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RESEARCH ARTICLE

SOME PROTECTIVE EFFECTS OF GINGER AGAINST CCl_4 INDUCED TOXICITY IN THE ADRENAL CORTEX OF ADULT WISTAR RATS (*Rattus norvegicus)*

***[1]Fakunle, P. B., [1]Ajibade, A. J., [2]Oyewo E. B. and [1]Olojede S. O.**

[1]Anatomy Departmment, Ladoke Akintola University of Technology, Ogbomoso, Oyo-state, Nigeria
[2]Biochemistry Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo-state, Nigeria

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ABSTRACT

Aims: To investigate some protective effects of ginger on CCl_4 (Carbon tetrachloride) induced toxicity in the adrenal cortex of adult wistar rats.
Study design: Histological and Biochemical study.
Place and Duration of study: Department of Anatomy, Faculty of Basic Medical Sciences, LAUTECH, Nigeria between September 2012 and December 2012.
Methodology: Twenty-four adult healthy wistar rats of both sexes of average weight 210±4.22g were randomly assigned into 4 groups four groups (N=6) such that T_1, T_2 and T_3 served as treatment groups, while C served as the control group. T_1 received 2g of ginger and 2mls of CCl_4, T_2 were given 2mls of CCl_4 Carbon tetrachloride while T_3 received 2g of ginger. The control group C was given distilled water. All the animals were exposed for 7 days. At the end of administration, all the rats were sacrificed cervical dislocation and processed immediately for histological techniques and bioassay of some antioxidant enzymes as well as lipid peroxidation.
Results: Oxidative stress enzymes Superoxide dismutase, Glutathione reductase and Glutathione peroxidase as well as Glutathione levels were significantly ($p<0.05$) reduced in T_2 compared to the control but relatively increased in T_1 and T_3 while Lipid peroxidation level was drastically reduced in T_3 and to some extent in T_1 compared to increased level in T_2. The histoarchitecture in the treatment group T_3 and control C revealed distinct and normal pyramidal cells and freely anastomosing polyhedral cellular distribution in the cellular zones of adrenal cortex. Treatment group T_1 also showed same normal histological presentation with few distortions. However the pictorial representation in treatment group T_2 showed pyknotic pyramidal cells characterized with vacoulations specifically in the zonal fasciculata and to lesser extent in the zonal reticulosa.
Conclusion: Ginger offers some ameliorative protections to the pyramidal and polyhedral cells of the adrenal cortex following CCl_4 induced toxicity in wistar rats and also further affirms its antioxidative potentials

INTRODUCTION

Medicinal plants have continued providing valuable therapeutic agents, both in modern and in traditional medicine (Krentz *et al*, 2005). With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions (Gupta and Briyal, 2004). Medicinal plants, their fractions and bioactive compounds play crucial role in detoxification of such toxins and scavenge free radicals (Sahreen *et al*., 2010). Ginger (*Zingiber officinale*) which belongs to the family *Zingiberaceae* is an example of botanicals that is vastly gaining popularity among modern physicians and its underground rhizomes are the medicinally useful part (Mascolo *et al*., 1989). Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals as its roots contain polyphenol compounds (6-gingerol and shogaols), which have a high antioxidant activity (Stoilova *et al*., 2007). It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali *et al*., 2008). In addition, ginger reported as detoxifying agent against alcohol abuse (Shati and Elsaid, 2009) and bromobenzene intoxication (El-Sharaky *et al*, 2009). The adrenal gland is reported to be the most common endocrine organ associated with chemically induced lesions (Ribellin, 1984). The adrenal gland is exquisitely sensitive to toxic assault as it has been reported that the most frequently observed site of endocrine lesion is the adrenal gland (Ribelin, 1984 and Harvey, 1999). There are two features of the adrenal gland which make it vulnerable to toxic assault (Hinson and Raven, 1999). It is a discrete gland and its high vascularity, facilitates the delivery of toxins and metabolic substrates as well as the efficient removal of steroid products (Vinson and Hinson, 1992). Carbon tetrachloride (CCl_4) is known to induce damage in liver, lungs, kidneys, adrenals and central nervous system in humans and experimental animals (Rechnagel *et al*.,1989). Carbon tetrachloride (CCl4) is a potent hepatotoxic agent causing hepatic necrosis and nephrosis, and is widely used in animal models for induction of acute and chronic injury (Khan *et al*., 2010). It has been found that metabolism of CCl_4 involves the production of highly fatal trichloromethyl radical (CCl3•) and proxy trichloromethyl (•OOCCl3) free radicals through P450 bioactivation (Weber *et al*., 2003; Khan and Ahmed, 2009). CCl_4 is capable of causing lipid peroxidation and decreases activity of antioxidant enzymes (Adewole *et al*., 2007). Endogenous antioxidants such as polyphenolic compounds, ascorbic acid and monosaccharides in medicinal plants may constitute antioxidative defense by scavenging free radicals possibly increase the longevity of biological systems (Khan and Ahmed, 2009). This study was carried out to evaluate some protective effects of Ginger (*Zingiber officinale*) against CCl_4 induced adrenal toxicity.

MATERIALS AND METHODS

Twenty-four adult healthy wistar rats of both sexes of average weight 210±4.22g were maintained under standard laboratory conditions for an acclimatization period of 2 weeks in the animal holdings of

Corresponding author: fakuns@yahoo.com

Anatomy Department, Ladoke Akintola University of Technology Ogbomoso. During which they were fed with standard laboratory mouse chow (Ladokun feeds, Ibadan) and provided water *ad libitum*. Daily weights were taken and documented. At the end of acclimatization, the rats were randomly assigned into four groups (N=6) such that T_1, T_2 and T_3 served as treatment groups, while C served as the control group. T_1 received 2g of ginger by oral administration and 2mls of Carbon tetrachloride, intraperitoneally, T_2 were given 2mls of Carbon tetrachloride, intraperitoneally while T_3 received 2g of ginger by oral administration. The control group C was given distilled water. All the animals were exposed for 7 days. At the end of administration, all the rats were sacrificed cervical dislocation and processed immediately for histological techniques and bioassay.

Enzyme Assay

For the measurement of the enzymes antioxidant activities, GSH and Lipid peroxidation, part of the adrenal gland specimens were weighed and homogenized in a sucrose buffer (0.25M Sucrose, 10Mm HEPES,1mMEDTA,pH 7.4) and the homogenate was centrifuged at 1000 x g for 60min at 4°C for the assay of superoxide dismutase (SOD). The activity of Superoxide dismutase (SOD) was measured by the method of Marklund *et al*, 1974 with some modification, an assay based on the ability of SOD to inhibit the autoxidation of pyrogallol by 50% . The assay mixture of 1 ml contained in final concentration, 50 mMSodium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.48 mMPyrogallol and appropriate amount of tissue extract containing 7-10 µg of protein. The change in absorbance of assay mixture was monitored spectrophotometrically at 420 nm for 3 min at 25°C against blank. One unit of enzyme activity is defined as the amount of enzyme that causes 50% maximal inhibition of pyrogallol autoxidation. The remaining brain tissues were homogenized in cold phosphate buffered saline (PBS) with 1mM EDTA and the homogenate was centrifuged at 1200 x g for 15 minutes at 4°C and the supernatant were analyzed for the activities of the following parameters:

Glutathione reductase (GR)

The Glutathione reductase (GR) activity was measured by the modified method of Erden *et al*, 1984. The reaction mixture of 1ml contained in final concentration, 4.1 mMTris-HCl (pH 7.5), 15mM MgCl2, 5.7 mM EDTA, 60 mMKCl, 2.6 mM Glutathione (oxidized) and 0.1 mM NADPH. The reaction was started by the addition of tissue extract containing approximately 100 µg of protein. One unit of enzyme activity is defined as 1 µmol of NADPH oxidized/min/mg protein. The decrease in absorbance was monitored at 25°C at 340 nm.

Glutathione peroxidase (GPx) which was determined using modified method as described by Lawrence and Burk.1976. The assay mixture of 1 ml contained in final concentration, 10 mM Potassium phosphate buffer (pH 7.0), 25 mM EDTA, 0.5mM Glutathione (reduced), 2mM Sodium azide, 1.5 IU Glutathione reductase, 0.1 mM NADPH and the cytosolic fraction containing about 50 µg of protein. The reaction was started by the addition of t-butyl hydroperoxide and the decrease in absorbance was monitored at 25°C at 340 nm. One unit of enzyme activity is defined as 1 µmol of NADPH oxidized/min/mg protein.

Glutathione (GSH) using commercially available kit (NWK-GSH01, North West Life science specialities, LLC., as previously described by Teitze,1969.

Malanoaldehyde (MDA)

The level of lipid peroxidation was assessed in the adrenal gland tissue by measuring the formed malondialdehyde (MDA), an end product of fatty acid peroxidation, using thiobarbituric acid reactive substance(TBARS) method (Genet,*et al*,2002).10% tissue homogenate was centrifuged at 1000xg for 10 min and deproteinized with half volume of 20% trichloroacetic acid (TCA). The supernatant in 10 mM Potassium phosphate buffer (pH 7.4) was incubated at 80°C for 15 min in water bath with 0.53% Thiobarbituric acid in glacial acetic acid and centrifuged. The concentration of MDA-TBA complex was determined spectrophotometrically at 532nm against blank.

Statistical analysis

The data were analyzed using the computerized statistical package 'SPSS Version 11'. Mean and standard error of mean (SEM) values for each experiment group was determined. The means were compared by analysis of variance at a level of significance of 95%.

RESULTS

Biochemical quantification

Biochemical quantification revealed a slightly decreased values in the specific activities of SOD in the treatment group T2 with Mean±SEM (0.48±0.28)*U/mg protein* compared to the control group C with Mean±SEM (1.71±1.1)*U/mg protein* while group T1 and T3 recorded significantly ($P < 0.05$) increased values Mean±SEM (1.82±1.32 and 1.88±1.22)*U/mg protein* respectively. The results obtained for GR showed that the specific activity was drastically reduced in group T2 with Mean±SEM (11.49±0.27)*mU/mg protein* compared to the control group C with Mean±SEM(18.55±4.03)mU/mg protein but on the contrast the groups T1and T3 recorded significantly ($P < 0.05$) increased values Mean±SEM (27.01±2.81 and 31.80±3.11)*mU/mg protein* respectively while the specific activity of GPx was decreased significantly($P<0.05$) for treatment group T2 with Mean±SEM (0.38±0.26)*mU/100mg protein* compared to the control group C with Mean±SEM(1.79±1.39)*mU/100mg protein* while group T1and T3 recorded significantly ($P < 0.05$) increased values Mean±SEM (3.59±0.92 and 3.82±0.71)*mU/100mg protein* respectively as shown in Table 1.

Table 1. Mean ± SEM of Antioxidant Enzyme Activities in the adrenal gland

GROUPS	SOD(U/mg protein)	GPxmU/100mg protein	GRmU/mg protein
C	1.71±1.10	1.79±1.39	18.55±4.03
T1	1.82±1.33	3.59±0.92	27.01±2.81
T2	0.48±0.28	0.38±0.26	11.49±0.27
T3	1.88±1.22	3.82±0.71	31.80±3.11

Significantly reduced values ($P<0.05$) was recorded in the GSH levels of Mean±SEM (0.86±0.13) *µM/mg protein* in groups T2 when compared to Mean±SEM (2.72±1.29) *µM/mg protein* obtained in the control group while group T1 and T3 recorded significantly ($P < 0.05$) increased values Mean±SEM (3.19±1.09 and 4.15±1.23) *µM/mg protein* respectively while MDA level was drastically reduced in T3 with Mean±SEM (0.62±0.22)*µM/mg protein* and to some extent in T1 with with Mean±SEM (0.95±0.82)*µM/mg protein* compared to increased level in T2 with Mean±SEM (2.37±0.46)*µM/mg protein* while control group value stood at Mean±SEM (1.51±0.11)*µM/mg protein* as shown in Table 2.

Table 2. (Mean ± SEM)*µM/mg protein* of GSH and LPO Levels in the adrenal gland

GROUPS	GSH	LPO
C	2.72±1.29	1.51±0.11
T1	3.19±1.09	0.95±0.82
T2	0.86±0.13	2.37±0.46
T3	4.15±1.23	0.62±0.22

Histological Findings

The histoarchitecture in the treatment group T3 and control C revealed distinct and seemingly normal cellular distribution of cellular zones of zona grannulosa (ZG), zona fasciculata (ZF) and zona reticulosa (ZR) with no histoarchitectural distortions. Treatment group T_1 also showed same normal histological presentation with few distortions. However

the pictorial representation in treatment group T2 showed pyknotic polyhedral cells with vacoulations in the zona fasciculata and to lesser extent in the zonal reticulosa.

DISCUSSION

There is increasing evidence that various chemicals introduced into the environment have the potential to disrupt the endocrine system in humans and wildlife (Waslat and Hanaa, 2011).

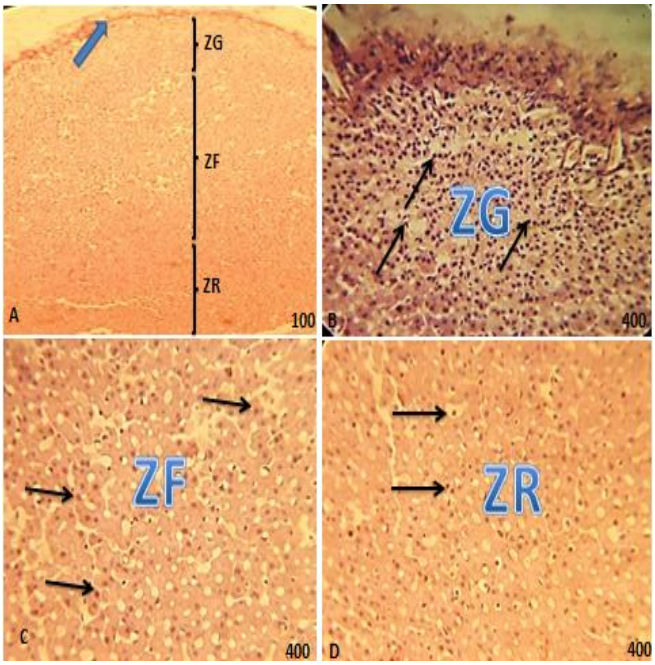

Figure 1: Photomicrograph of adrenal cortex (control section C) showing the different cellular zones of zona grannulosa (ZG), zona fasciculata (ZF) and zona reticulosa (ZR). Note the blue arrow pointing to the connective tissue capsule as well as the Black arrows pointing to the normal and well distributed pyramidal cells in ZG, polyhedral cells in ZF and ZR. H&E stain x400.

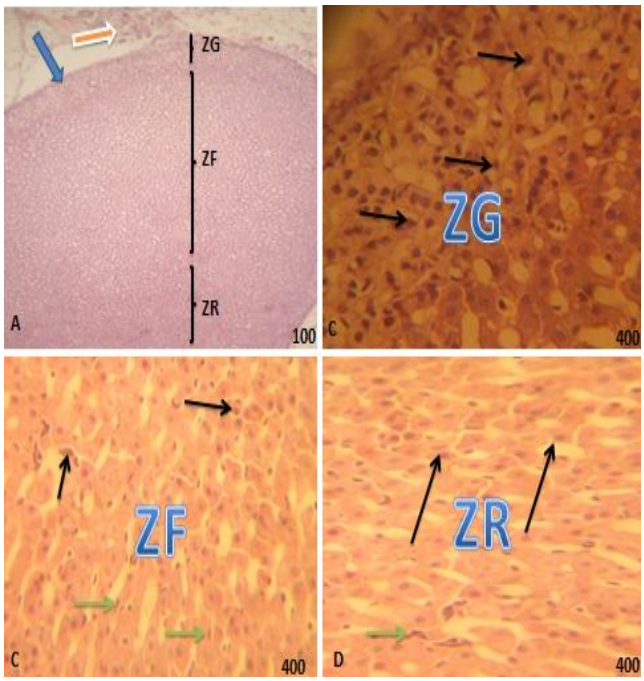

Figure 2. Photomicrograph of adrenal cortex (Treatment section T_1) showing the different cellular zones of zona grannulosa (ZG), zona fasciculata (ZF) and zona reticulosa (ZR) . Note the orange arrow pointing to the adipose tissue surrounding the adrenal gland while the blue arrow is pointing to the connective tissue capsule .The Black arrows point to the normal and well distributed pyramidal and polyhedral cells while the green arrows show the few distorted cells. H&E stain x400.

As clearly evident from results obtained in this study, Carbon tetrachloride (CCl_4) induced alterations in the histoarchitecture, antioxidant enzymes and lipid peroxidation which thus clearly suggest that CCl_4 is able to cause oxidative stress in adrenal gland. The histological presentations in this study as evident from Figure 3 revealed distorted and seemingly pyknotic cells with vacoulations in the zona fasciculata and the effects were less pronounced in zona reticulosa and zona glomerulosa which could have been due to damaging effects of CCl_4.However Figures 1 and 4 revealed normal histoarchitecture while the effects were less pronounced in Figure 2 and this is most likely due to a protective action of Ginger. Reports have it that the most frequently implicated lesion site of adrenal cortex is the zona fasciculate (Rosol *et al*, 2001).

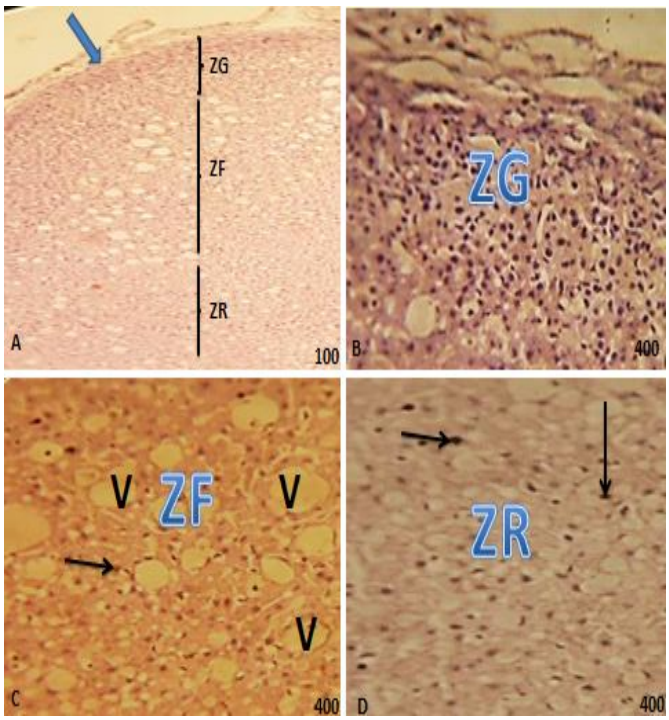

Figure 3. Photomicrograph of adrenal cortex (Treatment section T_2) showing the different cellular zones of zona grannulosa (ZG), zona fasciculata (ZF) and zona reticulosa (ZR) . Note the blue arrow pointing to the connective tissue capsule .The Black arrows point to the distorted polyhedral cells in the zona fasciculata and zona reticulosa with vacoulation (V) sites . H&E stain x400.

The distorted cells and vacoulations seen in this study is in conformation with the earlier findings of Sakr, *et al*, 2002 where atrophy and vacoulation of the zona fasciculate was observed following exposure to methylpredinysolone. The histological findings seen here revealed a seemingly protective potential of ginger which is ameliorating the damaging effects of CCl_4 in the ginger exposed group. Determination of activity level of these antioxidant enzymes is an appropriate indirect way to assess the pro-oxidant antioxidant status in tissues (Priscilla and Prince, 2009). It is a known fact that generation of highly ROS such as superoxide radicals, hydrogen-peroxide, hydroxl radicals and LPO in the presence of heavy metals ions are known to damage various cellular components including proteins, membrane lipids and nucleic acids (Halliwell and Gutheridge, 1989) as both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS (Frie *et al*, 1988). Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. In this study, determined activities of the antioxidant defense system like SOD, GR and GPx showed decreased values in the CCl_4 exposed group compared to the control but higher values were recorded in the Ginger exposed group as seen in Table 1 and this is also in conformity with earlier findings of Moreira *et al*.2001. Superoxide dismutases are specific for catalytic removal of superoxides by converting them into

H_2O_2 (Halliwell and Gutteridge, 2007). The decreased activity of SOD may be a response to increased production of H_2O_2 and O_2 as well as decreased protein expression levels as reported by Argano *et al*. 1997. The decreased activity of enzymes could also be due to their decreased protein expression levels from CCl_4 toxicity condition as reported earlier by Sindhu *et al*, 2004. An antagonistic effect between selenium (as a cofactor) and CCl_4 may affect GPx activity and it can thus render GPx a potential target for CCl_4 toxicity because a reduction in selenium uptake may increase the susceptability of cell to oxidative stress. It is clear that GPx needs GSH to decompose H_2O_2 or other peroxides with the simultaneous oxidation of GSH into GSSG, however, GR which is another component of the antioxidant defense system will reduce GSSG back to GSH and thereby will support the antioxidant defense mechanism indirectly. The presence of disulfide at the active site of GR as earlier reported by [Quinlan, 1988], may be a target for CCl_4 and will result in inhibition of GR activity. GSH is a predominant endogenous antioxidant and used as a cofactor to remove hydrogen peroxide and lipoperoxides by the GSH-Px family during which GSH is converted into oxidized form of glutathione (GSSG) (Muhammad and Tahira, 2011). In this study as seen from Table 2, significantly reduced level of GSH was recorded in the CCl_4 exposed group T2 compared to the control while the Ginger exposed groups T1 and T3 recorded values at par with the control and this clearly portrayed Ginger as an ameliorative oxidant. Oxidized glutathione is converted back into GSH by another rate controlling enzyme the glutathione reductase (GSR) thereby maintain the intracellular GSH levels. This optimum level of GSH is an utmost criterion in maintaining the structural integrity and physiology of cell membranes.

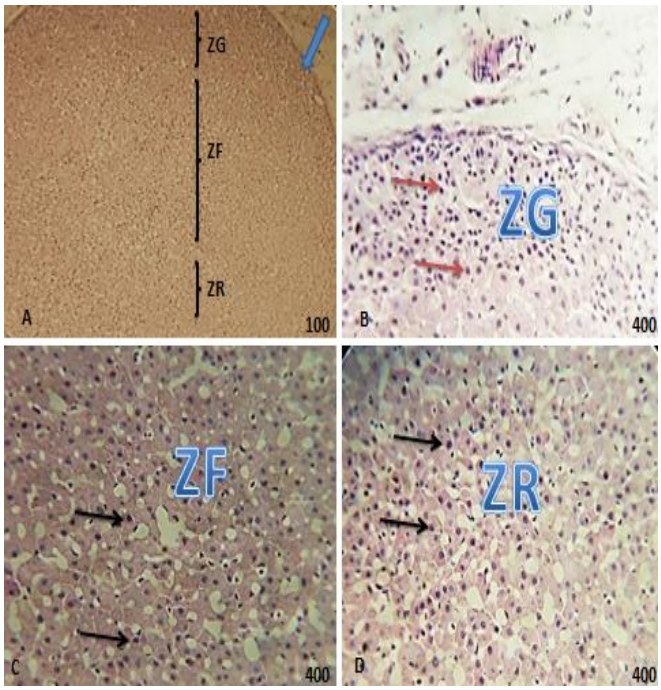

Figure 4. Photomicrograph of adrenal cortex (Treatment section T_3) showing the different cellular zones of zona grannulosa (ZG), zona fasciculata (ZF) and zona reticulosa (ZR). Note the blue arrow pointing to the connective tissue capsule as well as the Brown arrows pointing to the normal and well distributed pyramidal cells in ZG, while the Black arrows point to the normal polyhedral cells in ZF and ZR. H&E stain x400.

Lipid peroxidation is a free radical process involving a source of secondary free radical, which further can act as second messenger or can directly react with other biomolecules, enhancing biochemical lesions. Lipid peroxidation occurs on polysaturated fatty acid located on the cell membranes and it further proceeds with radical chain reaction. Hydroxyl radical initiates ROS and removes hydrogen atom, thus producing lipid radical and further converted into diene conjugate. Due to lipid peroxidation, a number of compounds are formed, for example, alkanes, malonaldehyde (MDA), and isoprotanes. When compared to the control and other groups in this study, significantly $P<0.05$ high level of MDA was recorded with extremely low values obtained in the Ginger exposed groups. Reactive oxygen species react with lipids and cause peroxidative changes that result in elevated lipid peroxidation. The increased lipid peroxidation with CCl_4 may be an indication of a decrease in non-enzymatic antioxidant of defense mechanism (Shanmugam *et al*, 2010). The MDA, another end product of lipid peroxidation has been demonstrated to be a mutagenic and genotoxic agent that can contribute to the development of human cancers (Feron *et al*, 1991). Hence, agents that can inhibit lipid peroxidation in organs with variations in the level of polyunsaturated fatty acids (PUFA) and antioxidants status will be an addition to the concept of functional food. Although previous studies have demonstrated the protective effect of *Z. officinale* against anticancer drugs induced organ damages in experimental animals (Ajith *et al*,2007,2008),the findings obtained from this study has also added to the available informations on the antioxidative potentials of ginger

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