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The Effect of Ginger (*Zingiber officinale* Roscoe) Extract on Liver Histopathology and Alanine Aminotransferase Serum Level in Carbofuran-Induced Rats

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Abstract: The aim of this study is to investigate the hepatoprotective effect of ginger extract in different doses against hepatotoxicity caused by carbofuran in male wistar rats from the histological and biochemical aspects. Twenty five male rats were divided into five groups, each representing as A: control negative (normal saline), B: control positive (0.8mg of carbofuran 10%), C: first experimental dose (0.8mg of carbofuran ang 500mg of ginger extract), D: second experimental dose (0.8mg of carbofuran (10%) and 750mg of ginger extract), E: third experimental dose (0.8mg of carbofuran and 1000mg of ginger extract). Study duration was one month. Liver specimen were examined by light microscope and the serum from all groups were examined by spectrophotometry. Resulted in a significant reduction in the levels of alanine aminotransferase (ALT) enzymes in comparison with the carbofuran treated animals; ginger extract also improves the histological changes produced by carbofuran in the liver tissues. The present study indicated that ginger had protective effect against liver damage induced by carbofuran and this is caused by antioxidant activities.
Key words: alanine aminotransferase, carbofuran, ginger extract, liver histopathology.

Introduction

Carbofuran (2, 3-dihydro -2.2-dimethyl-7- benzofuranyl methylcarbamate) generally called Furadan is a broad-spectrum carbamate pesticides that kill insects, mites and nematodes on contact or after ingestion[1].Carbofuran is most commonly used in agriculture today[2]. It has high toxicity by inhalation and ingestion, moderate toxicity by dermal absorption[1]. Some workers have speculated in reactive oxygen species (ROS) production that induced by carbofuran may have role inhibition of cytochrome c oxidase and creatine kinase[3]. Also, the carbofuran induced nitric oxide synthase activity has been implicated in the overproduction of superoxide anions[4]. Carbofuran can increased lipid peroxidation level, decreased GSH contents and antioxidant enzymes activity in the liver[2].

Liver is the main organ that has function to metabolite biological toxin and medicinal agent. Hence, metabolism is always associated with the interference of hepatocyte biochemistry and ROS production. ROSs are involved in liver damage[5], some studies have been reported that carbofuran induced liver tissue damage and alanine transaminase (ALT) serum elevation[6, 7].

Herbal medicine is commonly used for various clinical diseases. Plant extract that have natural antioxidants against chemically induced toxicities is got more attention[8].Ginger is one of the world's best known spices, belongs to Zingiberaceae family. It has also been universally used throughout history for its health benefits[9]. It contains many medicinal properties and widely used for treating the disease such as cold, headaches, nausea, stomach upset, diarrhea, digestive, gastrointestinal disorders, rheumatic complaints, diarrhea, nausea, asthma, parasitic infections, arthritis and muscular discomfort[10].

The main component of ginger are volatile oil, phenolic derivatives (zingerone) and oleoresin (gingerols and shogaols) which have antioxidant effect. It can scavenge superoxide anion and hydroxyl radicals. *Z. officinale* can inhibit the activity of lipoxygenase and peroxydase. Barakat and Mohamed (2011)[11] showed the potency of ginger for protecting liver against oxidative stress and hepatocellular injury that follows a supra therapeutic dose of acetaminophen[12]. In addition, it has been shown that this plant has a number of analeptic properties as an antioxidant, anti-lipidemic, antihyperglycemic, anti-inflammatory and anti-cancer agent. This medicinal herb is excellent for oral therapy as it is effective, non-toxic and without serious side effects[13].

So far, there is no information exists concerning the beneficial effects of ginger extract against carbofuran-induced toxic effect on the liver. Hence, the present study has been undertaken to evaluate the possible ameliorative effect of ethanolic ginger extract on liver of carbofuran-induced rats.

Experimental

Chemical

Carbofuran; Furadan 10% [2, 3-dihydro -2, 2-dimethyl-7-benzofuranyl methyl carbamate]. Were obtained from pharmacology laboratory, Faculty of medicine, Brawijaya University, Malang, Indonesia

Experimental design

Healthy, adult male Wistar albino rats weighing 100-150 g, aged 2-3 months were used in this study. The animals were provided by the pharmacological laboratory in the medical faculty of Brawijaya University in Malang, divided into five groups as A, B, C, D and E each comprising of five animals, had free access to a standard commercial diet, water and were kept in rooms maintained at 25 ± 1°C. Five animals of each subgroup were kept in one cage and treated for 30 days. Animals of group A (control -ve) were given normal saline orally. Group B (control +ve) were administered 0.8 mg/kg bw of carbofuran. The experimental animals of group C were administered combination of 0.8 mg/kg bw of carbofuran with 500mg/kg bw of ginger ethanolic extract, group D were administered combination of 0.8 mg/kg bw of carbofuran with 750mg/kg bw of ginger ethanolic extract and group D were administered combination of 0.8 mg/kg bw of carbofuran with 1000mg/kg bw of ginger ethanolic extract.

Extraction of plant material

Ginger (*Z. officinale Roscoe*) rhizomes were provide by Dinas Kesehatan Propinsi Jawa Timur-Materia Medica Batu City-East Jawa Timur Indonesia. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into pieces, air dried and powdered 100g of powder were macerated in 1000ml ethanol for 12 h at room temperature and then filtered through 5 µm filter to obtain the final extract. The concentrations of the ginger extract are 500mg/ml, 750mg/ml and 1000mg/ml. Each animal in the present study was given 1ml of the final ginger ethanolic extract orally[14].

Blood sampling

The blood from each animal was collected into a clean centrifuge tube. The blood left to coagulate and was centrifuged at 3000 rpm for 30 minutes to separate the serum. The separated serum was stored at -20°C for subsequent biochemical and enzyme analyses.

Alanine aminotransferase Serum Measurement

Colorimetric Determination of Alanine Transaminase activity. The EnzyChromTM ALT Assay Kit (Cat# EALT-100) were purchased from Bioassay Systems, Place, Hayward California USA. It was used for the determination of serum ALT following to the analytical protocol supplied by the kit manufacturer.

Histopathological Examination Method of the Liver

Liver samples were processed histologically for paraffin sections of 5µm thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. Liver specimens were evaluated using

light microscope (Nikon Eclipse Ci equipped with Digital Camera Optilab plus 12 Megapixel), Histopathological method for examination in liver used Histology Activity Index (HAI)[15]. Histopathological examination field divided two field of view, area 1 and area 2. Area 1 consist of zone 3 (around vena centralis/centrolobularis), have two parameters: confluent necrosis and Focal (spotty) lytic necrosis, apoptosis and focal inflammation and area 2 consist perilobular zone and lobular zone have three parameters: Periportal or periseptal interface hepatitis (piecemeal necrosis), Portal inflammation and Ishak Stage. Each area was observed with five field of view with magnification 400x. Where one slide per animal total 25 slides. Grading and staging (HAI) with different area.

Result from every field of view grading and staging (HAI) inserted in formula:

$$\text{Mean of HAI} : = \frac{[\text{Score HAI in field of view 1} + \cdots \text{Score HAI in field of view N}]}{\text{Number field of view examined}}$$

Where: N= Number of field of view

Ishak score for each sample group obtained from formula below:

$$\text{Ishak Score} : = \frac{[A+B+C+D+E]}{4}$$

Where: A. Confluent necrosis

B. Focal (spotty) lytic necrosis, apoptosis and focal inflammation

C. Periportal or periseptal interface hepatitis (piecemeal necrosis)

D. Portal inflammation

E. Ishak Stage

4 = Number HAI

Statistical analysis

Data analysis of histopathological examination of liver was conducted using Mann– Whitney U-test for histopathological scores were used to analyze the significance of differences among groups. The differences were accepted as statistically significant when $P < 0.05$.

Data analysis of Alanine aminotransferase was conducted using one way ANOVA statistic. Data analysis is done at the trusted rate 95 % ($\alpha = 0.05$) and probability rate of ($p=0.05$).

Results

Effect of different Doses of Ginger ethanolic extract on liver histopathology in carbofuran-induced rats.

A Histopathology of the liver observed by light microscopic examination. Area 1 Centrilobularis area (Zone 3) from each group.

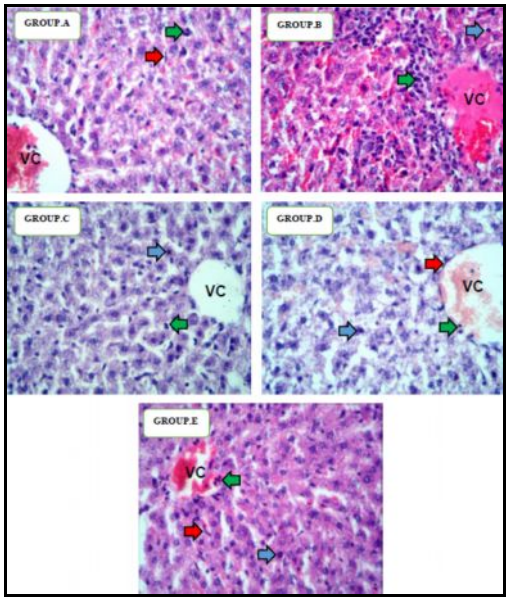

Figure 1. Histopathological Changes in rat's Liver (Area 1).Centrilobularis area (Zone 3) from all groups (H.E stain, 400x).

Description: blue arrow=necrotic hepatocyte green arrow= inflammatory cell (mononuclear) and red arrow = fibroblast cell VC=Vena centralis.

Group A (control –ve) showed absence necrotic hepatocyte, but showed mild infiltration inflammation cell and fibroblast cell in sinusoid and on the edge vena centralis. **Group B** showed necrotic hepatocyte with massive severe inflammation cell infiltration around vena centralis, and sinusoid. **Group C** showed moderate necrotic hepatocyte with mild inflammation cell infiltration and fibroblast cell. **Group D** showed mild necrotic hepatocyte with mild inflammation cell infiltration and fibroblast cell around vena centralis. **Group E** showed mild necrotic hepatocyte with mild inflammation cell infiltration around vena centralis and fibroblast cell at sinusoid.

B. Histopathology of the liver observed by light microscopic examination. (Area 2 (Lobularis area) form all groups).

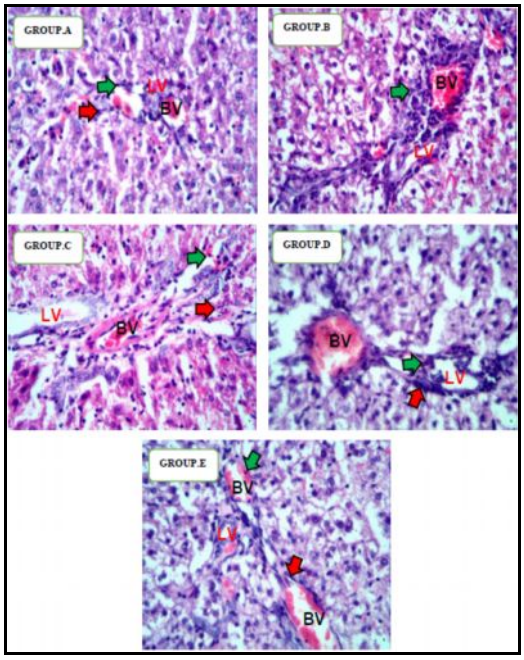

Figure 2. Histopathological Changes in rat's liver (Area 2). Lobularis area from all groups (H.E stain, 400x).

Description: green arrow = inflammatory cell (mononuclear) and red arrow = fibroblast cell BV=Blood Vessel, LV=Lymphatic Vessel.

Group A (Control **–ve**) showed mild infiltration inflammation cell and fibroblast cell around portal triad. **Group B** (control **+ve**) showed severe (massive) infiltration inflammation cell and fibroblast cell around portal triad. **Group C** And **Group D** showed mild infiltration inflammation cell and fibroblast cell around portal triad, **Group E** showed very mild infiltration inflammation cell and fibroblast cell around portal triad.

C. The histopathological scoring of liver sections from each group

Table 1. Mean of histology Activity Index (HAI) (ishak Score) from each group

Groups		Ishak Score					Mean
		Area 1		Area 2			
		Confluent necrosis	Portal inflammation	Periportal or periseptal interface hepatitis (piecemeal necrosis)	Focal (spotty) lytic necrosis, apoptosis and focal inflammation	Ishak Stage	
Group A	1	1,2	1,2	1,6	1,6	1,6	1,44
	2	1	1,8	1,6	1	1	1,28
	3	1	1,4	1,2	1,6	1	1,24
	4	2	1,4	1,6	1	1,2	1,44
	5	2	1,4	1,4	1,4	1,6	1,56
						Mean	**1,392**
Group B	1	2,4	2	1,6	2	2	2
	2	2	1,2	2	2	2	1,84
	3	1,8	1,8	2	2	2,2	1,96
	4	1,8	1,2	1,4	1,4	2,2	1,6
	5	2,6	1,8	3	2	3	2,48
							1,976
Group C	1	1,8	1,6	1,2	1,6	1,6	1,56
	2	1,6	1,2	1,6	1,2	1,4	1,4
	3	1,2	1,4	1,8	2	2	1,68
	4	1,6	1,2	1,2	1,2	1,8	1,4
	5	2,6	1,6	2,4	1,6	2,4	2,12
						Mean	**1,632**
Group D	1	1,4	1,4	1,4	1,4	2	1,52
	2	2	1,4	2	0,6	2	1,6
	3	1,6	1,6	2	2	2,4	1,92
	4	2	2	0,8	0	2	1,36
	5	1,6	1,2	1,4	1,4	2	1,52
						Mean	**1,584**
Group E	1	1,6	1,6	1,8	1	2	1,6
	2	1,8	1,8	1,8	1	1,6	1,6
	3	1,2	1	2	2	2,2	1,68
	4	1,2	0,8	1	2	2,2	1,44
	5	1,4	1	1,2	1,2	1,2	1,2
						Mean	**1,504**

Table 1 describe about Mean of histology Activity Index (HAI) (ishak Score) for each group which shows that the ginger extract at a dose of group E (1000 mg) can lower mean of (ishak Score) to the average of the lowest rather than the provision of Ginger extract at a lower dose is group D (750 mg), so doses of 1000 mg was more effective in lowering mean (ishak Score) than the dose of 750 mg. However, the provision of ginger extract at a dose of 750 mg can reduce the average mean of (ishak Score) better than a dose of group C (500 mg , so a dose of 750 mg was more effective in lowering mean of (Ishak score) than a dose of 500 mg . The average of (ishak Score) in granting ginger extract at a dose of 500 mg was more effective in lowering mean of (ishak Score) rather than the group B positive control group

Table 2. Result histopathological scoring of liver sections from each group

Score	Group	Comparative group	Significance
Ishak Score	**Group 1**	**Group 2**	0.009**
		Group 3	0.206
		Group 4	0.115
		Group 5	0.243
	Group 2	**Group 3**	0.116
		Group 4	0.036*
		Group 5	0.026*
	Group 3	**Group 4**	0.753
		Group 5	0.833
	Group 4	**Group 5**	0.916

****Significance at p<0.01 and *Significance at p<0.05**

From table 2 result of analysis of Histology Activity Index (HAI) of histopathology changes of liver. The histopathological scoring of liver sections from all groups through a Mann-whitney test, we obtained the following results:

Group A Control -ve with normal saline was significantly differing compared to group B: Control +ve carbofuran with dose 0.8mg ($p< 0.01$).

Concurrent treatment with carbofuran and ginger significantly attenuated the extent and severity of the histological features of liver damage induced by carbofuran alone.

Confluent necrosis, Portal inflammation, Periportal or periseptal interface hepatitis (piecemeal necrosis), Portal inflammation and Ishak Stage) scores in the liver sections from carbofuran treated rats group B were significantly reduced in rats treated with ginger and carbofuran group E , D

($p<0.05$).

Effect of different Doses of Ginger ethanolic extract on liver histopathology in carbofuran induced rats.

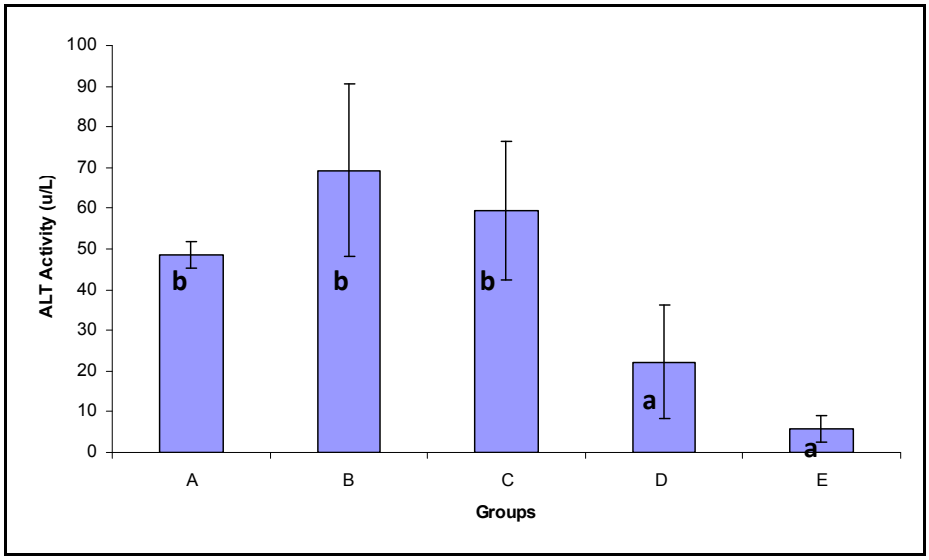

Figure 3. Bar Graph of mean of Alanine aminotransferase in serum for each group

Figure 3 shows the difference in dose of ginger extract influence or different effects on ALT serum. The presence of the effect of ginger extract is starting to look when the concentration of ALT serum levels in carbofuran-induced rats becomes lower, after the treatment was given in the form of ginger extract started at a dose of 500 mg (group C), compared with ALT serum in the (group B) positive control group. Then the ALT

serum decreased at higher dosage. Thus, based on the assessment descriptively according to the mean ALT serum is, it can be said that the administration of treatment in the form of ginger extract at a dose of 500 mg (group C), 750 mg (group D) and 1000 mg (group E) showed different influences, where the higher dose of ginger extract provided will further lowering the ALT serum.

Based on the results of analysis of variance for ALT serum data, showed a significance value of 0.000 ($p < 0.05$), and it can be concluded that there are differences in ALT serum data in each treatment group of ginger extract.

Discussion

The present study was carried out to determine the protective effect of the orally administered aqueous ginger extract against hepatotoxicity and liver histopathology change induced by carbofuran in adult male wistar rats. In this research we found that on liver histopathology in the group E treated has highest rate of effect compared to other groups, followed by group D and group C. This indicates that the ginger extract at high dose led to decrease liver histopathology change, increasing the ginger extract doses will reduce the histopathology changes on liver. Even though the highest doses of ginger extract, gives the best effect on decreasing liver changes, it showed the mildest necrotic hepatocyte with mild inflammation cell infiltration around vena centralis and fibroblast cell at sinusoid and around portal triad. This condition may related to the bioactive of molecules of ginger like gingerols that shown has antioxidant activity in various modules[16]. Antioxidant are formed to protect the cells and organ systems of the body against reactive oxygen species thus preventing them from causing damage[17]. In addition to antioxidant effects, ginger may also exert its hepatoprotective effect by means in different ways. For example in the mechanism of carbofuran toxicity it was shown that carbofuran induces liver cells damage. Carbofuran disturb antioxidant balance leading to oxidative stress. Mitochondria are the major cellular sources of ROS and key contributors to many diseases. Almost all intracellular ATP is known to be generated in the mitochondria and about one-third of the cellular adenine nucleotides are located in this organelle. Therefore, chemicals causing mitochondrial dysfunction may deplete ATP, leading to excessive generation of ROS. accumulation of oxidative damage to mitochondria in brain may lead to neural and cognitive dysfunction. Recent studies have shown that mitochondrial-formed oxidants are mediator of molecular signaling and implicated in mitochondrial-dependent apoptosis. In a previous investigation, the role of mitochondrial dysfunction and oxidative stress in causing carbofuran is correlated with the change at the biochemical level[18]. carbofuran is liphophilic in nature, its chronic exposure is reported to be responsible for oxidative injury leading to perturbations in membrane structure and functions[19]. Some workers have speculated that the enhancement in the generation of reactive oxygen species by carbofuran may be due to the inhibition of cytochrome c oxidase and creatine kinase[3]. Also, the carbofuran induced nitric oxide synthase activity has been implicated in the overproduction of superoxide anions[4]. Indicate that the carbofuran induces oxidative stress as evidenced by increased levels of lipid peroxidation and decreased GSH contents, and lowered activities of antioxidant enzymes[2].when lipid peroxidation occurs, changes in cellular membrane permeability and even membrane leakage can be manifested[20].

ROS are involved in liver damage[5].Results obtained in the present study indicated that carbofuran induced many histological alterations in liver tissue and elevate transaminases serum. This result has been supported by previous studies[6, 7]. However, by giving ginger extract that contain antioxidant could neutralize free radical as a result liver damage can be prevented[21]. This result has been supported by previous study which showed the protective effects of ginger extract or its constituents, through their antioxidant properties and improve the hepatic dysfunctions and hepatic damage that induced by hepatotoxicants, CCl4 , acetaminophen, cisplatin and adriamycin[9,21,22,23,24]. Ginger treatment has potency for hepatoprotective effect, by enhancing the activity of antioxidant enzyme, SOD, and diminished amount of lipid peroxidation against the carbofuran-induced hepatotoxicity in rats. Ginger was scavenging free radical by its potent antioxidant. Ginger-free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, lipid peroxidation inhibition, DNA protection and reducing power abilities indicating strong antioxidant properties[25]. Ginger ameliorated cisplatin-induced nerphrotoxicity and this protection is mediated either by preventing the cispaltin induced decline of renal antioxidant defense system or by their direct free radical scavenging activity[23].

Transaminases are the most sensitive biomarkers directly implicated in the extent of cellular damage and toxicity because they are cytoplasmic in location and are released into the circulation after cellular damage[26]. Elevation of the serum levels of the hepatic enzymes are the indicators for impaired liver functions[27].

To know further the effect of ginger extract in concentration of Alanine aminotransaminase (ALT) which can be used in the assessment of liver function we used spectrophotometry.

We found the result that the difference in dose of ginger extract influences on ALT serum. The existence of the effect of ginger extract is starting to look where the concentration of ALT serum levels in rats induced carbofuran becomes lower, after the treatment was given in the form of ginger extract started at a dose of 500 mg, compared with ALT serum in the group B. Then the ALT serum decreased when given higher doses. Thus, based on the assessment descriptively according to the mean ALT serum is, it can be said that the administration of treatment in the form of ginger extract at a dose of 500 mg, 750 mg and 1000 mg showed different influences, where the higher dose of ginger extract provided will further lowering the ALT serum. The result of this spectrophotometry supported the result of the histopathology of liver And these may be attributed to ginger components which may stabilize hepatocytes plasma membrane and prevent delivery of ALT to the extracellular fluid[23].Ginger can serve as an Antioxidants by significantly reduces the extent of lipid peroxidation and improves plasma antioxidant capacity[28].

Conclusion

The present results provide in vivo evidence of direct hepatotoxicity caused by carbofuran. Histopathological evaluations showed that ginger protects and reduces the damage caused by carbofuran in the liver and also decrease Alanine aminotransferase concentration. The protective properties of ginger and the fact that ginger has been used by humans in food, makes it a potential therapy for carbofuran induced hepatic toxicity. The results can further suggest the possible use of ginger against oxidative stress induced organ toxicities.

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References

1. Osama Eshtewi AE, Setyawati K, Hidayat S. The Effect of Carbofuran on Testosterone Serum Concentration and Histological Change of Leydig Cell in Mice. IOSR J Pharm Biol Sci., 2013, 7(6); 1-4.
2. Chandra D, Uma Nath T, Shalini S, Amit S. Carbofuran induced biochemical toxicity in mice: Protective role of Momordica charantia. Eur J Exp Biol., 2011, 1(1); 106-112.
3. Milatovic D, Zivin M, Gupta RC, and Dettbarn W. Alterations in cytochrome c oxidase activity and energy metabolites in response to kainic acid-induced status epilepticus. Brain Res., 2001, 912(1); 67–78.
4. Pou S, Pou WS, Bredt DS, Snyder SH and Rosen G M. Generation of superoxide by purified brain nitric oxide synthase. J Biol Chem., 1992, 267(34); 24173–24176.
5. Yesmin F, Rahman Z, Dewan JD, Helali AM, Rahman NA, Alattraqchi AG. Ahmed A,Yousuf R, Salam A, Haque M. Hepato-protective role of the aqueous and n-hexane extracts of nigella sativa linn. In experimental liver damage in rats. Int. Res. J. Pharm., 2013, 6(3); 205-209.
6. Soufy H, Soliman MK, EL Manakhy EM, Gaafar AY. Some biochemical and pathological investigations on monosex tilapia following chronic exposure to carbofuran pesticide. Glob Vet., 2007, 1(1); 45-52.
7. El-Sayed MA El-Damaty,Abdel-Razik H Farrag, Gamal Rowayshed and Hany M Fahmy. Biochemical and Histopathological Effects of Systemic Pesticides on Some Functional Organs of Male Albino Rats. J App Sci Res., 2012, 8(11);5459-5469.
8. Heikal T Abdel Tawab H Mossa, Mona A Abdel Rasoul, Gehan I Kh Marei.The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats. Asian J Pharm Clin Res., 2013, 6(1); 48-55.
9. Kazeem MI, Bankole HA and Fatai AA. Protective effect of ginger in normal and carbon-tetrachloride induced hepatotoxic rats. Ann Biol Res. 2011, 2(1); 1-8.
10. Haniadka R, Saxena A, Shivashankara AR, Fayad R, Palatty PL, Nazreth N, Francis A, Arora R and Baliga MS. Ginger Protects the Liver against the Toxic Effects of Xenobiotic Compounds: Preclinical Observations. J Nutr Food Sci., 2013, 3: 226.

11. Barakat LAA, Maha MM, Ginger, Cumin. Mustard Seeds Modulate Acetaminophen-Induced Acute Hepatic Injury in Rats. J App Sci Res., 2011; 7(9): 1368-1374.
12. Saber A Sakr, Hoda A Mahran and Hawazen A Lamfon. Protective effect of ginger (Zingiber officinale) on adriamycin - induced hepatotoxicity in albino rats. J Med Plant Res., 2010, 5(1); 133-140.
13. Ahmed MAAS. The Protective Effect of Ginger (Zingiber Officinale) Against Adriamycin-Induced Hepatotoxicity in Rats: Histological Study. Life Science Journal, 2013, 10(1); 1412-1422.
14. Kamtchouing P, Mbongue Fandio GY, Dimo T, Jatsa HB. Evaluation of androgenic activity of Zingiber officinale and Pentadiplandra brazzeana in male rats. Asian J. Androl. 2002, 4(4); 299-301.
15. Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol., 2007, 47; 598–607.
16. Afzal M, Al-Hadidi D, Menon M, Pesek J & Dhami M S. Ginger: An Ethnomedical, Chemical and Pharmacological Review. Drug Metabol, Drug Interact., 2001, 18; 159–190.
17. Diplock AT, Charleux JL, Crozier-Willi G. Functional food science and defence against reactive oxygen species. Br J Nutr., 1998, 80(Suppl 1); S77-S112.
18. Kamboj SS, Kumar V, Kamboj A, Sandhir R. Mitochondrial Oxidative Stress and Dysfunction in Rat Brain Induced by Carbofuran Exposure. Cell Mol Neurobiol., 2008, 28(7); 961-969.
19. Rai DK, Sharma B. Carbofuran-induced oxidative stress in mammalian brain. Mol Biotechnol., 2007, 37(1); 66-71.
20. Schafer FQ, Buettner GR. Acidic pH amplifies iron-mediated lipid peroxidation in cells. Free Radic Biol Med., 2002, 28; 1175–1181.
21. Ahmed M, Attyah, Sajida H Ismail. Protective Effect of Ginger Extract Against Cisplatin-Induced Hepatotoxicity and Cardiotoxicity in Rats. Iraqi J Pharm Sci., 2012, 21(1); 27-33.
22. Omoniyi KY, Matthew CI. Protective Effects of *Zingiber officinale* (Zingiberaceae) against Carbon Tetrachloride and Acetaminophen-induced Hepatotoxicity in Rats. Phytother. Res. 2006, 20; 997–1002.
23. Ajith TA, Hema U, Aswathy MS. Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant Status. Food and Chemical Toxicology. 2007, 45; 2267–2272.
24. Saber A Sakr, Hoda A Mahran and Hawazen A Lamfon.Protective effect of ginger (Zingiber officinale) on adriamycin - induced hepatotoxicity in albino rats. J Med Plant Res. 2010, 5(1); 133-140.
25. Siddaraju MN, Dharmesh SM. Inhibition of gastric H^+, K^+-ATPase and Helicobacter pylori growth by phenolic antioxidants of,Zingiber officinale. Mol. Nutr. Food Res., 2007, 51(3); 324-332.
26. Stockham SL, Scott MA. Fundamentals of Veterinary Clinical Pathology. Ames. Iowa State University Press. 2002, 434–459.
27. Iseri S, Ercan F, Gedik N, Yuksel M, Alican I. Simvastatin attenuates cisplatin-induced kidney and liver damage in rats. Toxicology. 2007, 230; 256–264.
28. Afshari A T, Shirpoor A, Farshid A, Saadatian R, Rasmi Y, Saboory E. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chemistry. 2007, 101; 148–153.

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