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Study of the hepatoprotective effect of ginger aqueous infusion in rats

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ABSTRACT

The present study aimed to evaluate the hepatoprotective effect of ginger aqueous infusion on the paracetamol induced hepatotoxicity in rats. Different groups (1, 2, 3) of rats were given ginger in three doses (100,200 and 400 mg /kg at 12 hours intervals for 48 hours prior to single paracetamol dose (640 mg /kg), group 4 rats were given silymarin (25mg/kg) as reference hepatoprotective drug, group 5 rats were given paracetamol alone(positive control group),group 6 rats were given distilled water(negative control group). Blood was collected from all teated groups for determination of liver enzymes:- alanine aminotransferase (ALT), alanine aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin after that rat were sacrificed and the livers were excised for the histopathological study in which the examination of liver tissue for rat treated with paracetamol and extract at dose of 200,400 mg/kg and also with silymarin revealed normal hepatic architecture than liver for rats treated with extract at a dose 100 mg. In vitro bioassay on primary culture of rat hepatocytes monolayer revealed that the LC_{50} of ginger extract was at 750µg/ml while the hepatoprotective activity of the extract concentration at which extracts exhibit a hepatoprotective activity was of (15 µg/ml).

Keywords: Ginger, paracetamol, liver, LC_{50} , rats.

INTRODUCTION

Researches involve the plants traditionally used to support the liver functions and treat diseases of the liver. Ginger is known to possess antioxidant properties. There has been a substantial

increase in the use of so-called complementary and alternative therapies by patients with liver disease. Although many such modalities are available, herbal therapies are the most popular, and of these remedies, silymarin extracted form milk thistle plant (*silybum marianum*) is most widely subscribed to as a remedy for liver disease [1].

Drug metabolism and formation of reactive toxic metabolites by the hepatic microsomal enzyme system plays a role in the hepatotoxic mechanism [2].Xenobiotics are metabolized to inert metabolites that are excreted by the kidney, but some are metabolized to more reactive compounds that are more toxic than the parent compound **.**Examples of these drugs are **:** paracetamol ,isoniazid and methotrexate [3]. Paracetamol {acetaminophen (N-acetyl-para-aminophenol [APAP])} is an extensively used analgesic and antipyretic drug and though safe when used at therapeutic doses [4], intentional or unintentional overdose has become the most frequent cause of acute liver failure [5].Haematological factors play an important role in the development of hepatotoxicity .Since blood passes in the liver from zone 1 to zone 3 in a sequential manner[6],hepatocytes of zone 3 are the last to receive oxygen and nutrients and the least resistant to hypoxic and xenobiotic injuries. When cells of zone 3 die, the formed picture is called centrilobular necrosis as with paracetamol hepatotoxicity [7].

Botanical medicines have been used traditionally by herbalists and indigenous healers worldwide for the prevention and treatment of liver disease. [8].They include:Curcuma longa (turmeric), Camellia sinensis (green tea), Glycyrrhiza glabra (licorice) , Silybum marianum (milk thistle) and Picrorhiza kurroa (kutkin) [9]. The ginger has been listed in "Generally Recognised as Safe" (GRAS) document of the US FDA[10]. In the fresh ginger rhizome, the polyphenolic compounds gingerols ; i.e. 6-gingerol, 8-gingerol and zingerone were identified as the major active components and [6]gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl)decan-3-one is the most abundant constituent in the gingerol series and also responsible for its characteristic pungent taste [11].

MATERIALS AND METHODS

2.1. Animals

Sprague-Dawley albino rats weighing (175-200gm) were used for in vivo study and Wistar male rats (275-300gm) were used for in vitro study. All animals were housed in standard metal cages in an air conditioned room at 22 ± 3°C, 55 ± 5% humidity and were provided with a standard laboratory diet and water *ad libitum.* They were obtained from animal house colony of the National Research Center, Dokki, Giza, Egypt. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) [12].

2.2. Drugs and chemicals:

a- Ginger in form of powder obtained from MEPACO
b- Paracetamol (acetaminophen) in form of powder obtained from EIPICO.
c- Silymarin in form of powder was obtained from SEDICO, pharmaceutical Co., 6 October City-Egypt

2.3. Diagnostic kits:

– ALT (alanine aminotransferase) and AST (aspartate aminotransferase) according to Reitman and Frankel [13].
– ALP (alkaline phosphatase) according to King and King [14].
– Total bilirubin according to Walter and Gerarde [15].

2.4. Method

2.4.1. In vitro study:

In vitro bioassay on primary culture of rat hepatocytes monolayer:

Primary culture of rat hepatocytes was prepared according to Seglen method [16], modified by Kiso et al.,[17], using Wistar male rats (250-300 g),Then biological screening of aqueous extract of Zingiber Officinale on primary cultures of rat hepatocytes was performed to achieve the following objectives:Determination of the LC_{50} of extracts according to the method of Mosmann [18] modified by Carmichael et al.,[19],evaluation of the hepatoprotective activity of the extracts and determination of the concentration at which the extracts exhibit a biological activity.

2.4.2. In vivo study:

The rats were divided into three groups and three subgroups, eight animals each:

1-Negative control group: Rats received daily oral dose of 3 ml distilled water , served as negative control.

2-Positive control group: Rats received paracetamol orally in a dose of 640 mg/kg [20].

3-Reference group: Rats received silymarin orally (25 mg /kg) [21] in four divided doses at 12 h intervals for 48 h, one hour after the last dose administration; paracetamol (640 mg/kg) was given orally.

4-Treated groups: Rats were divided into thee subgroups as fellows: - Rats received ginger aqueous infusion orally (100,200,400 mg /kg) at 12 h intervals for 48 h one hour after the last dose of ginger administration, paracetamol (640 mg/kg) was given orally.

2.4.2.1. Biochemical parameters

After the last dose of treatment of all rats , blood was obtained from the retro-orbital plexus of veins over night fasting after being lightly anesthetized with ether, the blood was allowed to flow into clean dry centrifuge tube and left to stand for 30 min before centrifugation to avoid hemolysis. Samples were centrifuged for 15 min at 3000 rpm. The clear supernatant, serum was separated and collected by pasteur pipette into a dry clean tube for the following biochemical tests {(alanine amino transferase (ALT) aspartate amino transferase (AST), alkaline phosphatase (ALP) and total bilirubin }**.**

2.4.2.2. Histopathological study

Animals were sacrificed 24 hours after the last treatment, the thoracic cavities opened, livers rapidly and carefully excised and all attached vessels and ligaments trimmed off. The removed livers were washed with cold saline, dried with filter papers and weighed. Liver slides were prepared and stained with hematoxylin and eosin (H & E) staining [22].

2.5. Statistical analysis:

Values were expressed as means $\pm$ SE. results were analysed using one way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test to compare all groups.

RESULTS

3.1. In vitro bioassay test results

The results of in vitro bioassay on primary culture of rat hepatocytes monolayer revealed that the $\mathbf{LC_{50}}$ of ginger extract was at 750 µg/mL while the hepatoprotective activity of the extract concentration at which extracts exhibit a hepatoprotective activity was of 15µg/ml (Table 1, 2, 3 & Figures 1, 2)

*3.2. Biochemical results***:**

In this study the percentage of the hepatoprotective of efficacy of ginger treated rat in its three dose level (100,200,400 mg/kg) compared to silymarin is respectively 16.88, 67.89, 80 % for

ALT serum level , 59, 86, 81 % for AST serum level, 7, 34, 67 % for ALP and 103,105,106 % for serum total bilirubin level (Table 4 & Figures 3,4,5,6)

3.3. Histopathological results:

Histopathological examination of liver tissue of rats received distal water (negative control group) shows apparently normal hepatic tissue (Fig.7) while that of liver tissue of rats treated with paracetamol revealed partially disturbed lobular architecture, portal tracts were infiltrated by mononuclear inflammatory cells and show mild periportal fibrosis, hepatic parenchyma showed also distortion of liver cell plates particularly around central hepatic veins which demonstrated endothelitis, congestion, engorgement with RBCs and mononuclear inflammatory cells and hepatocytes showed scattered foci of spotty necrosis and minimal steatosis, perivenular hepatocytes exhibited hydropic degeneration, swelling and ballooning of hepatocytes (Fig.8).Examination of liver tissue of rats treated with paracetamol and extract at dose "200 mg/kg" (Fig .11) and silymarin "25mg/kg" (Fig 9) before paracetamol adminestration showed better hepatic architecture than liver of rats treated with extract "100 mg /kg" (Fig.10).

Table.1.Hepatoprotective and hepatotoxic concentrations of ginger and silymarin.

	Hepatoprotection (µg/ ml)	**Hepatotoxicity (LC_{50}) (µg/ ml)**
Ginger	**15**	**750**
Silymarin	**50**	**500**

Table .2.Sample without paracetamol

Concentration µg/ml	**Mean absorbance percentage of viable cells**	
	Ginger	**Silymarin**
100	73%	94%
150	69%	70%
200	69%	70%
250	61%	68%
500	37%	60%
750	49%	53%
1000	34%	45%
100% cell control	100%	
50% cell control	50%	

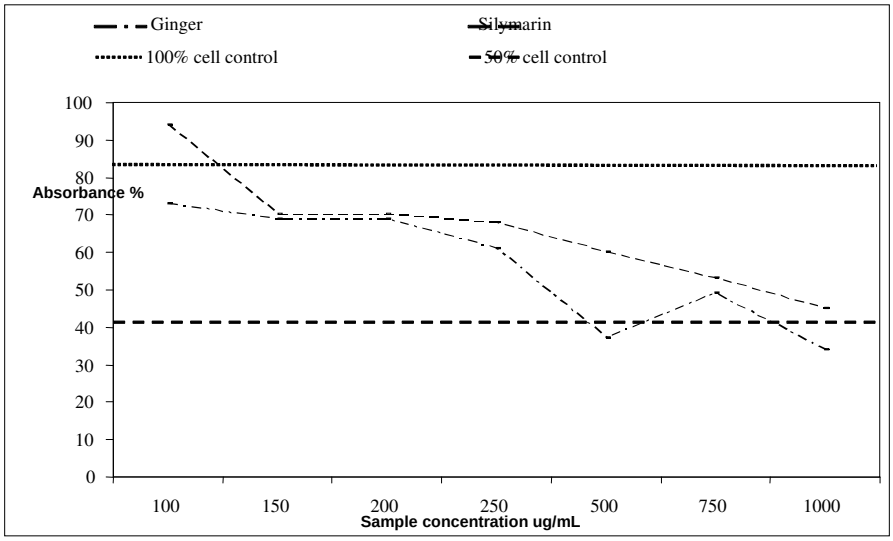


Fig .1. Effects of ginger and silymarin on liver cells (sample without paracetamol).

Table .3. Sample with paracetamol

Concentration ml	Mean absorbance percentage of viable cells	
	Ginger	**Silymarin**
10	93%	84%
15	98%	92%
25	94%	95%
50	88%	102%
75	87%	91%
100	70%	82%
250	60%	76%
100% cell control	100%	
50% cell control	50%	
Paracetamol (20 mM)	68%	

In the hepatoprotection experiment the hepatocytes were intoxicated using a concentration of 20 mM paracetamol.

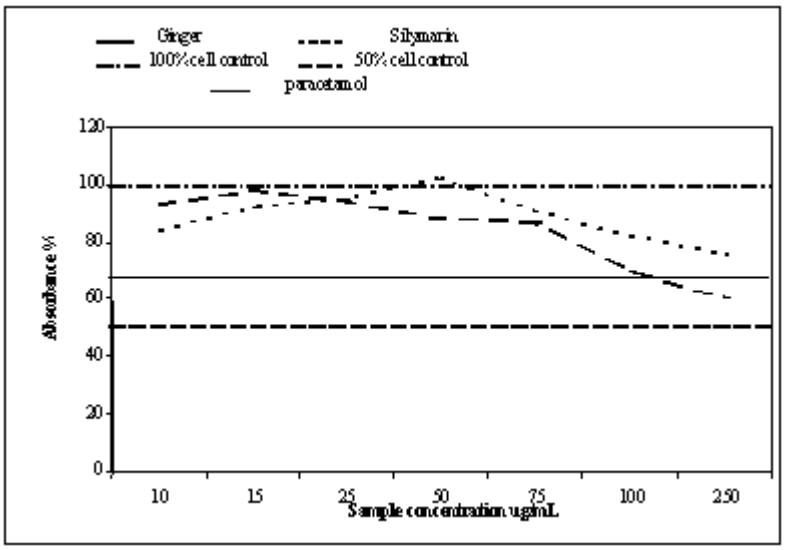


Fig .2. Effect of ginger and silymarin on liver cells(sample with paracetamol).

Table.4.Effect of ginger aqueous extract (100,200 and 400 mg/kg) and Silymarin (25 mg /kg) administration in hepatic damage rats induced by single paracetamol (640 mg/kg) in serum levels of ALT, AST, ALP and total bilurbin(N=8).

Group / Parameter	Neagtive control	Paracetamol 640mg/kg	Ginger 100mg/kg	Ginger 200mg/kg	Ginger 400mg/kg	Silymarin 25mg/kg
ALT (U/ml)	20.24 ±2.16	48.22# ±4.79	43.70† ±1.68	30.04*† ±2.8	26.78* ±2.51	21.44* ±1.52
AST (U/ml)	65.42 ±2.12	94.77# ±3.14	81.51*† ±3.11	75.47* ±2.05	76.63* ±2.57	72.28* ±1.38
ALP (U/ml)	99.62 ±2.51	458.48# ±23.65	444.08† ±40.48	390.64† ±36.67	326.73* ±12.26	261.52* ±21.19
Total Bilirubin (mg/dl)	0.42 ±0.03	3.16# ±0.138	0.69*‡ ±0.07	0.64*‡ ±0.05	0.61*‡ ±0.06	0.76* ±0.07

Values were represents a mean of 8 rats ± S.E of the mean. Results were analyzed using one way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test to compare all groups.
#Significance at P < 0.01 for paracetamol group versus negative control(normal group),
**Significance at P < 0.01 versus paracetamol (hepatotoxic) group,*
† Significance at P < 0.01 .‡ Significance at P < 0.01 ,where serum level of total bilirubin for all ginger groups is significantly less than silymarin group.

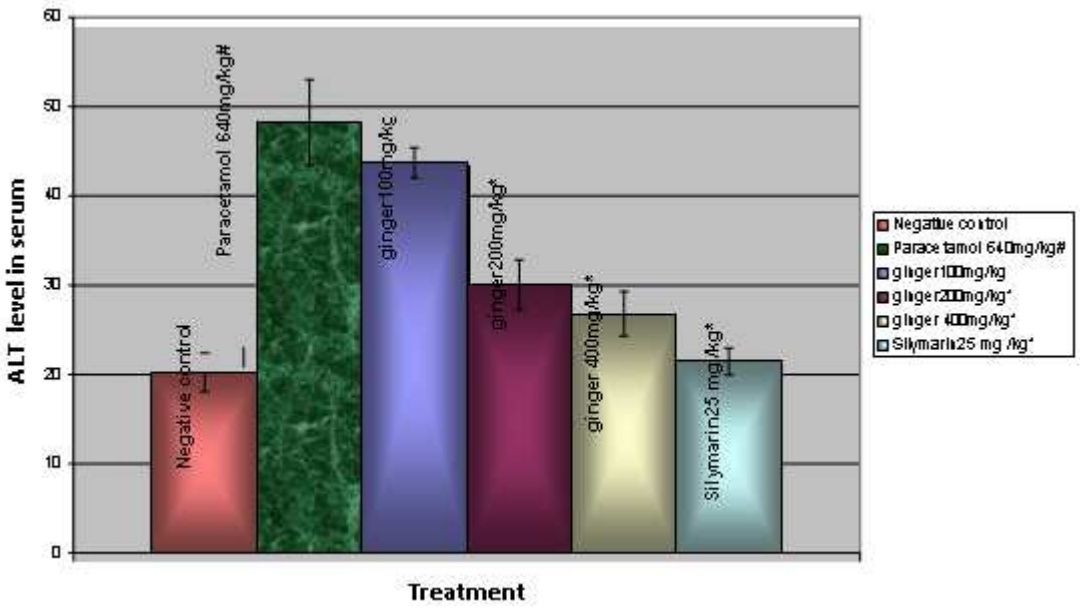


Fig .3. Effect of ginger and silymarin pretreatment on ALT (N=8).
 # Significance at P < 0.01 versus negative control (normal) group.
** Significance at P < 0.01 versus poaracetamol (hepatotoxic) group.*

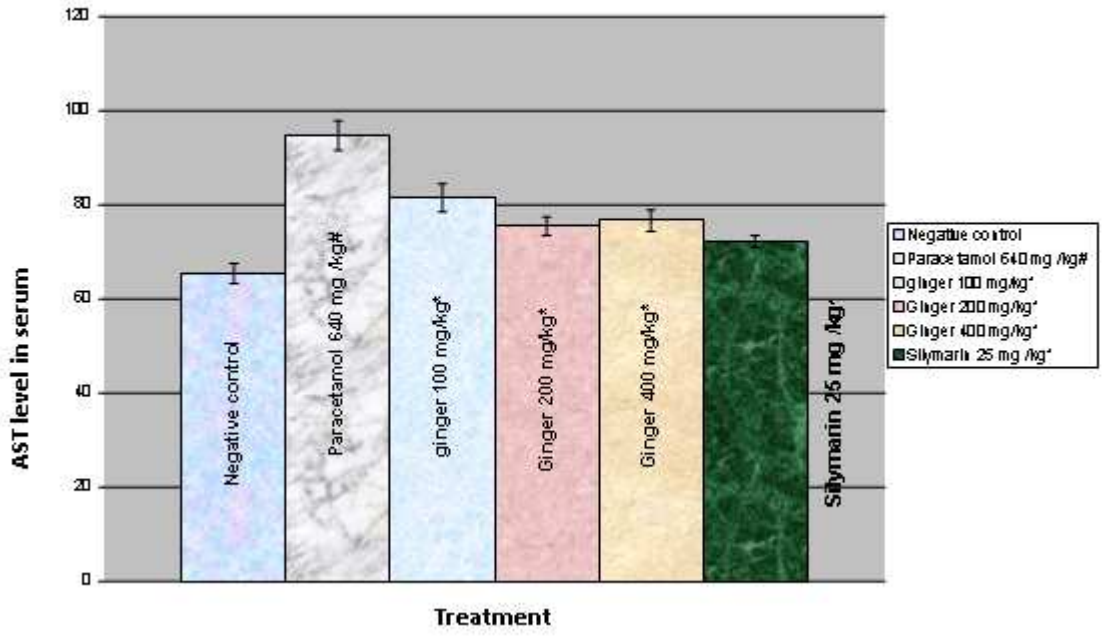


Fig.4.Effect of ginger and silymarin pretreatment on AST (N=8).
 # Significance at P < 0.01 versus negative control (normal)group.
** Significance at P < 0.01 versus paracetamol (hepatotoxic) group.*

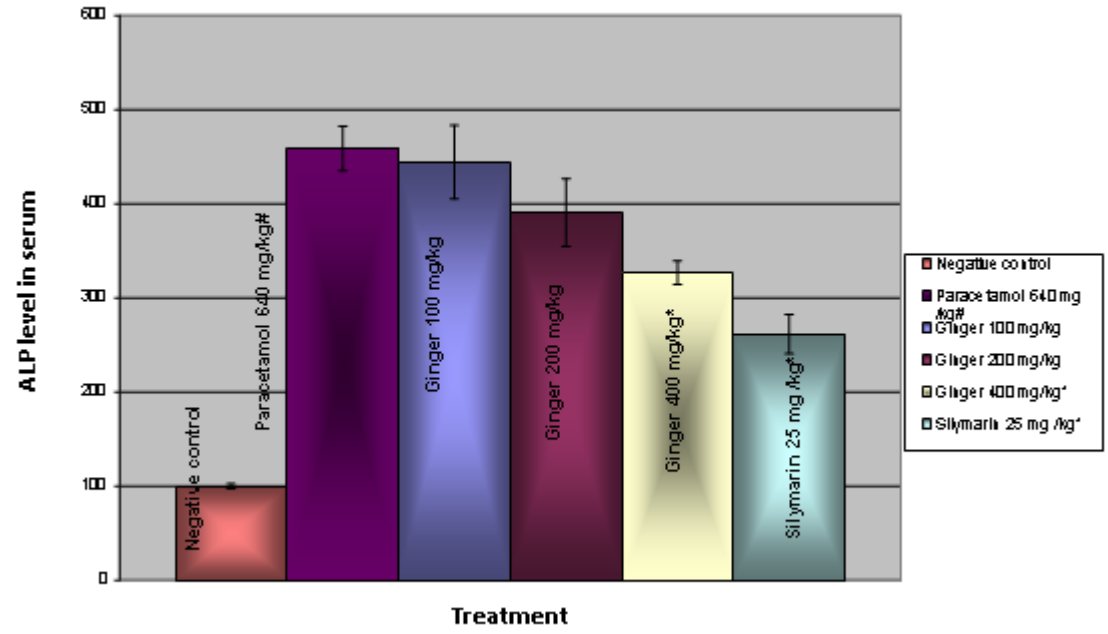


Fig.5. Effect of ginger and silymarin pretreatment on ALP (N=8).
 # Significance at P < 0.01 versus negative control (normal) group.
** Significance at P < 0.01versus paracetamol (hepatotoxic) group.*

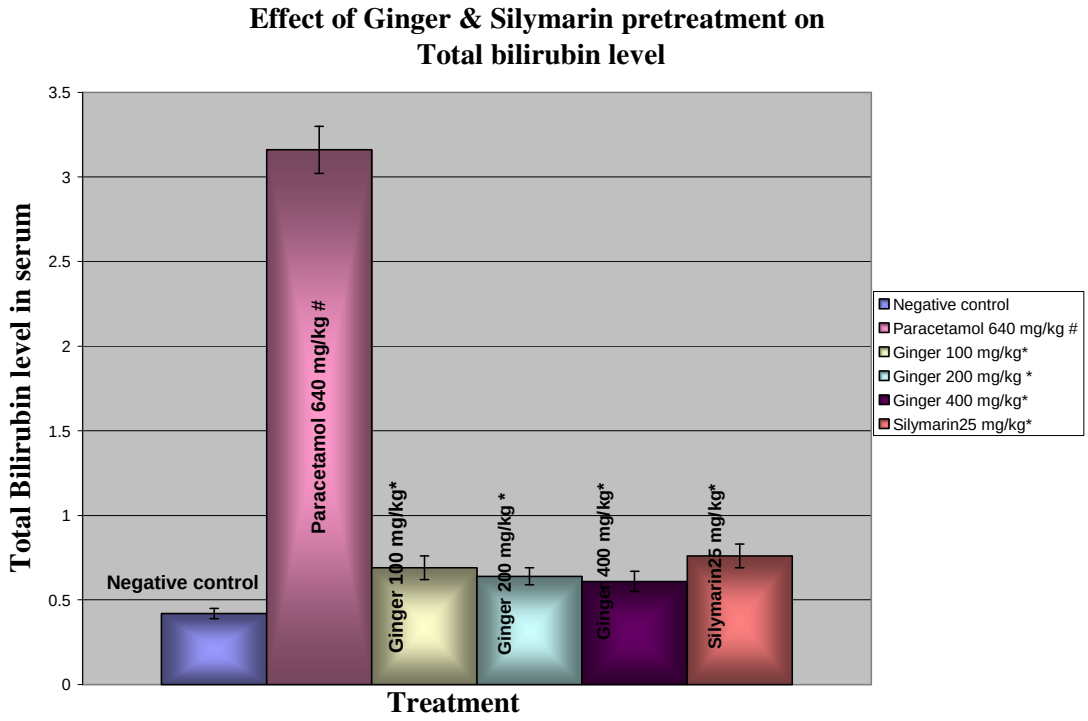


Fig.6.Effect of ginger and silymarin pretreatment on total bilirubin(N=8).

 # Significance at P < 0.01 versus negative control (normal)group.

** Significance at P < 0.01 versus paracetamol (hepatotoxic) group.*

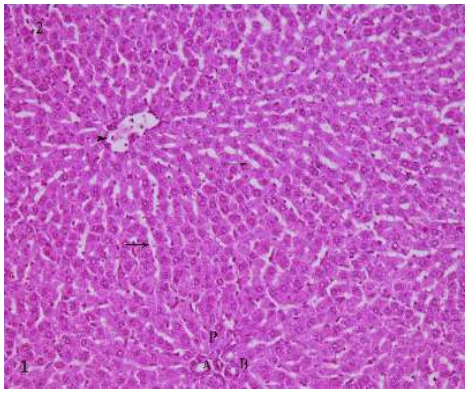


Fig .7. A photomicrograph of a liver section of a control rat apparently showed normal hepatic tissue (Hx. & E. X 200)

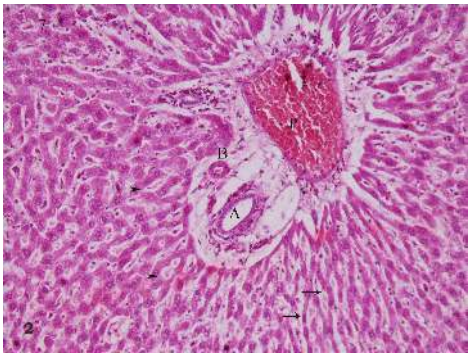
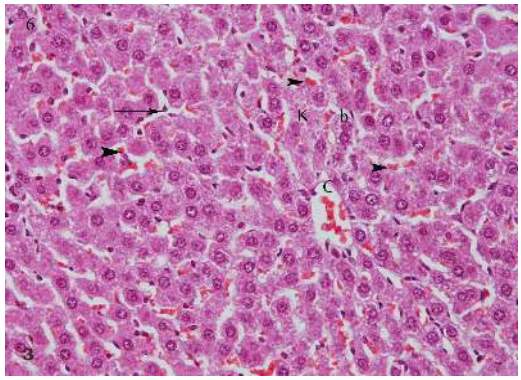


Fig.8. A photomicrograph of a section of liver tissue of rat treated with paracetamol showing partially disturbed lobular architecture and portal tracts were infiltrated by mononuclear inflammatory cells and show mild periportal fibrosis (Hx. & E. X 200).



Fi g .9. A photomicrograph of a section of liver tissue from a rat treated with silymarin before paracetamol injection showing a normal central vein (C) and showed better hepatic architecture (Hx. & E. X 200).

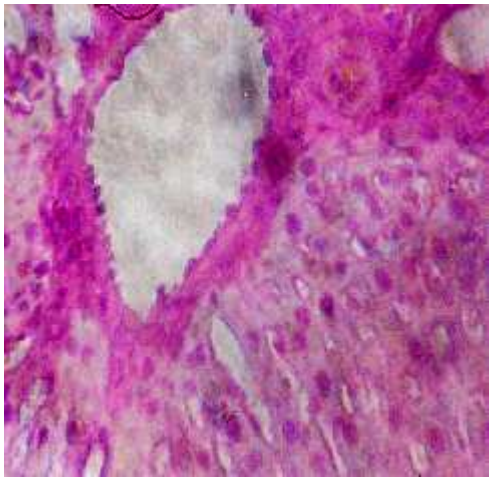


Fig .10. Photomicrograph of rat liver treated with ginger 100 mg followed by paracetamol showing better liver architecture. No inflammatory cellular infiltrate (Hx. & E. X 200)

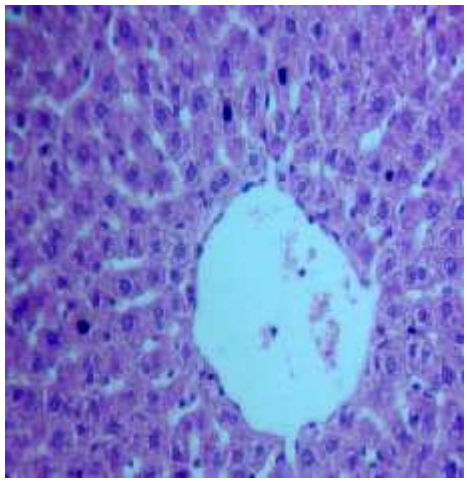


Fig .11. Photomicrograph of rat liver treated with ginger 200mg followed by paracetamol showing better hepatic architecture and normal hepatic vein morphology(Hx. & E. X 200)

DISCUSSION

Both ginger and silymarin reduced serum ALT,AST and ALP indicating membrane stabilization and antioxidant properties of ginger [21].It is widely accepted that the induction of phase II hepatic enzymes for drug biotransformation results in protection against toxicity and chemical carcinogenesis, especially during the initiation phase. Phase II xenobiotic metabolizing enzyme glutathione S-transferase (GST), plays a major role in the cellular detoxification of oxidative damaging, genotoxic and carcinogenic xenobiotics [23]. GSTs are a family of soluble proteins, which conjugate xenobiotics with glutathione. Metabolites after glutathionylation are more hydrophilic and thus biologically inactive. Therefore, they are readily excreted in bile or urine as conjugates. This action is thus believed to be a major mechanism for the detoxification of xenobiotics [24]. It was suggested that intake of ginger oil may affect the host enzymes associated with activation and detoxication of xenobiotic compounds [25].Zerumbone(ZER), a major sesquiterpene compound of a tropical ginger Zingiber zerumbet Smith, induces nuclear localization of the transcription factor (Nrf2) that binds to antioxidant response element (ARE) of the phase II enzyme genes, suggesting that ZER is a potential activator of the Nrf2/ARE-dependent detoxification pathway [26].

Water and alcoholic extracts of ginger have been shown to possess potent antioxidant activity on fats and oils and prevent lipid peroxidation .Zingerone present in ginger inhibits liver microsomal lipid peroxidation at concentrations greater than 150 mM. In addition zingerone functions as an effective scavenger of superoxide anions as measured by nitro-blue-tetrazolium reduction in a xanthine-xanthine oxidase system [27].Besides these findings**,** ginger has antioxidant activity due to its polyphenolic components which have the capacity to be donors of hydrogen atoms or electrons and to capture the free radicals so act as radical scavengers [28]. This is due to the presence of zingeberene and oleoresin [29]. Also since the mechanism of hepatic injury may be due inflammatory process, so the hepatoprotective activity of ginger may be due to its content of volatile oils, which showed anti-inflammatory, analgesic and immunomodulatory effects. Volatile oil of ginger is capable of inhibiting T lymphocyte dependent immune reactions [30].Moreover the anti-inflammatory activity of ginger is due to its ingredients as the gingerols and gingerol analogs (shogaols and paradols). Previous reports have documented the ability of these compounds to directly inhibit prostaglandin and leukotriene synthesis [31]. [6]-Gingerol 1 besides having various pharmacological and physiological effects including anti-inflammatory, analgesic, antipyretic, gastroprotective activities, is also reported to have cardiotonic and antihepatotoxic activities [32]. The hepatoprotective plant curcuma longa (turmeric) is a member of the Zingiberaceae family which is the same family of ginger is hepatoprotective due to its active constituents, which are :the flavonoid curcumin and volatile oils including :tumerone, atlantone, and zingiberone ,all are present in ginger also. The ratio of curcumin increases with storage time. In vitro and in vivo animal studies provide evidence for the hepatoprotective effects of turmeric has been found to protect animal livers from a variety of hepatotoxic substances, including carbon tetrachloride and paracetamol [33]. Dietary supplementation of turmeric in rats was found to significantly protect against iron-induced lipid peroxide formation. In addition to its antioxidant effects, curcumin has also been shown to enhance liver detoxification by increasing the activity of glutathione S-transferase [34]**.**Therefore the hepatoprotective effects of turmeric may stem from its potent antioxidant effects [35].Ginger aqueous extracts is able to reduce platelet thromboxane formation from exogenous arachidonate which confirms its anti-inflammatory activity [36].Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase- 1 and cyclooxygenase-2. It also suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase. This pharmacological property distinguishes ginger from nonsteroidal anti-inflammatory drugs.

This discovery preceded the observation that dual inhibitors of cyclooxygenase and 5-lipoxygenase may have a better therapeutic profile and have fewer side effects than non-steroidal anti-inflammatory drugs. The characterization of the pharmacological properties of ginger entered a new phase with the discovery that a ginger extract (EV.EXT.77) derived from Zingiber officinale (family Zingiberaceae) and Alpina galanga (family Zingiberaceae) inhibits the induction of several genes involved in the inflammatory response. These include genes encoding cytokines, chemokines, and the inducible enzyme cyclooxygenase-2. This discovery provided the first evidence that ginger modulates biochemical pathways activated in chronic inflammation [37].

In the present study, the hepatoprotective effect of ginger aqueous infusion when given orally to the rats was evident by significant reduction in ALT, AST, ALP and total bilirubin in rats groups receiving ginger before giving paracetamol when compared to the rats group receiving paracetamol only.

So from this study as well as what was mentioned in other previous studies, we can conclude that ginger appears to be a herb that can be used for several purposes besides its use for its aroma in cooking, because it has nutrient and medical values. But further studies involving animals and more studies involving human volunteers should be done before approving its use as a supplement for treatment of the liver diseases. Moreover precautions should be done before its trial on patients who have diseases that ginger may worsen, this is because natural substances can interact with medicine, be inappropriate for many health conditions and be harmful in high doses.

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