Research Article

Ameliorating Effects of Aqueous Extract of *Zingiber officinale* Against Dichlorovos Induced Liver Toxicity in Male Albino Rats

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ABSTRACT

An indiscriminate application of organophosphate (OP) pesticides has led to environmental pollution and severe health problems. The aim of the present study was to evaluate the protective effect of an aqueous extract of *Zingiber officinale* (AGE) against hepatotoxicity and oxidative damage induced by dichlorovos in male rats. Rats were divided into four groups containing six rats in each group. Group I served as control. Group II, III and IV were treated with aqueous extract of *Zingiber officinale* (200 mg /kg bwt); dichlorovos 20 mg/kg bwt (1/20 LD_{50}); dichlorovos (20 mg/kg bwt) plus *Zingiber officinale* (200 mg/kg bwt) respectively. Rats were orally administered with their respective doses daily for 45 days. Dichlorovos administration to rats resulted in significant reduction in body weight and elevation in liver weight compared to control. Dichlorovos caused significant elevation of serum transaminases (AST & ALT), alkaline phosphatase (ALP), total protein, total bilirubin and decrease of serum albumin. Furthermore, significant depletion of hepatic superoxide dismutase (SOD), catalase (CAT), glutathione reduced (GSH) and elevation of lipid peroxidation (LPO) expressed as malondialdehyde (MDA) content were noticed in dichlorovos treated rats. Oral administration of dichlorovos induced various histological changes in the liver. These changes include congestion of blood vessels, leucocytic infiltration, cytoplasmic vacuolization of the hepatocytes and fatty infiltration. Histopathological studies of liver revealed that supplementation of AGE resulted in mild degeneration and necrosis of the hepatocytes. Treated animals with aqueous extract of ginger and dichlorovos led to an improvement in the histological changes induced by dichlorovos together with significant decrease in AST, ALT, ALP, total protein and total bilirubin level. Furthermore, AGE had normalized CAT, SOD and GSH content, whereas attenuated albumin and LPO. The results of the present work indicated that *Zingiber officinale* (ginger) possess protective effect against liver damage induced by dichlorovos possibly due to its antioxidant activities.

Keywords: Dichlorovos, *Zingiber officinale*, Lipid peroxidation, Liver, Hepatocytes, Oxidative damage, Histopathology.

INTRODUCTION

Dichlorvos (2, 2-dichlorovinyl dimethyl phosphate; DDVP), is a member of the most commonly used organophosphorus insecticide.[1]Dichlorvos is rapidly absorbed through the gastrointestinal and respiratory tracts and skin, it enters human system via inhalation, dermal, and oral routes, and it is metabolized by the liver and excreted by the kidney.[2] Like other organophosphate (OP) compounds, dichlorovos is known to produce toxic effects through the inhibition of acetyl cholinesterase (AChE) activity.[3-4] DDVP is a nervous system poison, and its toxicity involves its ability to act as potent acetyl cholinesterase inhibitor, inhibition of this enzyme leads to the accumulation of acetylcholine in synapses and disruption of the nerve function, and finally death by poisoning.[5]

Studies support a role for reactive oxygen species (ROS) in the mechanism of dichlorvos toxicity.[6] Oxidative stress occurs when the production of ROS is beyond the antioxidant capability of the tar-get cell, resulting in oxidative damage because of ROS interaction with critical macromolecules. Oxidative stress is an imbalance of oxidation and antioxidation and results in damages to membrane lipids, protein, DNA, and tissues in the body.[7-8]Besides inhibiting cholinesterase (ChE), oxidative stress has been recently proposed as a main toxicity mechanism for organophosphorus (OPs) both in acute and chronic poisoning cases.[9] Antioxidant systems are composed of enzymatic and non-enzymatic systems. The enzymatic system includes superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) which scavenge free radicals and ROS.[10-11] The non-enzymatic mechanism involves endogenous and exogenous compounds are administered into the body, such as flavonoids, vitamins E and C, urate, and melatonin. These substances may prevent ROS formation and exhibit pro-oxidative effects. A number of studies were conducted on the toxicity of dichlorovos on different organisms and indicated that it is a potent hepatotoxic, neurotoxic, mutagenic, carcinogenic, and cytotoxic compound.[12]Hepatic metabolism converts drugs into products that are more easily excreted.[13]The central role of liver in drug metabolism predisposes them to toxic injury.

Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as they have fewer adverse effects as compared with modern medicines.[14] *Zingiber officinale* (Ginger) is known for anti-oxidant,[15] anti-inflammatory[16] and curing effects for different diseases,[17] is among mostly used herb for treatment of reproductive toxicity since antiquity.[18] It considered safe with little or no side effects compared to

synthetic drugs.[19] The aqueous extracts of *Zingiber officinale* (ginger) has been shown to be effective in treating hepatotoxic issues.[20]

The liver was chosen to focus in the present study of the toxic effect of dichlorovos. To the best our knowledge, the possible modulating structure effect of *Zingiber officinale* extract in the presence of dichlorovos has not been yet investigated. Hence, the present study aimed to show the biochemical, histopathological and antioxidant changes occurred post-application of dichlorovos on the rat liver and to evaluate the possible protection of *Zingiber officinale* against dichlorovos induced biochemical and hepatic injury.

MATERIALS AND METHODS

Animals

Twenty four male albino rats (150-200 gm) of the Wistar strain were obtained from the animal house of the University of Rajasthan, Jaipur, India for the experimentation. The rats were housed in polypropylene cages at room temperature (25 ± 3°C) with 12/12 hours light and dark cycle. Animals were fed with a balanced diet and tap water *ad libitum*. The rats were allowed to acclimatize for a period of three weeks before the commencement of the experiment. The guidelines for care and use of animals for scientific research[21] were strictly followed throughout the course of investigation. The experimental protocol has approval of the Institutional Animal Ethics Committee (IAEC). (UDZ-13/18.12.13)

Chemical and plant material

Technical grade dichlorvos (2, 2-dichlorovinyl dimethyl phosphate) obtained from Sudarshan Crop, Ltd., Jaipur (Raj.) (India) was used for experimentation. The insecticide was dissolved in olive oil and administered to animals through oral intubations. All other chemicals used in the study were of analytical grade.

Fresh *Zingiber officinale* (ginger) rhizome were collected from the local market of Jaipur, Rajasthan, India and authenticated at the Herbarium, Department of Botany, University of Rajasthan, Jaipur in comparison with the pre existing voucher specimen **(RUBL 211509)**. Rhizome (underground stem) of *Zingiber officinale* (ginger) were washed thoroughly, shed dried and powdered. Shed dried rhizome of ginger was extracted with distil water for 36-48 hours by Soxhlet extraction method. The extract was filtered and then water was separated under reduced pressure to obtain a solid mass. Olive oil was used as a vehicle for the dichlorovos pesticide and distilled water for aqueous extract of ginger (AGE).

Experimental design

The rats were randomly divided into four groups comprising 6 rats each and were subjected to the following treatments:

Group-1 (control rats) received olive oil only as a vehicle.

Group-2 Aqueous extract of *Zingiber officinale*(AGE) was given at the dose of 200 mg per kg body weight/day.

Group-3 Dichlorovos was given at the dose of 20 mg per kg body weight/day.

Group-4 (AGE supplemented, dichlorovos-treated rats) received in addition to exposure of 20 mg per kg body weight/day dichlorovos, dose of aqueous extract of *Zingiber officinale* **(**AGE) at 200 mg/kg bodyweight/day.

The drug and extract administration were given by oral intubation and lasted for a period of 45 days. On 46th day of drug administration, the animals were anaesthetized under light ether anesthesia and the blood samples were collected for estimation of biochemical and hematological parameters. At the end of the study, liver and other vital organs were dissected out and washed with 0.9% NaCl and stored at -4°C for further processing.

Body and liver weight measurements

The body weight has been recorded on the initial day of experiment and also on the day of sacrifice (46th day), both the control and experimental groups, by using automatic balance. Blood was collected in sterile tubes by cardiac puncture for hematological studies and serum was separated for serological studies. Similarly the weight of different vital organs (liver, kidney heart and pancreas) was also recorded.

Biochemical Analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST),[22] alkaline phosphatase (ALP),[23] total bilirubin,[24] total protein[25] and albumin[26]were determined in serum by using standard methods.

Oxidative stress and antioxidant parameters in liver

Lipid peroxidation (LPO),[27] Catalase,[28] Glutathione,[29] and Superoxide dismutase (SOD)[30] were assessed by standard methods.

Histopathological Examination

Specimens of the liver tissues were fixed immediately in Bouins solution for histological studies. Then the tissues were treated with conventional grades of alcohol and xylol, embedded in paraffin and sectioned at 4 to 6 µm thickness. The sections were stained with hematoxylin and eosin (H and E) stain for studying the histopathological changes.[31]

Statistical Analysis

The results are expressed as mean± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) and the significance of differences was set at P≤0.05 (significant) and P≤0.01 (highly significant) levels.

RESULTS AND DISCUSSION

In the vertebrate body, liver is the largest organ and it is the major site of excretion and xenobiotic metabolism.

Liver damage is a general pathological feature which exists in many hepatic diseases. Since cirrhosis, liver fibrosis and even liver cancer could result from the long existence of hepatic injury. Therefore, treatment and prevention of liver damage is a key to treat hepatic diseases clinically.[32-33]

Dichlorovos is one of the most toxic pesticide that is widely used to control the internal and external parasites of farm animals, and to eliminate insects threatening the household, public health, and stored products[34] but it is associated with toxic side effects on different body organs. The oxidative stress through formation of free radicals is one of the mechanisms of dichlorovos-induced toxicity.[35] The free radical scavengers, that prevent the formation and/or scavenge the reactive hydroxyl free radicals, can provide protection against dichlorovos-induced toxicity.[36] Different natural products and dietary compounds have been recently investigated and evaluated as potential protective antioxidant agents against dichlorovos-induced hepatotoxicity.[37] *Zingiber officinale* (ginger) reveals a number of analeptic properties as an antioxidant, anti-lipidemic, antihyperglycemic, anti-inflammatory and anti-cancer agent.[38-39] This medicinal herb is excellent for oral therapy as it is effective, non-toxic and without serious side effects.[40] The present study aimed to investigate the possible protective effect of AGE against dichlorovos-induced toxicity in hematological, biochemical, oxidative stress and histopathological changes in rat liver.

General observations

During the experiment, no death was observed in any of the experimental groups. Rats in the control group and in AGE treated group did not show any sign of toxicity. However, dichlorovos and dichlorovos + AGE treated rats showed varying degrees of clinical signs few minutes after dosing. The signs included huddling, mild tremor and diarrhea. The observed signs were related to the cholinergic crisis; a consistent sign in organophosphate poisoning. Except for the huddling, no other significant clinical manifestation was observed following AGE supplementation.

Body and Liver Weights

Data of final body weights and liver weights of male rats subjected to different treatments are shown in (Table 1). At the end of the experimental course, there was no significant difference in body and liver weights between AGE and control rats. However a significant (P<0.05) loss of body weight accompanied by a significant increase in the liver weights were recorded in rats treated with dichlorovos compared to the control. The administration of AGE (Aqueous extract of *Zingiberofficnale*) to pesticide treated groups has an ameliorated effect either in the loss of body weight or in the increase of liver weights (Table 1).

Table1: Effect of dichlorovos and *Zingiber officinale* on body weight and liver weight

Group		Body weight (gm)		liver weight (mg/gm)
		Initial	Final	
Control	G1	181.37 ±1.05	181.37 ±1.05	3322.46 ±33.31
Zingiber officinale (200 mg/kg bwt/ day)	G2	182.68 ±1.24	182.68 ±1.24	3208.34^{ns} ±26.94
Dichlorovos (20 mg/kg bwt/ day)	G3	178.25 ±0.82	178.25 ±0.82	3920.02* ±26.40
Dichlorovos (20 mg/kg bwt/ day) + *Zingiberofficinale* (200 mg/kg bwt/ day)	G4	180.68 ±1.43	180.68 ±1.43	3598.48* ±23.14

(Mean±SEM of 6 animals); Group II & III compared with Group I; Group IV compared with Group III)

ns = non significant; * = Significant (P<0.05); ** = Highly significant (P<0.01)

In toxicological studies, body and organ weights are important criteria for evaluation of organ toxicity.[41] Changes in body and liver-weight could be due to the toxic effects of xenobiotics, which results in hepatic injury.[42] In the present study, oral administration of dichlorovos resulted in a significant reduction and elevation in body and liver weights of rats, respectively. The reduction in body weight may be due to the combined action of cholinergic and oxidative stress[43] and/or due to the overall increased degradation of lipids and proteins as a result of the direct effects of dichlorovos as an organophosphate compound.[44] Moreover, the increase in liver weight could be attributed to the relationship between liver weight increase and various toxicological effects or to the reduction in body weight gain of experimental animals.[45] These results are

consistent with many previous investigators.[46-47] However, co-administration of ginger attenuated body and liver weights of intoxicated rats.[44]

Biochemical Analysis

ALT, AST, ALP, total bilirubin, total protein and albumin are indicators of hepatic function. The oral administration of AGE to normal rats produced significant (P<0.05) changes in ALT, AST and ALP and non significant changes in bilirubin, albumin and total protein compared to the normal control rats (Tables 2). Exposure to dichlorovos caused a significant elevation in the activities of ALT, AST and ALP serum enzymes, compared to the normal control rats. However, the co-administration of AGE attenuated the increment in serum enzymes ALT, AST and ALP levels (Table 2). Treatments with dichlorovos induced a significant increase ($p < 0.05$) in the total protein levels and highly significant decrease ($p < 0.01$) in albumin levels compared to the control rats. However, co-administration of AGE to the treated rats resulted in a partial recovery in the above mentioned parameters (Table 2). The estimated bilirubin concentrations in serum of dichlorovos treated rat were recorded in Table 2. Bilirubin concentration was increased in intoxicated rats; however mitigation was recorded following administration of AGE (Table 2).

Table 2: Effect of dichlorovos and *Zingiber officinale* on liver enzymes, bilirubin, albumin and total protein.

Group		Alanine amino transfrase (ALT)	Aspartate amino transferase (AST)	Alkaline phophatase ALP	Bilirubin	Albumin (mg/dl)	Total Protein (mg/dl)
		(U/l)					
Control	G1	127.30 ±3.79	70.75 ±2.00	63.42 ±2.21	0.29 ±0.19	4.10 ±0.26	18085.20 ±564.34
Zingiberofficinale (200 mg/kg bwt/ day)	G2	115.20* ±4.52	61.14* ±4.26	66.43* ±4.69	0.23 ns ±0.24	4.56 ns ±0.41	17875.25 ns ±561.34
Dichlorovos (20 mg/kg bwt/ day)	G3	155.63* ±5.15	88.65* ±4.34	80.51* ±2.30	2.76* ±0.51	2.76** ±0.32	24143.62* ±1122.93
Dichlorovos (20 mg/kg bwt/ day) + *Zingiberofficinale* (200 mg/kg bwt/ day)	G4	134.27* ±3.95	75.28* ±2.81	70.85* ±1.15	1.23* ±0.34	3.71* ±0.30	20819.15* ±469.60

(Mean±SEM of 6 animals); Group II & III compared with Group I; Group IV compared with Group III
ns = non significant; * = Significant (P<0.05); ** = Highly significant (P<0.01)

Enzymes, such as ALT, AST and ALP are mainly monitored for the evaluation of liver damage and toxicity.[48-49] AST and ALT are the most sensitive biomarker enzymes used in evaluation of the function and integrity of liver cells. Both enzymes are present mainly in the cytoplasm of hepatic cells.[50] Previous studies have shown that OP pesticides may cause the release of hepatic enzymes into blood circulation. In this study, enzymatic activities of ALT, AST and ALP were increased in dichlorovos group as compared to control group. These results indicate the increased enzyme activities are associated with ecrotic lesions in the liver cells or hepatocyte degeneration. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation, indicating a necrosis and inflammatory reactions.[51]In this study, supplementation of ginger extract could prevent the liver damage caused by dichlorovos as revealed by the remarkable decrease in the activities of ALT, AST and ALP. The reduction of the liver enzymes in AGE pre-treated rats may be due to its antioxidant effect that reduces the free radical-induced oxidative damage in the liver, thereby stabilizing the membrane permeability and reducing the leakage of enzymes into the blood.[52]

Administration of dichlorovos caused a significant rise in serum total protein and total bilirubin levels. Total protein is done as a routine test to evaluate the toxicological nature of various chemicals. An increase of total protein was observed in the present study following dichlorovos treatment. The increase of total protein in treated groups may be due to production of enzymes lost as a result of tissue necrosis or to increased demand of detoxifying the pesticide might necessitate enhanced synthesis of enzyme proteins.[53] Bilirubin which is a product of haemoglobin degradation is a marker of hepatobiliary injury.[48] The increase of total bilirubin in serum is attributable to the impairment of hepatocellular function in acute or subacute hepatic necrosis.[54] AGE could reduce the total bilirubin and total protein level in AGE+dichlorovos treated group. In the initial stage of tissue reaction following OP pesticide exposure, growth protein may be stimulated and acute phase protein will be generated causing an increase in total protein level.[55]

Previous studies also reported that high-protein level is reflected by the increase in globulin level.[56] In this study, there was remarkable decrease in total protein level in dichlorovos rats supplemented with AGE.

Antioxidant parameters in liver

Liver lipid peroxidation (LPO)

Administration of dichlorovos led to a significant (P<0.05) increment in lipid peroxidation as evidenced by the increase in serum MDA levels by as compared to the control group. However, co-administration of AGE to treated rats significantly (P<0.05) reduced the augmentation in serum MDA levels for dichlorovos treated rats (Table 3). No significant changes were observed between control and AGE treated groups.

Liver glutathione (GSH)

Glutathione, in the reduced form (GSH), acts as one of the major detoxifiers in the body. A highly significant (P<0.01) decrease of glutathione (GSH) level in liver was evident in dichlorovos treated groups when compared to control. However, co-administration of AGE to treated rats significantly (P<0.05) ameliorated the decrease in liver GSH levels for dichlorovos treated rats. No significant changes were observed between control and AGE treated groups (Table 3).

Table 3: Effect of dichlorovos and *Zingiber officinale* administration on the level of antioxidant enzymes in the liver of male rats.

Group		Catalase (n mole of h_2o_2 consumed/min/mg protein)	SOD (Units/mg protein)	GSH(n mole/gm)	LPO (n mole MDA/mg protein)
Control	G1	85.26 ±3.16	27.31 ±2.48	36.87 ±3.57	1.59 ±0.04
Zingiberofficinale (200 mg/kg bwt/ day)	G2	87.25^{ns} ±2.54	28.97^{ns} ±2.06	38.12^{ns} ±3.11	1.42^{ns} ±0.07
Dichlorovos (20 mg/kg bwt/ day)	G3	68.39* ±2.84	17.46** ±2.37	16.59** ±2.43	2.34* ±0.09
Dichlorovos (20 mg/kg bwt/ day) + *Zingiberofficinale* (200 mg/kg bwt/ day)	G4	81.53* ±3.22	24.58* ±3.14	31.26* ±2.73	1.81* ±0.05

(Mean±SEM of 6 animals); Group II & III compared with Group I; Group IV compared with Group III

ns = non significant; * = Significant (P<0.05); ** = Highly significant (P<0.01)

Super oxide dismutase (SOD) and Catalase (CAT) levels in liver

Table 3 also shows the effect of chronic treatment of dichlorovos and dichlorovos + AGE on SOD and catalase. Subacute levels of the tested pesticides resulted in a state of liver injury and extensive oxidative damage in rats as manifested by the significant alteration in these enzymes. In fact, in dichlorovos treated rats, a significant (P<0.05) decrease was noted in the activities of CAT and SOD when compared to the control animals. However, the co-administration of AGE mitigated the significant (P<0.05) alteration in the activities of SOD and CAT.

In this study, we demonstrated that dichlorovos intoxication induces oxidative stress in rat liver in vivo through the generation of free radicals and alterations in the cellular antioxidant defense system as well as the protection of the ginger in alleviating such oxidative stress in rats. The toxicity of dichlorovos may be due to dichlorovos -induced alterations in membrane integrity via the formation of reactive oxygen species and the perturbation of antioxidant defense mechanisms. Antioxidant enzymes include LPO, SOD, Catalase, and glutathione (GSH).

MDA is one of the major oxidation products of peroxidized polyunsaturated fatty acids, and increased MDA content is an important indicator of LPO.[57-58] Previous studies found that chlorpyrifos increased MDA levels in various rat tissues.[59] In this study, MDA levels in liver were also increased in the dichlorovos-only group, suggesting that MDA level could be used as a marker for dichlorovos-injury. This increase also suggested that dichlorovos can accelerate the increase in free oxygen groups when adminis- trated at the indicated dose. The treatment of cells with ginger attenuated the increase MDA level. Therefore, the ginger extract may have a beneficial effect in reducing dichlorovos-based toxicity.

Glutathione is a small tripeptide protein synthesized in the liver. It is a potent antioxidant with high redox potential and it also serves as a co-factor for several oxidative stress detoxifying enzymes (glutathione peroxidase and glutathione transferase).[60] It can be assumed that the reduction in tissue glutathione levels

was as a result of increased oxidative stress and lipid peroxidation occasioned by the effect of dichlorovos.[61] In the present study, after oral administration of ginger extract, dichlorovos treated animals showed a significantly elevated level of GSH. It is possible that extract might have reduced the extent of oxidative stress, leading to lesser GSH degradation or increase in the biosynthesis of GSH.[62]

Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species;[63] organisms contain intracellular antioxidant enzymes such as SOD and CAT, that work together to prevent oxidative damage to the cell.[64] SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide, while CAT converts hydrogen peroxide into water. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS.[65] In this study, liver SOD and CAT activities were decreased in the dichlorovos -treated groups. The decrease in the activity of SOD in OP-treated groups may be attributed to the saturation of SOD during the process of converting O2- to H_2O_2. The CAT decrease may involve CAT saturation during the breakdown of free radicals and hydrogen peroxide (H_2O_2), or the inhibition of CAT by these radicals.[66] In this study, SOD and CAT activities significantly decreased in the dichlorovos-treated groups. The currently noted elevated levels of both SOD and CAT levels could be due the influence of ginger extract.[67]

Hepatoprotective potentials of medicinal plants against pesticides induced hepatotoxicity remain an area that needs extensive scientific research. Results of the current study revealed that ginger extract reversed the elevation of lipid peroxidation. Hence, it is possible that the mechanism of hepatoprotection of ginger extract may be attributed to phenolic compounds (e.g. gingerol) that scavenge a wide range of free radicals including the most active hydroxyl radical, which initiate lipid peroxidation.[68] Therefore, it may decrease the concentration of lipid free radicals.[69] It was reported previously that phenolic compounds chelate metal ions, especially iron and copper, which, in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides.[70-71]

Histopathological Examination

The representative figures of histopathological examination in the liver tissue are shown in Figure 1 (A-D). In control rats (Group I) the histopathological examination of liver showed no pathological alterations. Liver tissues of rats showing normal structure of hepatocytes with normal nuclei and normal blood sinusoids appeared between the liver cords. They formed of hepatocytes radiating from central vein to the periphery of the lobules (Fig. 1A). Histopathological examination of liver of AGE treated rats (Group II) hepatocytes was more or less similar to control group (Fig. 1 B). However, liver tissues of animals intoxicated with (20 mg/kg b.w) dichlorovos (Group III), showed vacuolization of hepatocytes, congested blood sinusoids in between the liver cords with intense mononuclear inflammatory cellular infiltration. Liver cells appeared swollen and areas of hemorrhage were noticed between the cells. Nuclei of the cells become flattened-shaped and Liver lobules of dichlorovos treated rats showed degeneration and coagulative necrosis in hepatocytes (Fig. 1C). These histopathological changes were mitigated by rats treated with aqueous extract of *Zingiberofficinale*. The number of rats displaying histopathological changes in liver was decreased in (Group IV) which had received dichlorovos plus *Zingiberofficinale* as the rats of this group revealed only mild necrotic changes in the liver tissues (Figure 1D).

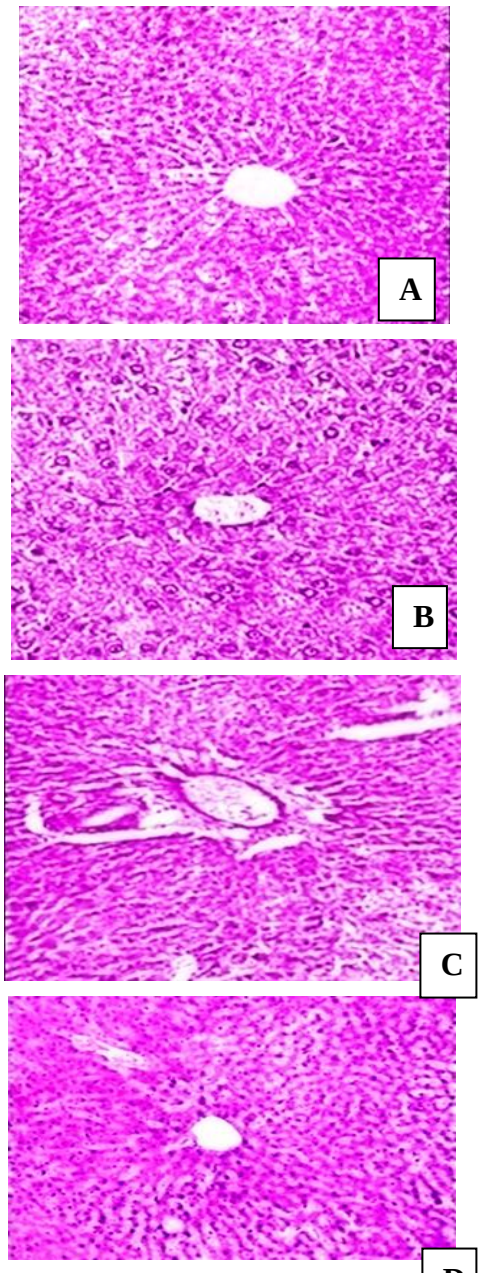

Figure 1: Paraffin sections stained by haema in and eosin (H&E, X 400) for histopathological examination of hepatocytes: (A) Liver tissue of control showing normal structure, central vein, normal arrangement of hepatic cords, normal blood sinusoids and hepatocytes; (B) Liver tissue of aqueous extract of *Zingiber officinale* treated rats showing normal histology was more or less similar to

control group; (C) Liver tissue of dichlorovos treated rats showing hemorrhage in the central vein, necrosis and showing dilation of blood sinusoids; (D) Liver of dichlorovos with *Zingiber officinale* treated rat showing mild necrotic and degenerative changes.

In the present study, dichlorovos caused histopathological alterations in hepatic tissue. Free radicals produced from LPO in the liver, were probably responsible for sever tissue damage, leading to necrosis of hepatocytes, marked damage of the liver tissues in the form of dilated veins, hemorrhagic spots and some degenerative signs of the hepatocytes. These changes are entirely consistent with the changes in various biochemical parameters that were also observed. In the same respect, Gokcimen*et al.* (2007)[72] mentioned that, such liver damage may arise from the toxic effect of dichlorovos, which disturbs the detoxification mechanisms of the liver. In addition, it is possible that dichlorovos, like several other insecticides, adversely affects the cytochrome P450 system or the mitochondrial membrane transport system of hepatocytes. These results are coincide with El-Halwgy*et al.* (2008)[73] who found that administration of fenitrothion OP to the rats showed pathological signs in liver tissues varying from dilated veins, hemorrhagic spots and distorted nuclear membrane. In this study all of biochemical and histological changes that were induced by dichlorovos exposure were almost normalized when *Zingiber officinale* (ginger) was given together with dichlorovos. Moreover, our light microscopic analysis revealed that the *Zingiber officinale* treated dichlorovos exposed animals did not exhibit the hepatic calcification, vacuolar degeneration and necrosis seen in the liver of the dichlorovos treated group. Thus, *Zingiber officinale* could ameliorate the liver damage induced by dichlorovos exposure.

The biochemical alterations accompanied by histopathological changes resulted from dichlorovos exposures were alleviated following ginger extract administration. This could be attributed to the antioxidant capacity of ginger that attenuates the lipid peroxidation and liver antioxidant enzymes capacity which in turn restore the integrity of the cell membrane and improve the disturbance in permeability.

CONCLUSION

In conclusion, the results of the present study showed that dichlorovos induced oxidative damage and hepatotoxicity in male albino rats. In contrast, AGE reduces oxidative stress by virtue of its antioxidant properties thus improving the structural integrity of cell membrane and eventually alleviates the histopathological changes as well as the biochemical perturbations. These beneficial effects of AGE were able to ameliorate dichlorovos induced hepatotoxicity and oxidative damage. Based on our present observations, we propose that AGE may provide a cushion for prolonged therapeutic option against pesticide induced hepatotoxicity without harmful side effects.

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