Evaluation of Immunostimulatory and Antioxidant Activities of Ginger and Moringa Extracts Against Paracetamol Induced Hepatic Damage in Rats.

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Abstract

The present study was conducted to investigate the ameliorative effect of both *Zingiber officinale* and *Moringa oleifera* ethanolic extracts on rats intoxicated by paracetamol through some immunological and antioxidant studies.

One hundred and eighty apparently healthy male albino rats were divided into 12 equal groups, each of 15 rats. Gp. (1) kept as control, Gp. (2) was orally given paracetamol (2g/kg) for 4 weeks, Gp. (3) was orally given ginger ethanolic extract (200 mg/kg) for 4 weeks, Gp. (4) was orally given moringa ethanolic extract (200 mg/kg) for 4 weeks, Gp. (5) was orally given silymarin (200 mg/kg) for 4 weeks. Gps. (6,7,8) (prophylaxis groups) were administered ginger, moringa ethanolic extracts or both extracts respectively for 2 weeks then paracetamol for another 2 weeks by the previous doses. Gps. (9, 10, 11) were firstly given paracetamol for 2 weeks then the plant extracts for the following 2 weeks. Gp. (12) was orally given paracetamol for 2 weeks then silymarin for the other 2 weeks. Blood samples were collected at the end of the 2nd and 4th week of experiment. Liver, kidney and spleen specimens were collected and immediately fixed in 10% neutral buffered formalin for histopathological examination.

The results showed lymphopenia and granulocytosis in gp. (2) all over the experiment with increase phagocytic index only at the 2nd week in addition to increase hepatic MDA and reduction in catalase levels compared to the normal control. The prophylactic groups (6,7,8) manifested good amelioration in the fore mentioned parameters in comparison to treatment ones (9-12). It could be concluded that the natural plants are effective as a prophylaxis theraby against paracetamol toxicity not as a treatment ones. Combination of plant extracts with chemical drug (paracetamol) may give a new challenge in the face of diseases.

Key words: paracetamol over dose, ginger, moringa and hepatic damage.

Introduction

Liver diseases are still a worldwide health problem. Considering the hazards of treatment failure such as drug resistance and heavy costs of therapy ***(Magda et al., 2011).*** Remediation of liver injuries by natural plants and herbs has been applied in India since ancient times and distributed all over the world by prime pharmaceuticals ***(Arpita et al., 2011).*** Some plants and herbs used today are valued for their antioxidant and hepato-protective effects as *Zingiber officinale* (ginger) and *Moringa oleifera* (moringa). Ginger has many medicinal effects that proved by many recent scientific researches such as antioxidant, hepatoprotective **(*Yemitan and Izegbu, 2006)*** and immuno-stimulatory effects ***(Zhou et al., 2006).*** *Moringa oleivera* is the most widely spread species of a monogeneric family Moringaceae ***(Anwar et al., 2007).*** Several active components as glucosinolates, isothiocyanates, thiocarbamates and flavonoids supported the antioxidant activity of *Moringa oleifera* ***(Santos et al., 2012).*** Hepatic damage caused by overdose or long use of paracetamol exist important practical troubles that may reach to about 40 % of acute liver failure cases ***(Wallace, 2004).***

The aim of the present work was to investigate the prophylactic and curative effects of both *Z. officinale* and *M. oleifera* extracts against paracetamol toxicity through evaluation of the immune response and antioxidant status of rats.

Materials and methods

I. Materials

1- Experimental animals:

A total number of 180 apparently healthy adult male albino rats (100-150 g average body weight) were obtained from the laboratory animal housing, Faculty of Veterinary Medicine, Zagazig University. The animals were housed in metal cages under hygienic conditions. They were given balanced ration with water *ad-libitum* and observed for 7 days before starting of experiment.

2- Plants:

Ginger rhizomes were obtained from the local markets. *M. oleifera* leaves were obtained from herbalist. They used at a dose of 200 mg/kg b.wt orally according to ***Ajith et al. (2007) and Fakurazi et al. (2008)*** respectively**.**

3- Drugs:

Silymarin powder (Livamarin) was obtained from European Egyptian Pharm. IND. (Alexandria, Egypt). It used by the dose of 200 mg/kg b.wt orally for 4 weeks ***(Kanchanai and Sadiq, 2011).*** Acetaminophen (paracetamol) cetal was obtained from PHARCO pharmaceuticals (Alexandria, Egypt) and used at a dose of 2g/kg b.wt orally for 4 weeks ***(Eesha et al., 2011).***

II. Methods

Blood samples:

At the end of the 2nd and 4th week of experiment, blood sample was collected from the retro-orbital

venous plexus of each rat and divided into two parts. The 1st part (1ml) was taken in clean EDTA tube for hematological studies (leukogram) ***(Coles, 1986),*** while the 2nd one (3 ml) was taken in heparinized tube for phagocytic assay.

Tissue specimens:

Samples from the liver, kidney and spleen were collected from all groups in 10 % formalin for histopathological studies. Also, specimens of liver were taken and homogenized for measurement of MDA and CAT activities.

Hematological techniques:

Leukogram:

The total and differential leukocytic counts were carried out by using automatic cell counter ***(Feldman et al., 2000)***

Evaluation of phagocytic activity & phagocytic index

This method is based on the uptake of *C.albicans* by monocytes over a certain period of time. Under the oil immersion lens of a light microscope, a total number of 100 phagocytic cells were counted randomly in about ten microscopic fields. The total number of phagocytes which ingested yeast cells was determined to calculate the percentage of phagocytosis and phagocytic index ***(Wilkinson, 1977)***

Phagocytic (%): is the number of monocytes ingesting *Candida albicans.*

$$\frac{\text{Number of monocytes containing } \textit{Candida albicans}}{\text{Total number of counted monocytes}} \times 100$$

Phagocytic index: is

$$\frac{\text{Total number of counted monocytes}}{\text{The number of } \textit{C. albicans} \text{ ingested by 100 monocytes.}}$$

Antioxidant studies:

Hepatic malondialdehyde (MDA) and catalase (CAT) activities were performed according to ***Ohkawa et al. (1979) and Aebi (1984)*** respectively using test kits of spectrum.

Statistical analysis:

The obtained data were statistically analyzed by F- test as stated by ***Tamhane and Dunlop (2000).*** Means at the same column followed by different letters were significantly different and the highest value was represented with the letter a.

Experimental design (Table 1): *One hundred and eighty (180) adult male rats were divided into 12 groups; each group contained 15 rats.*

Groups	Treatments (dose and duration)				Sampling
	Paracetamol (2g/kg b.wt orally)	Ginger (200mg/kg b.wt orally)	Moringa (200mg/kg b.wt orally)	Silymarin (200mg/kg b.wt orally)	At the end of 2nd and 4th weeks of experiment
1	-	-	-	-	
2	For 4 weeks	-	-	-	
3	-	For 4 weeks	-	-	
4	-	-	For 4 weeks	-	
5	-	-	-	For 4 weeks	
6	For the last 2 weeks	For the first 2 wks	-	-	
7		-	For the first 2 wks	-	
8		For the first 2 wks	For the first 2 wks	-	
9	For the first 2 weeks	For the last 2 wks	-	-	
10		-	For the last 2 wks	-	
11		For the last 2 wks	For the last 2 wks	-	
12		-	-	For the last 2 wks	

RESULTS

Regarding to the leukogram, Gp. (2) showed highly significant decrease in lymphocytic count with graulocytosis all over the experiment in addition, leukopenia observed only at the 4th week of experiment in this group. These parameters statistically changed for better in plant prophylactic groups at the end of 4th week of experiment with the best value in Gp. (8). While, only gps. (11, 12) showed slight improvement in leukogram picture compared to paracetamol treated group (Tables 2 and 3).

Moreover, highly significant increase in phagocytic index was observed in all groups with non-significant changes in monocytic count and phagocytic % compared to normal control at the 2nd week of experiment, while at the end of 4th week, Gp. (8) showed highly significant increase in monocytic count and phagocytic % and groups (6, 8, 11) showed highly significant increase in phagocytic index compared to gp. (2) , the highest value observed in gp. (8) (Tables 2 and 3).

Concerning the results of lipid peroxidation and antioxidant

enzymes, hepatic MDA showed highly significant increase with reduction in catalase enzyme in paracetamol treated group (gp.2) at both periods of experiment compared to normal control. Groups (6,7,8) and gps (9-12) showed highly significant reduction in hepatic MDA with elevation in CAT activity compared to gp. (2) at the end of 4th week of experiment (Table 4).

Table 2: *Leukogram (χ 10^3 / μl) and phagocytic activity of experimental groups (Mean values±S.E) at the 2nd week of experiment.*

Periods	2 weeks					
Groups	**TLC**	**Lymphocytes**	**Monocytes**	**Granulocytes**	**Phagocytic %**	**Phagocytic index**
Gp.1	10.07±0.48 **b**	8.65±0.36 **c**	1.01±0.03	0.44±0.09 **b**	82.4±0.40	2.98±0.06 **d**
Gp.2	14.50±1.19 **a**	4.05±0.37 **d**	0.95±0.04	9.5±0.88 **a**	80.8±0.49	4.82±0.04 **a**
Gp.3	15.53±1.54 **a**	13.82±1.44 **ab**	0.98±0.06	0.73±0.05 **b**	82.8±0.49	4.24±0.08 **b**
Gp.4	17.07±0.98 **a**	15.62±0.96 **a**	0.91±0.05	0.54±0.04 **b**	81.6±0.93	4.34±0.14 **b**
Gp.5	13.43±1.18 **ab**	11.64±1.05 **b**	0.94±0.05	0.85±0.03 **b**	83.6±0.40	3.50±0.2 **c**
Gp.1	10.07±0.48 **b**	8.65±0.36 **c**	1.01±0.03	0.44±0.09 **b**	82.4±0.40	2.98±0.06 **d**
Gp.2	14.50±1.19 **c**	4.05±0.37 **d**	0.95±0.04	9.5±0.88 **a**	80.8±0.49	4.82±0.04 **b**
Gp.6	15.60±0.71 **ab**	13.99±0.64 **b**	0.88±0.03	0.73±0.03 **b**	82.4±0.75	4.16±0.12 **c**
Gp.7	16.67±1.06 **ab**	15.03±0.89 **ab**	1.04±0.08	0.60±0.13 **b**	82.6±1.03	4.02±0.04 **c**
Gp.8	18.50±1.23 **a**	16.71±1.05 **a**	0.87±0.05	0.93±0.14 **b**	83.2±0.49	5.22±0.05 **a**
Gp.1	10.07±0.48 **b**	8.65±0.36 **a**	1.01±0.03	0.44±0.09 **b**	82.4±0.40	2.98±0.06 **d**
Gp.2	14.50±1.19 **a**	4.05±0.37 **c**	0.95±0.04	9.5±0.88 **a**	80.8±0.49	4.82±0.04 **b**
Gp.9	16.33±0.75 **a**	6.04±0.12 **b**	0.95±0.05	9.34±0.63 **a**	84±0.63	5.92±0.06 **a**
Gp.10	15.27±0.74 **a**	4.79±0.48 **bc**	1.02±0.09	9.46±0.25 **a**	81.2±1.02	4.80±0.1 **b**
Gp.11	16.12±1.28 **a**	3.92±0.87 **c**	1.04±0.11	11.14±0.43**a**	83±1.41	4.34±0.22 **c**
Gp.12	15.3±0.75 **a**	4.54±0.33 **bc**	0.95±0.06	9.81±0.66 **a**	82.8±0.49	4.34±0.07 **c**
F test	**	**	**N.S**	**	**N.S**	**

Means at the same column at the same period followed by different letters were significantly different and the highest value was represented with the letter a ****:** High significant at 0.01 probability

Table 3. *Leukogram (χ 10^3 /µl) and phagocytic activity of experimental groups (Mean values±S.E) at the 4th week of experiment.*

Periods	4 weeks					
Groups	**TLC**	**Lymphocytes**	**Monocytes**	**Granulocytes**	**Phagocytic %**	**Phagocytic index**
Gp.1	13.67±1.17 **b**	11.16±1.01 **b**	1.40±0.07 **b**	1.1±0.09 **d**	79.2±0.49 **c**	3.0±0.05 **c**
Gp.2	8.10±0.21 **c**	1.91±0.13**bc**	1.08±0.09 **b**	5.12±0.09 **a**	80.4±0.93 **bc**	3.06±0.05**c**
Gp.3	24.93±0.38 **a**	16.54±0.32 **a**	3.70±0.24 **a**	4.35±0.12 **b**	82±0.63 **ab**	4.40±0.07 **b**
Gp.4	25.93±1.94 **a**	17.55±0.96 **a**	4.30±0.92 **a**	4.09±0.09 **bc**	82.8±0.49 **a**	4.46±0.37 **b**
Gp.5	15.33±0.68 **b**	10.32±0.75 **b**	1.23±0.15 **b**	3.89±0.07 **c**	79±0.71 **c**	5.06±0.05 **a**
Gp.1	13.67±1.17 **a**	11.16±1.01**a**	1.40±0.07 **b**	1.1±0.09 **d**	79.2±0.49 **b**	3.0±0.05 **c**
Gp.2	8.10±0.21 **b**	1.91±0.13**c**	1.08±0.09 **b**	5.12±0.09 **a**	80.4±0.93 **b**	3.06±0.05 **c**
Gp.6	12.50±0.38 **a**	8.18±0.14 **b**	1.18±0.09 **b**	3.13±0.15 **bc**	80±0.63 **b**	3.54±0.09 **b**
Gp.7	13.19±1.20 **a**	8.67±1.04 **b**	1.16±0.05 **b**	3.36±0.21 **b**	79.2±0.49 **b**	3.32±0.11 **bc**
Gp.8	14.63±0.87 **a**	9.77±0.59 **ab**	2.13±0.18 **a**	2.74±0.29 **c**	84.8±0.49 **a**	4.80±0.21 **a**
Gp.1	13.67±1.17 **a**	11.16±1.01 **a**	1.40±0.07**ab**	1.1±0.09 **d**	79.2±0.49 **ab**	3.0±0.05 **bcd**
Gp.2	8.10±0.21 **d**	1.91±0.13 **d**	1.08±0.09 **b**	5.12±0.09 **a**	80.4±0.93 **ab**	3.06±0.05 **bc**
Gp.9	9.90±1.07 **cd**	4.51±0.59 **c**	1.99±0.35 **a**	3.39±0.26 **c**	80.2±0.66 **ab**	3.18±0.04 **ab**
Gp.10	10.40±0.72**bcd**	4.31±0.41 **c**	1.52±0.35**ab**	4.57±0.12 **ab**	79±0.71 **ab**	2.8±0.1 **d**
Gp.11	11.47±0.93**abc**	5.94±0.13 **bc**	1.65±0.34**ab**	3.87±0.49 **bc**	81.4±0.69**a**	3.38±0.11 **a**
Gp.12	13.03±1.12**ab**	7.0±0.61 **b**	2.15±0.26 **a**	3.88±0.31 **bc**	78.2±1.07 **b**	2.90±0.1 **cd**
F test	**	**	**	**	**	**

Means at the same column at the same period followed by different letters were significantly different and the highest value was represented with the letter a ****:** High significant at 0.01 probability

Table 4: *Hepatic catalase and MDA activities of experimental groups (Mean values±S.E) at the 2nd and 4th week of experiment.*

Periods	2 weeks		4 weeks	
Groups	**CAT U/g**	**MDA Nmol/g**	**CAT U/g**	**MDA Nmol/g**
Gp.1	1.49±0.02 **b**	26.91±0.78 **b**	1.55±0.02 **c**	25.90±0.67 **b**
Gp.2	1.13±0.02 **c**	69.15±2.16 **a**	1.06±0.01 **d**	75.39±1.79 **a**
Gp.3	1.94±0.008 **a**	16.68±0.27 **c**	2.40±0.06 **ab**	14.95±0.50 **c**
Gp.4	1.88±0.01 **a**	15.96±0.86 **c**	2.48±0.06 **a**	14.19±0.74 **c**
Gp.5	1.91±0.02 **a**	18.08±1.16 **c**	2.32±0.02 **b**	16.45±0.45 **c**
F test	**	**	**	**
Gp.1	1.49±0.02 **c**	26.91±0.78 **b**	1.55±0.02 **b**	25.90±0.67 **c**
Gp.2	1.13±0.02 **d**	69.15±2.16 **a**	1.06±0.01 **c**	75.39±1.79 **a**
Gp.6	1.93±0.02 **ab**	14.42±1.37 **c**	1.79±0.02 **a**	31.09±1.26 **b**
Gp.7	1.85±0.03 **b**	18.29±1.22 **c**	1.76±0.02 **a**	33.0±1.97 **b**
Gp.8	1.96±0.03 **a**	15.15±1.23 **c**	1.82±0.02 **a**	30.13±1.04 **bc**
F test	**	**	**	**
Gp.1	1.49±0.02 **a**	26.91±0.78 **c**	1.55±0.02 **a**	25.90±0.67 **d**
Gp.2	1.13±0.02 **c**	69.15±2.16 **a**	1.06±0.01 **d**	75.39±1.79 **a**
Gp.9	1.10±0.01 **c**	67.71±1.76 **ab**	1.39±0.01 **c**	60.37±1.29 **b**
Gp.10	1.18±0.008 **b**	70.63±1.51 **a**	1.42±0.01 **c**	62.6±1.81**b**
Gp.11	1.17±0.02 **bc**	62.43±2.39 **b**	1.50±0.008 **b**	55.07±1.05 **c**
Gp.12	1.14±0.02 **bc**	65.24±0.85 **ab**	1.47±0.01 **b**	53.86±0.96 **c**
F test	**	**	**	**

Means at the same column at the same period followed by different letters were significantly different and the highest value was represented with the letter a

****:** High significant at 0.01 probability

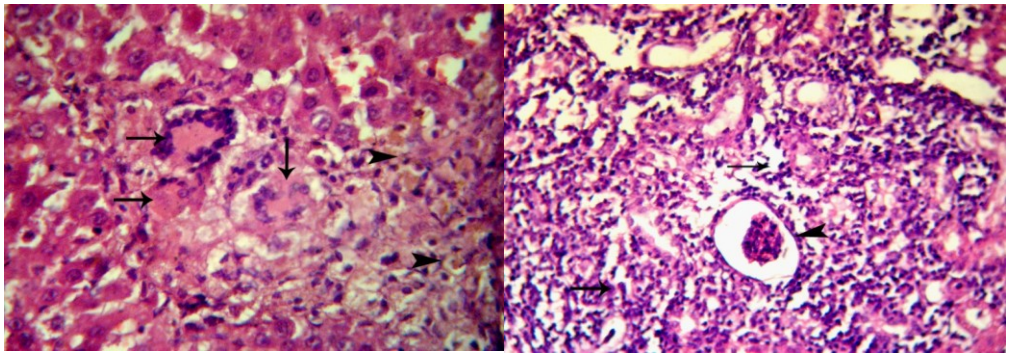

Fig. (1) : *Liver of Gp.(2), showing extensive necrosis surrounded with granulomatous reaction of giant cells (arrows) besides cholestasis of brown pigments (arrowheads) (H & E x 400).*

Fig. (2): *Kidney of Gp.(2) , showing extensive necrosis and aggregations of inflammatory cells replacing the renal tissue (arrows) besides shrunken glomerular tuft (arrowhead) (H & E x 400).*

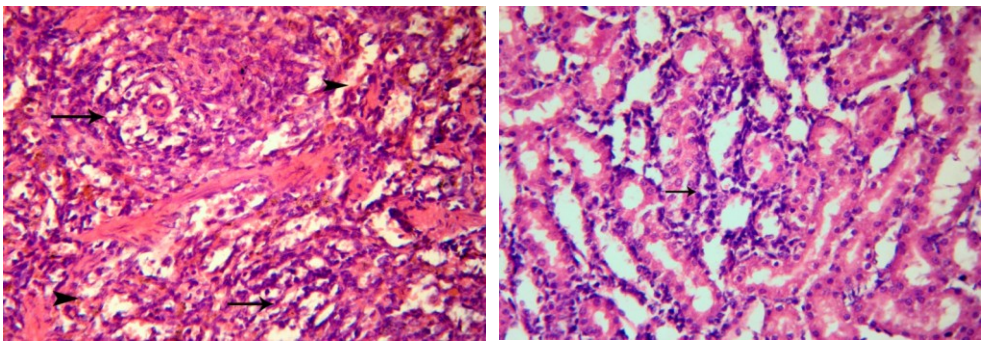

Fig. (3): *Spleen of Gp.(2), showing necrosis and depletion of the lymphoid cells in the white pulp (arrow) and increased the fibrous tissue in the red pulp (arrow head) (H & E x400).*

Fig. (4): *Kidney of Gp. (6), showing few lymphocytes infiltrations among the renal tubules (arrow) (H & E x 400).*

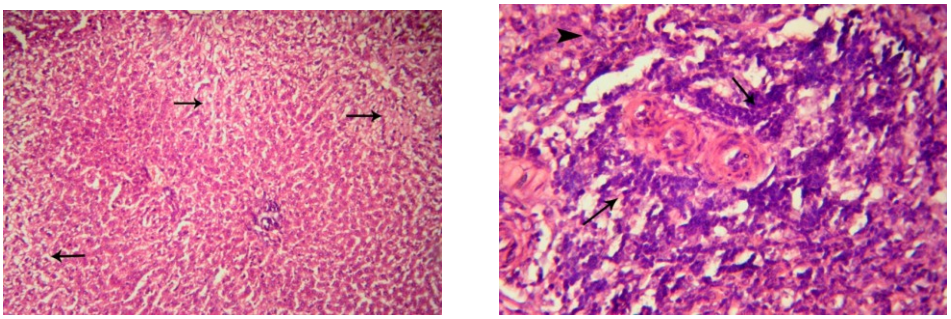

Fig. (5): *Liver of Gp. (8), showing normal hepatic tissue with mild hydropic degeneration (arrows). (H& E x 150).*

Fig. (6): *Spleen of Gp. (8), showing activation of the lymphoid cells in the white pulp with increased the mitosis (arrow and arrow head) (H & E x400).*

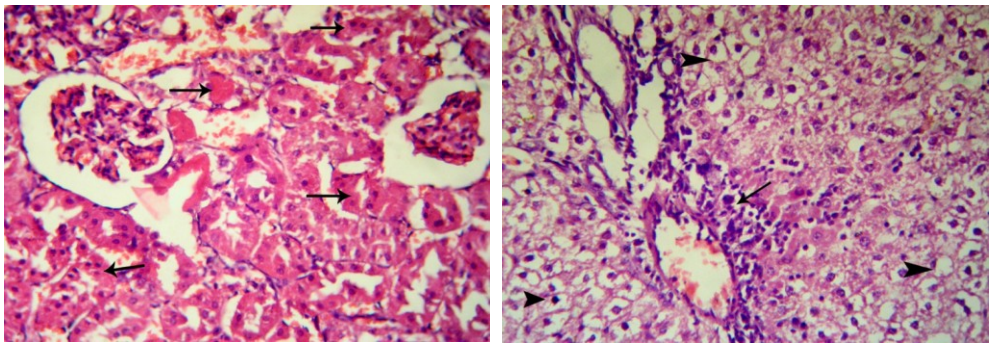

Fig.(7): *Kidney of Gp. (10), showing coagulative necrosis in renal tubular epithelia (arrows) (H & E x 400).*

Fig.(8): *Liver of Gp. (11), showing inflammed portal area with congested blood vessels and lymphocytes infiltrations (arrow) and hydropic degeneration (arrowheads) (H & E x 400).*

Discussion

The present study demonstrates granulocytosis with lymphopenia in paracetamol treated group at both periods of experiment that might be due to the stress condition coupled with inflammatory changes in the body tissue responsible for phagocytosis of toxic substances ***(Bhaumik and sharma, 2002).*** Granulocytosis may be due to neutrophilia that related to tissue destruction caused by toxic metabolite of paracetamol (NAPQI). Toxic chemicals and drug may result in tissue destruction with increasing the number of circulating neutrophils ***(Coles, 1974)***. Lymphopenia may be attributed to the immunosuppressive effect of paracetamol with necrosis of lymphoid tissues ***(Young et al., 1975)*** or could be due to the migration of lymphocytes to site of damaged tissue as a part of defensive mechanism of the immune system**.** Our results coincided with those of ***Ahur et al. (2013)*** who indicated that oral administration of paracetamol at 2g/kg b.wt to rats showed significant decrease in total leukocytic and lymphocytic counts with neutrophilia as compared to normal control. Furthermore, leukopenia observed in this group at the end of 4^{th} weeks may be due to the cumulative chemotoxic effect of paracetamol toxic metabolite on the lymphoid organs. This result correlates with the histopathological findings of spleen in figure (3). Similar findings were reported by ***Senthilkumar et al. (2014)*** who observed reduction in total leukocytic count of wistar rats received 2 g of paracetamol/kg b .wt for 14 days compared to control

group. Complement to the above, increase in the phagocytic index in gp. (2) at the end of 2nd week only, explaining the function of monocytes as phagocytic cell against any foreign substances and cellular debris that result from tissue necrosis caused by paracetamol toxicity. Our results are in agreement with ***Gordon and Taylor (2005)*** who reported that monocytes and tissue macrophage cooperate in the phagocytosis of pathogens and removal of damaged tissue by means of proteolytic digesion.

Prophylactic groups (6-8) showed significant improvement in leukogram manifested by leukocytosis and lymphocytosis with granulocytopenia compared to gp. (2) at the end of 4th week that indicate the effectiveness of both ginger and moringa extracts as prophylactic agents against paracetamol toxicity and their ability to alleviate the stress condition applied on rats. It was found that *M. oleifera* extracts play a plausible role in enhancing the immunity, may be due to the vitamins and minerals such as arginine and vitamin C content in this plant ***(Banji et al., 2012).*** Also the presence of variety of flavonoids with antioxidant activity in *M. oleifera* is responsible for improvement of blood picture ***(Kaviarsan et al., 2004).*** Similar findings as improvement in the blood parameters and total leukocytic count were reported in rats prophylactic with mixtures of different plants (Roselle, Moringa, Ginger and Ugwu) for 180 days before exposure to cement dust ***(Yahaya et al., 2012)***

Plant treated groups did not show any enhancement in leukogram except gp. (11) that might be due to the synergistic beneficial effect of *Z. officinale* and *M. oleifera* together. This partially agree with the results presented by ***Osman et al. (2012) and Tende et al. (2012)*** who recorded non-significant changes in total and differential leukocytic count of normal rats administered daily with moringa and ginger extracts respectively for nearly 4 weeks. Silymarin treatment (gp.12) clarified some degree of amelioration as the silymarin is a potent protein inducer via stimulation of RNA and DNA synthesis which is necessary for regeneration after toxic and inflammatory insults ***(Machicao and Sonnenbichler ,1977)***

Increase in hepatic MDA concentration with decrement in catalase level in paracetamol treated group may be attributed to over production of free radicals and reactive oxygen species during paracetamol metabolism that leads to exhaustion of natural body antioxidant system and enhanced lipid peroxidation. Covalent binding of NAPQI, an oxidative product of paracetamol to sulphydryl group of protein cause rapid depletion of intracellular GSH resulting in cell necrosis and lipid peroxidation

(Jollow et al., 1974). This is in agreement with ***Yanpallewar et al. (2002)*** who indicated that administration of paracetamol at 2g/kg for 7 days caused significant increase in MDA level.

Treatment with plant extracts abrogated the paracetamol induced decrease in catalase activity and elevation of lipid peroxidation marker. This suggests the ability of their antioxidant constituents to break lipoperoxidation chain reaction and facilitate the removal of reactive oxygen species generated by paracetamol over dose. Our results harmonize with the preceding findings of ***(Hamid et al., 2011 and Fakurazi et al., 2012)*** who mentioned that administration of ethyl acetate extract of *Zingiber Zerumbe* and *M.oleifera* hydro-ethanolic extract at doses of 200 and 400mg/kg protected the rats from paracetamol hepatotoxicity by inhibition of liver MDA elevation and restoring the level of antioxidant enzymes (CAT and SOD).

It can be concluded that, Combination of two plants as ginger and moringa may provide a novel therapeutic strategy against paracetamol induced hepatic damage better than each alone as demonstrated by enhancing the immune status and antioxidant defense mechanism of rats.

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الملخص العربى

تقييم النشاط المناعى والمضاد للاكسدة لمستخلصات الزنجبيل والمورينجا ضد العطب الكبدى المحدث بالباراسيتامول فى الفئران

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أجريت هذه الدراسة لتقييم استخدام كلا من نبات الزنجبيل والمورينجا ضد العطب الكبدى المحدث بالباراسيتامول عن طريق بعض الدراسات المناعية ومضادات الاكسدة.

لقد استخدم فى هذه الدراسة عدد مائة و ثمانين من ذكور الفئران البيضاء السليمة ظاهريا تم تقسيمهم الى اثنتى عشرة مجموعة متساوية كل مجموعة خمسة عشر فأرا. المجموعة الاولى تركت بدون معاملة كمجموعة ضابطة. المجموعة الثانية أخذت الباراسيتامول بجرعة ٢ جم / كجم من وزن الجسم عن طريق الفم من اليوم الاول من التجربة ولمدة ٤ اسابيع متتالية. المجموعة الثالثة اخذت المستخلص الكحلى لنبات الزنجبيل(٢٠٠ مجم/كجم) لمدة ٤ اسابيع. المجموعة الرابعة اخذت المستخلص الكحلى لنبات المورينجا(٢٠٠ مجم/كجم) لمدة ٤ اسابيع.المجموعة الخامسة أخذت السليمارين بجرعة ٢٠٠ مجم/كجم لمدة ٤ اسابيع. المجموعات ٦و٧و٨ تم تجريعها بمستخلصات النباتات اولا لمدة اسبوعين ثم اخذت الباراسيتامول فى الاسبوعين التاليين من التجربة بنفس الجرعات السابقة. المجموعات ٩و١٠و١١ اخذت الباراسيتامول فى اول اسبوعين ثم تم اعطاؤها مستخلصات النباتات فى الاسبوعين التاليين. اما المجموعة ١٢ اخذت الباراسيتامول فى اول اسبوعين ثم اخذت دواء السليمارين فى الاسبوعين التاليين. تم تجميع عينات من الدم عند نهاية الاسبوع الثانى والرابع من التجربة.وقد اوضحت نتائج تحاليل الدم والمناعة الخلوية وجود نقص معنوى فى الخلايا الليمفاوية مع زيادة فى الخلايا متعددة النواة فى المجموعة الثانية على مدار التجربة مع زيادة فى معدل التهام العدوى فقط عند نهاية الاسبوع الثانى من التجربة مقارنة بالمجموعة الضابطة، كما لوحظ زيادة فى مستوى المالونديهيد مع نقص فى انزيم الكاتليز فى نسيج الكبد فى هذه المجموعة. ادى استخدام كلا من نبات الزنجبيل والمورينجا كوقاية للكبد ضد الباراسيتامول الى تحسن ملحوظ فى هذه القياسات مقارنة بالمجموعات العلاجية. لذا نستخلص من هذه الدراسة عدم كفاءة استخدام هذه النباتات كعلاج للعطب الكبدى ولكن قد يؤدى استخدامها كوقاية مع الادوية الكيميائية الى خلق تحديات جديدة فى مواجهة العديد من الامراض.