2. Al-Jassim, A.B., Jawad, H.A., Al-asoudi, E., and Jeedkhadim, S., (2015): biochemical and histological alterations in the liver due to repeated administration of ivermectin alone or with combination of vitamin c in local female rabbits. *J. Inter. Acad. Res. Multidiscip.*, 3; 349-364.
3. Atakisi, E., Atakisi, O., Topcu, B., and Uzun, M. (2009): Effects of therapeutic dose of ivermectin on plasma nitric oxide and total antioxidant capacity in rabbits. *Eu. Rev. Med. Pharmacol. Scie.*, 13(6); 425-429.
4. Atik, E., Onlen, Y., Savas, L., and Doran, F. (2008): Inducible nitric oxide synthase and histopathological correlation in chronic viral hepatitis. *International journal of infectious diseases: I.J.I.D.*, 12 (1); 12-15.
5. Aydemir, D., and Ulusu, N. N. (2020): Is glucose-6-phosphate dehydrogenase enzyme deficiency a factor in Coronavirus-19 (COVID-19) infections and deaths?. *Pathoge. Glob. Health*, 114 (3); 109-110.
6. Bachmann, M., Waibler, Z., Pleli, T., Pfeilschifter, J., and Mühl, H. (2017): Type I Interferon Supports Inducible Nitric Oxide Synthase in Murine Hepatoma Cells and Hepatocytes and during Experimental Acetaminophen-Induced Liver Damage. *Fronti. Immunol.*, 8, 890.
7. Bancroft, J.D. and Gamble, M. (2007): Connective tissue stains. In: Bancroft JD, Gamble M (eds). *Theory and practice of histological techniques. 6th ed. London, New York and Philadelphia: Churchill Livingstone. p. 150*
8. Boyd, E.M. and Berezky, G.M. (1966): Liver necrosis from paracetamol. *Br. J. Pharmacol.*, 26; 606-614
9. Braakman, I., Keij, J., Hardonk, M.J., Meijer, D.K., and Groothuis, G.M. (1991): Separation of periportal and perivenous rat hepatocytes by fluorescence-activated cell sorting: confirmation with colloidal gold as an exogenous marker. *Hepatology.*; 13: 73-82.
10. Bradley, B. T., Maioli, H., Johnston, R., Chaudhry, I., Fink, S. L., Xu, H., Najafian, B., Deutsch, G., Lacy, J. M., Williams, T., Yarid, N., and Marshall, D. A. (2020): Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series. *Lancet*, 396 (10247); 320-332.
11. Burrack, K. S., and Morrison, T. E. (2014): The role of myeloid cell activation and

- arginine metabolism in the pathogenesis of virus-induced diseases. *Fronti. Immunol.*, 5; 428.
12. Caly, L., Druce, J. D., Catton, M. G., Jans, D. A., and Wagstaff, K. M. (2020): The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral res.*, 178; 104787.
 13. Campbell, W.C., Fisher, M.H., Stapley, E.O., Albers-schonberg, G., and Jacob, T.A., (1983): Ivermectin: a potent new antiparasitic agent. *Science*; 221, 823-828.
 14. Cha, M.H., Regueiro, M., and Sandhu, D.S. (2020): Gastrointestinal and hepatic manifestations of COVID-19: A comprehensive review. *World J. Gastroenterol.*, 26: 2323-2332
 15. Chaccour, C., Hammann, F., Ramón-García, S., and Rabinovich, N. R. (2020): Ivermectin and COVID-19: Keeping Rigor in Times of Urgency. *AmerJ. Trop. Med. Hygiene*, 102 (6): 1156–1157.
 16. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. (2020): Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*; 395: 507-513
 17. Church, R. J., Kullak-Ublick, G. A., Aubrecht, J., Bonkovsky, H. L., Chalasani, N., Fontana, R. J., Goepfert, J. C., Hackman, F., et al., (2019): Candidate biomarkers for the diagnosis and prognosis of drug-induced liver injury: An international collaborative effort. *Hepatology (Baltimore, Md.)*; 69 (2): 760–773.
 18. Colerangle, J.B. (2017): Preclinical Development of Nononcogenic Drugs (Small and Large Molecules). In A Comprehensive Guide to Toxicology in Nonclinical Drug Development, 2nd Ed. pp: 659-683. Academic press. <https://doi.org/10.1016/C2015-0-00147-2>
 19. Dadarkar, S. S., Deore, M. D., and Gatne, M. M. (2007): Comparative evaluation of acute toxicity of ivermectin by two methods after single subcutaneous administration in rats. *Regul. Toxicol. Pharmacol., RTP*; 47 (3): 257–260.
 20. Day, R. O., Graham, G. G., and Whelton, A. (2000): The position of paracetamol in the world of analgesics. *Amer. J Therp.*, 7 (2): 51–54.
 21. Elzoghby R.R., Amin A. , Ahlam, F. H. , and Abdel Fatah, A. (2015): Toxicological and pathological studies of Ivermectin on male albino rats. *J. Amer. Scie.*,11(3):73-83
 22. Fiel, M. I., El Jamal, S. M., Paniz-Mondolfi, A., Gordon, R. E., Reidy, J., Bandovic, J., Advani, R., Kilaru, S., Pourmand, K., Ward, S., Thung, S. N., and Schiano, T. (2021): Findings of Hepatic Severe Acute Respiratory Syndrome Coronavirus-2 Infection. *Cellular. molec. Gastroenterol. Hepatol.*, (3): 763–770.
 23. Fosang AJ, Hey NJ, Carney SL, and Hardingham TE. (1990): An ELISA plate based assay for hyaluronan using biotinylated proteoglycan G1 domain (HA-binding region). *Matrix*; 10:306– 313.
 24. Frost GI, and Stern R. A. (1997): Microtiter-based assay for hyaluronidase activity not requiring specialized reagents. *Anal Biochem.*, 251:263– 269.
 25. Fu, S., Wu, D., Jiang, W., Li, J., Long, J., Jia, C., and Zhou, T. (2020): Molecular Biomarkers in Drug-Induced Liver Injury: Challenges and Future Perspectives. *Fronti. Pharmacol.*, 10: 1667.
 26. George, J., and Stern, R. (2004): Serum hyaluronan and hyaluronidase: very early markers of toxic liver injury. *Clin. Chem. Acta; I. J. Clin. Chem.*, 348 (1-2): 189–197.
 27. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, et al., (2020): China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N. Eng. J. Med.*, 382: 1708-1720
 28. Gupta, R. C., Lall, R., Srivastava, A., and Sinha, A. (2019): Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. *Fronti. Vet. Scie.*, 6: 192.
 29. Hanafy, A. S., and Abd-Elsalam, S. (2020): Challenges in COVID-19 drug treatment in patients with advanced liver diseases: A hepatology perspective. *World J. Gastroenterol.*, 26 (46): 7272–7286.
 30. Hellwig, M. D., and Maia, A. (2021): A COVID-19 prophylaxis? Lower incidence associated with prophylactic administration

- of ivermectin. *I. J. Antimicrob. Agents*, 57 (1): 106248.
31. **Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (2011):** Guide for the Care and Use of Laboratory Animals. 8th ed. National Academy Press, Washington DC, PP. 21-55.
<http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf>.
 32. **Iwakiri, Y., and Kim, M. Y. (2015):** Nitric oxide in liver diseases. *Trends in pharmacol. Sci.*, 36 (8): 524–536.
 33. **Iyanda, A. A., and Adeniyi, F. A. (2011):** Biochemical and histologic presentations of female Wistar rats administered with different doses of paracetamol/methionine. *Nig. J. Physiol. Sci.*, 26 (2): 151–160.
 34. **Jaeschke, H., and Naisbitt, D.J. (2018):** Immune mechanisms in drug-induced liver injury. In: Chen, M, Will, Y, editors *Drug-Induced Liver Toxicity. Humana Press; New York: 511–531.*
 35. **Kasarala, G., and Tillmann, H.L. (2016):** Standard liver tests. *Clin. Liver Dis.*, 8: 13–18.
 36. **Khalaf, M. AM., Younis, R. HA., and Elroby, F.A. (2017):** Accuracy of Using Glutamate Dehydrogenase (GDH) and High-Mobility Group Box-1 (HMGB1) Plasma Levels as Early Predictors of Acute Aluminum Phosphide Hepatotoxicity. *J. Pharmacol. Clin. Toxicol.*, 5(3):1076.
 37. **Kim, S.H., and Naisbitt, D.J. (2016):** Update on Advances in Research on Idiosyncratic Drug-Induced Liver Injury. *Allergy Asthma Immunol. Res.*, 8(1):3–11.
 38. **Kutlu, S., Colakoglu, N., Halifeoglu, I., Sandal, S., Seyran, A. D., Aydin, M., and Yilmaz, B. (2007):** Comparative evaluation of hepatotoxic and nephrotoxic effects of aroclors 1221 and 1254 in female rats. *Cell biochem. Function*; 25(2): 167–172.
 39. **Lankas, G. R., Minsker, D. H., and Robertson, R. T. (1989):** Effects of ivermectin on reproduction and neonatal toxicity in rats. *Food Chem. Toxicol.*, 27 (8): 523–529.
 40. **Lehrer, S., and Rheinstein, P. H. (2020):** Ivermectin Docks to the SARS-CoV-2 Spike Receptor-binding Domain Attached to ACE2. *In vivo (Athens, Greece)*, 34 (5): 3023–3026.
 41. **Leticia, A.A.1, Luiz, E.F., Caroline, L. R., Alzinei, S., Angélica, M. B. O., and Rita, B. (2021):** Drug-Induced Hepatotoxicity in COVID-19. *I. J. Avaced. Eng. Res. Sci. (IJAERS)*, 8 (9): 267-275.
 42. **Litwiniuk, M., Krejner, A., Speyrer, M. S., Gauto, A. R., and Grzela, T. (2016):** Hyaluronic Acid in Inflammation and Tissue Regeneration. *Wounds. Clin. Res. Pract.*, 28 (3): 78–88.
 43. **Lytvynets, A., Langrová, I., Lachout, J. et al. (2010):** Drinking water ivermectin treatment for eradication of pinworm infections from laboratory rat colonies. *Helminthol.*, 47:233–237.
 44. **Mahmoud, S, G., AbdEl-baset, E, El., Aziza, A, A., Marwa, M, D. (2017):** Pathological studies on effects of ivermectin on male and female rabbits. *B.V.M.J.*, 32 (1): 104-112
 45. **Mastorodemos, V., Zaganas, I., Spanaki, C., Bessa, M., and Plaitakis, A. (2005):** Molecular basis of human glutamate dehydrogenase regulation under changing Energy Demands. *J. Neurosci. Res.*, 79 (1-2): 65–73.
 46. **McGill M. R. (2016):** The past and present of serum aminotransferases and the future of liver injury biomarkers. *E.X.C.L.I. J.*, 15: 817–828.
 47. **Melquist, S., Estep, K., Aleksandrovich, Y., Lee, A., Beiseker, A., Hamedani, F. S., and Bassett, J. (2020):** COVID-19 presenting as fulminant hepatic failure: A case report. *Med.*, 99 (43): 22818
 48. **Meunier, L., and Larrey, D. (2019):** Drug-Induced Liver Injury: Biomarkers, Requirements, Candidates, and Validation. *Fronti. Pharmacol.*, 10: 1482.
 49. **Ministry of health and population (2020):** Management Protocol for COVID-19 Patients Version 1.4/30th May 2020 (MOHP), Egypt. Available from https://www.researchgate.net/publication/344078546_Management_Protocol_for_COVID19_Patients,Version:1430th.May_2020 Ministry of health and population (MOHP) Egypt

- 50. Molento M. B. (2020):** COVID-19 and the rush for self-medication and self-dosing with ivermectin: A word of caution. *One health (Amsterdam, Netherlands)*; 10, 100148.
- 51. Mühl, H., Bachmann, M., Pfeilschifter, J. (2011):** Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity. *Cell Microbiol.*, 13:340–348.
- 52. National institute of health (2021):** Covid 19 treatment guidelines. Available from <https://www.covid19treatmentguidelines.nih.gov/therapies/antiviral-therapy/>
- 53. Nesreen I. M, Basim A. S, Ali A. A. (2014):** Protective effect of allopurinol on paracetamol induced liver injury in rats. *Az. J. Pharm. Sci.*, 50 (2): 117-136
- 54. Neuman, M. G., Cohen, L. B., and Nanau, R. M. (2016).** Hyaluronic acid as a non-invasive biomarker of liver fibrosis. *Clin. Biochem.*, 49(3): 302–315.
- 55. O'Brien, P. J., Slaughter, M. R., Polley, S. R., and Kramer, K. (2002):** Advantages of glutamate dehydrogenase as a blood biomarker of acute hepatic injury in rats. *Lab. Animals*, 36(3): 313–321.
- 56. Oliveira, C.P.M.S., Coelho, A.M.M., Barbeiro, H.V., Lima, V.M.R., Soriano, F. and Ribeiro, C. et al. (2006):** Liver mitochondrial dysfunction and oxidative stress in the pathogenesis of experimental nonalcoholic fatty liver disease. *Braz. J. Med. Biol. Res.*, 39(2): 189-194.
- 57. Pautz, A., Art, J., Hahn, S., Nowag, S., Voss, C., and Kleinert, H. (2010):** Regulation of the expression of inducible nitric oxide synthase. *Nitric oxide: Boil. Chem.*, 23(2): 75–93.
- 58. Quispe-Cañari, J. F., Fidel-Rosales, E., Manrique, D., Mascaró-Zan, J., Huamán-Castillón, K. M., Chamorro-Espinoza, S. E., Garayar-Peceros, H., Ponce-López, V. L., Sifuentes-Rosales, J., Alvarez-Risco, A., Yáñez, J. A., and Mejia, C. R. (2021):** Self-medication practices during the COVID-19 pandemic among the adult population in Peru: A cross-sectional survey. *Saudi Pharmacol. J.*, 29 (1): 1–11.
- 59. Reagan-Shaw, S., Nihal, M., and Ahmad, N. (2008):** Dose translation from animal to human studies revisited. *F.A.S.E.B.J.* , 22 (3): 659–661.
- 60. Romano, S., Galante, H., Figueira, D., Mendes, Z., and Rodrigues, A. T. (2021):** Time-trend analysis of medicine sales and shortages during COVID-19 outbreak: Data from community pharmacies. *Res. Social Administ. Pharma., RSAP*, 17 (1): 1876–1881.
- 61. Rostami, S., and Parsian, H. (2013):** Hyaluronic Acid: from biochemical characteristics to its clinical translation in assessment of liver fibrosis. *Hepatitis Monthly*, 13 (12): e13787.
- 62. Schmidt, E.S., and Schmidt, F.W. (1988):** Glutamate dehydrogenase: biochemical and clinical aspects of an interesting enzyme. *Clin. Chim. Acta*, 173: 43–55.
- 63. Schomaker, S., Potter, D., Warner, R., Larkindale, J., King, N., Porter, A. C., Owens, J., Tomlinson, L., Sauer, J. M., Johnson, K., and Aubrecht, J. (2020):** Serum glutamate dehydrogenase activity enables early detection of liver injury in subjects with underlying muscle impairments. *PLoS one*, 15 (5): e0229753.
- 64. Stahl, S. H., Yates, J. W., Nicholls, A. W., Kenna, J. G., Coen, M., Ortega, F., Nicholson, J. K., and Wilson, I. D. (2015):** Systems toxicology: modelling biomarkers of glutathione homeostasis and paracetamol metabolism. *Drug discovery today. Technol.*, 15: 9–14.
- 65. US Food and Drug Administration. Acetaminophen information. Maryland: US Food and Drug Administration (2015):** [Online] (accessed JUNE 2021).
- 66. Veit, O., Beck, B., Steuerwald, M., and Hatz, C. (2006):** First case of ivermectin-induced severe hepatitis. *Trans.Royal Soci. Tropic. Med. Hyg.*, 100 (8): 795–797
- 67. Woolbright, B.L. and Jaeschke, H. (2018):** Mechanisms of Inflammatory Liver Injury and Drug-Induced Hepatotoxicity. *Curr. Pharmacol. Rep.*, 4 (5): 346–357.
- 68. Woolbright, B.L., Jaeschke, H. (2017 a):** The impact of sterile inflammation in acute liver injury. *J. Clin. Transl. Res.*, 3(1):170–88.
- 69. Woolbright, B.L., Jaeschke, H. (2017 b):** Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure. *J. Hepatol.*, 66 (4): 836–48.

70. World health organization (2020a): Covid19-global .Available from <https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON305>
71. World health organization (2020 b): Home care for patients with suspected or confirmed COVID-19 and management of their contacts. Interim guidance, 13 August 2020. Available at <https://apps.who.int/iris/rest/bitstreams/1292529/retrieve>. Last accessed 30 September 2020.
72. World health organization (2021): Egypt: WHO corona virus disease dashboard. Available from <https://covid19.who.int/region/emro/country/eg>
73. Wu, J., Song, S., Cao, H. C., and Li, L. J. (2020): Liver diseases in COVID-19: Etiology, treatment and prognosis. *World J. Gastroenterol.*, 26 (19): 2286–2293.
74. Xu, X. W., Wu, X. X., Jiang, X. G., Xu, K. J., Ying, L. J., Ma, C. L., Li, S. B., Wang, H. Y., Zhang, S., Gao, H. N., Sheng, J. F., Cai, H. L., Qiu, Y. Q., and Li, L. J. (2020): Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *B.M.J. (Clin. Res. ed.)*, 368: m606
75. Yang, S., Atkinson, S. C., Wang, C., Lee, A., Bogoyevitch, M. A., Borg, N. A., and Jans, D. A. (2020): The broad spectrum antiviral ivermectin targets the host nuclear transport importin $\alpha/\beta 1$ heterodimer. *Antiviral research*; 177: 104760.
76. Zhang, C., Shi, L., and Wang, F. S. (2020): Liver injury in COVID-19: management and challenges. *Lancet. Gastroenterol. Hepatol.*, 5 (5): 428–430.

دراسة بيوكيميائية ونسجية للتأثيرات السامة المحتملة على كبد ذكور الجرذان البيضاء عند دمج عقارى الباراسيتامول و الافرميكتين المستخدمين فى البروتوكول العلاجى لكوفيد -١٩

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المقدمة: يستخدم كلا من عقار الإفرميكتين والباراسيتامول لعلاج الحالات الخفيفة والمتوسطة من عدوى كوفيد -١٩. الهدف: هدفت هذه الدراسة إلى تقييم التأثيرات السمية الكبدية المحتملة من استخدام الباراسيتامول والإفرميكتين معا في ذكور الجرذان البيضاء البالغة. طريقة الدراسة: تم تقسيم خمسين جرذاً بالغاً من ذكور الجرذان البيضاء إلى مجموعة ضابطة (سلبية و إيجابية) ومجموعة معالجه بالافرميكتين والباراسيتامول سويا وقد تضمنت ثلاثين فأراً مقسمين بالتساوي إلى ٣ مجموعات فرعية (أ، ب، ج) وقد تلقى كل فأر ٣.٧ مجم / كجم / يوم من الإفرميكتين عن طريق الفم بالتزامن مع ٣٧٠ مجم / كجم / يوم من الباراسيتامول (تم التضحية بالمجموعات الفرعية أ، ب، ج بعد ٧ أيام و ١٤ يوماً و ٢٨ يوماً على التوالي) وتم تقييم مستويات المصل من الالانين امينوترانسفيراز ALT واسبرنتيت امينو ترانسفيريز AST وجلوتاميت ديهيدروجينيز GLDH و الهيالورونان Hyaloronan وقد خضعت أنسجة الكبد للفحص النسيجي والدراسة الهستوكيميائية المناعية لمعرض السينسيز لأكسيد النيتريك ودراسات القياس المورفومتري ، النتائج: كان مستوى الهيالورونان هو أول مؤشر حيوي تم زيادته مع ارتفاع مستواه بعد ٧ أيام من العلاج تليها GLDH التي زادت بعد ١٤ يوماً من العلاج بينما لم تزد مستويات ALT and AST إلا بعد ٢٨ يوماً من العلاج المشترك بينما اقتصررت التغيرات النسيجية المرضية المكتشفة في المجموعة المعالجة بـ الإفرميكتين والباراسيتامول بعد ٧ أيام على احتقان الوريد المركزي مع بعض الإخلاء في السيتوبلازم الكبدى. تفاقمت هذه التغيرات مع زيادة مدة العلاج وأظهرت احتقان وتليف في الوريد البابي ، تمدد الجيوب الأنفية في الدم ، تكاثر خلايا كوبر ، مع مناطق تسلل الخلايا وحيدة النواة والبؤر النخرية. الاستنتاج و التوصية: أدى الجمع بين الإفرميكتين والباراسيتامول إلى إصابة الكبد التي تم اكتشافها في وقت مبكر بعد ٧ أيام من العلاج بمستوى الهيالورونان ورافقها تغيرات نسيجية مرضية أصبحت أكثر حدة مع زيادة مدة العلاج. مما يوصى باتخاذ الاحتياطات الواجبه عند استخدام إفرميكتين و باراسيتامول سويا لتجنب احتمال تفاقم إصابة الكبد التي يُعتقد أنها إحدى مضاعفات كوفيد -١٩ .