ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Study of the Protective Effects of Ginger Extracts Against Hepatic Induced by Carbon Tetrachloride in Rats.

Haider Salih Jaffat*, Zainab Sajid Al-Shemmari and Muhsin C. Abbas.

Department of biology, Faculty of Science, Univeristy of Kufa, Najaf, Iraq.

ABSTRACT

The present study was designed to evaluate the possible hepatoprotective and antioxidant activity of ethanol and aqueous extracts of *Zingiber officinale* by using carbon tetra chloride induced hepatotoxicity in rats. In this study 42 male rats divided into 6 groups. The rates teated with various extracts in compare with control group. At the end of the treatment period that was 8 weeks, we obtained blood samples from the rats for assessment of oxidative stress parameters creatinine, urea, total protein and antioxidant parameters MDA , GSH and vitamin C. Additionally, the result indicated that ethanol extract at concentration 300mg/kg has the ability to a significant decrease ($p<0.05$) urea, creatinine, and MDA level. A significant increase ($p<0.05$) in GSH level, vitamin C, and total protein. The results of this study indicate that the two concentrations (150, 300) mg/kg of the ethanolic *Zingiber officinale* rhizomes extracts gave positive results, even better than aqueous extract.

Keywords: CCL4, *Zingiber officinale* **,** MDA , GSH , vitamin C, Rat

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Ginger (*Zingiber officinale Roscoe*;Zingiberaceae) has been used as spice for over thousand years (Bartley and Jacobs, 2000). Its roots contain polyphenol compounds (6-gingerol and shogaols) which have a high antioxidant activity (Stoilova *et al;* 2007). The antioxidant property of ginger has been proposed as one of the major possible mechanisms for the protective effects of the plant against toxicity and lethality of radiation (Jagetia *et al;* 2003) and a number of toxic agents such as carbon tetrachloride, arsenic and cisplatin (Amin and Hamza, 2006; Yemitan and Izegbu; 2006; Morakinyo *et al;* 2010). Liver is a major detoxifying organ in vertebrate body. It plays a central role in carbohydrate, protein and fat metabolism and allows the detoxification of various xenobiotics. Additionally, it regulates the synthesis and secretion of bile (Casarett and Doull's, 2008). Many xenobiotics such as acetaminophen, CCl4 and yellow phosphorus produce liver damage in a predictable and dose-dependent manner, the most frequent mechanism of hepatocellular injury involves production of injurious metabolites by the cytochrome p450 system (Tewari and Gupta, 2000). Toxic injury occurs in the liver more often than other organs because all ingested substances that are absorbed are first presented to the liver and that the liver is responsible for the metabolism and elimination of many substances (Casarett and Doull's, 2008).

Liver is central to the metabolic disposition of virtually all drugs and CCl4 (Matsumura, 1994). For the most part, this process is accomplished without injury to the liver itself or to other organs. A few compounds such as acetaminophen, CCl4 and toxins themselves or produce metabolites which cause liver injury (Wang *et al*., 1998). Peoples can be exposed to carbon tetrachloride from the air, drinking water, foodstuffs and from soil due to very low background levels, Exposures higher than background levels can occur near certain industrial sites where carbon tetrachloride is still used or there has been previous industrial contamination. However oxidative stress is implicated in different physiological condition, including respiratory chain reactions, killing of bacteria, and others. Such involevements of oxidative stress may lead to various diseases, such as Alzheimer disease, cardiovascular disease and others (Tanas *et al*., 2010). Herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy (Venukumar and Latha, 2002). Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases (Dash *et al*., 2007).

MATERIALS & METHODS

Ethanol Extract

20 g of the powder kept in thimble was extracted with 200 ml 90% ethanol in a soxhlet extractor for 24 hours. The extract was concentrated in a vacuum at 60 C° using rotary evaporator to evaporate the remaining solvent. The extract was kept in a freeze dryer for 24 hour yielding semisolid residues of EGE (David, 2006) .

Aqueous Extract

Aqueous extract of ginger was prepared by maceration method with slight modification. A total 10 g of the ginger powder was steeped in 100 ml of sterilized distilled water for one day, and then filtered through eight layers of cotton. It was further filtered by using filter paper (Whatman No.1) and centrifuged at 3000x for 10 minutes, and then the filtrated was kept in a freeze dryer for 48 hour yielding solid residues of AGE (Ladd et al; 1978).

Determination of Serum Glutathione Activity

The test is intended for quantitative of glutathione (GSH) concentration in serum through the immunosorbant assay (ELISA) using bioelisa reader EL x800 (biokit, USA).

Determination of Lipid Peroxidation Activity (MDA)

Lipid Peroxidation Activity determined by ELISA according to manufacture instructions (Cell Bio labs , USA).

Determination of Vitamin C

Quantitative determination of vitamin C in human serum was supplied by ALPCO company , USA external standard method (Falch, 1998).

Determination of urea , creatinine and total protein

Creatinine kit for quantitative determination of creatinine in serum , spinreact, S.A.U. Urea kit was supplied by biomerieux, France. Total protein was determined by Biuret method.

Animal experiment

Ethical approval for this study was obtained from the local medical ethics committee, Faculty of Science, University of Kufa. The study was designed and conducted in accordance with the tents of Declaration of Helsinki. Animal experiment was designed as 42 male rats divided into 6 groups (7 rats in each group) and treated as follows: groupI was treated with oral doses of normal saline and saved as a control, groupII was treated with 0.5 ml of volume dose oral doses of CCl4 suspended in olive oil (1: 1 v/v) 0.5ml at 3 days in week. Group III was treated with 0.5 ml of volume dose from CCl4 plus ethanolic extract at a concentration of 300 mg/kg at the same time, group IV was treated 0.5 ml of volume dose from with CCl4 plus ethanolic extract at concentration 150 mg/kg at the same time; group V was treated with 0.5 ml of volume dose from CCl4 plus aqueous extract at concentration 300 mg/kg at same time; group VI was treated with 0.5 ml of volume dose from CCl4 plus aqueous extract at concentration 150 mg/kg at the same time.

Statistical analysis

Data were analyzed using the program statistical package for the Social Science (SPSS for windows, version 10.0).

RESULTS

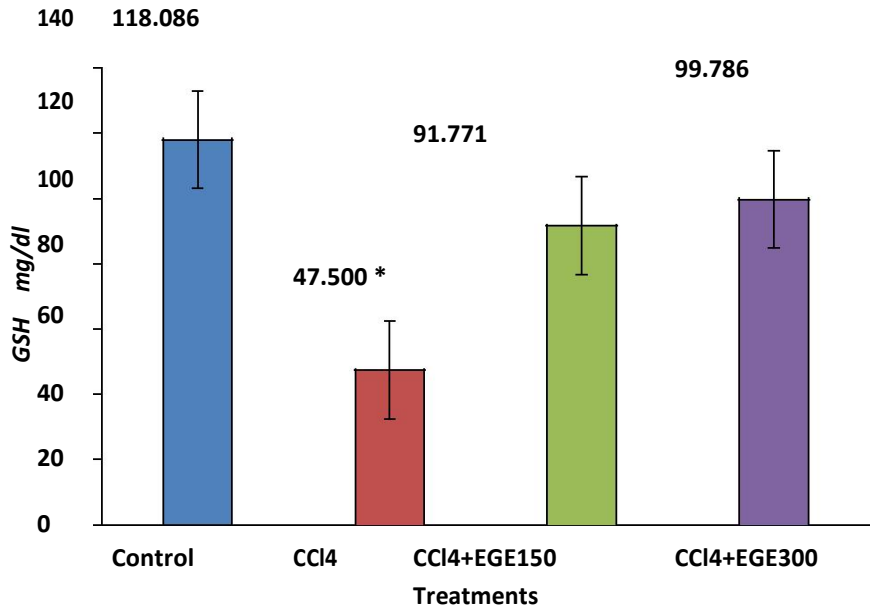


Figure (1): effect of ethanolic ginger extract on GSH levels in the rats treated with CCl4. *p<0.05

significant different to control group, ss= significant different to CCl4 group. Each value represents mean ±SD.

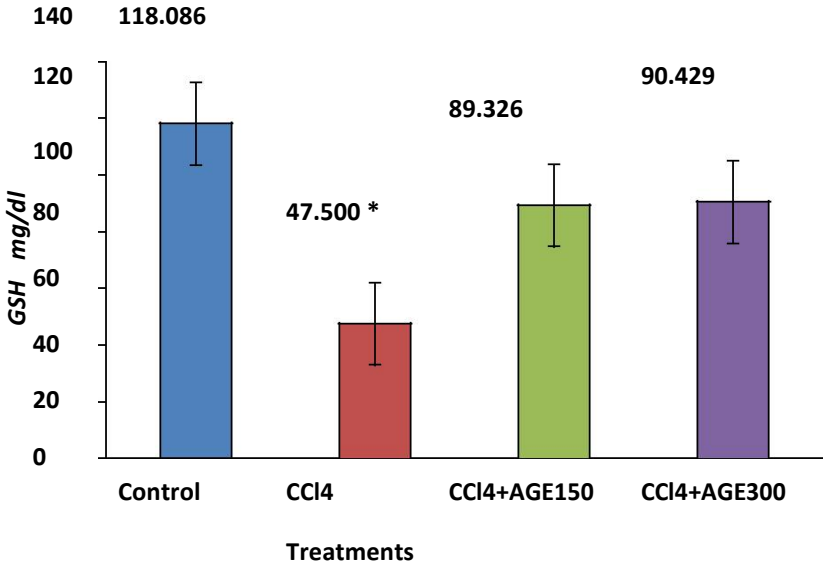


Figure (2): effect of aqueous ginger extract on GSH levels in the rats treated with CCl4 . *p<0.05 significant different to control group, ss= significant different to CCl4 group. Each value represents mean ±SD.

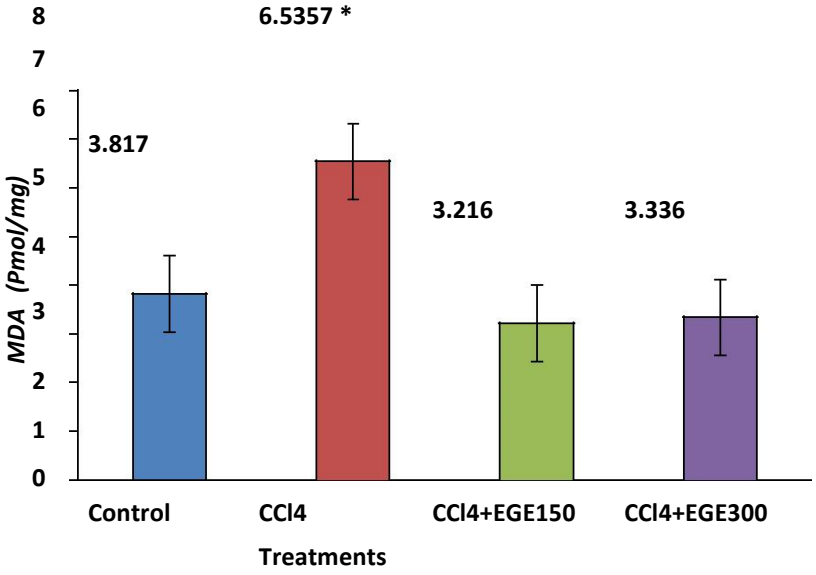


Figure (3): effect of ethanolic ginger extract on MDA levels in the rats treated with CCl4. *p<0.05 significant different to control group, ss= significant different to CCl4 group. Each value represents mean ±SD.

The results in **figure1** shows a significant decrease in serum level glutathione reductase (GSH) in animals treated with CCl4 for 8 weeks compared with control group. Treatment of the animals with EGE at concentration 150, 300 mg/kg of body weight along with CCl4for 8 weeks showed a significant increase in level of GSH compared with CCl4 group. Treatment of the animals

with AGE at concentration 150, 300 mg/kg of body weight along with CCl4 for 8 weeks ($p<0.05$) shows a significant increase in GSH levels compared with CCl4 group ($p<0.05$) this is shown in **figure 2**.

The results in **figure3** show a significant increase in lipid peroxidation products (MDA) within the group treated with CCl4 for 8 weeks compared to control ($p<0.05$). Treatment of the animals with EGE at concentration150, 300 mg/kg of body weight along with CCl4 for 8 weeks showed a significant decrease in level of MDA compared with CCl4 group. Treatment of the animals with AGE at concentration
150, 300 mg/kg of body weight) along with CCl4 for 8 weeks ($p<0.05$) showed a significant decrease in MDA levels compared with CCl4 group ($p<0.05$), this show in figure 4.

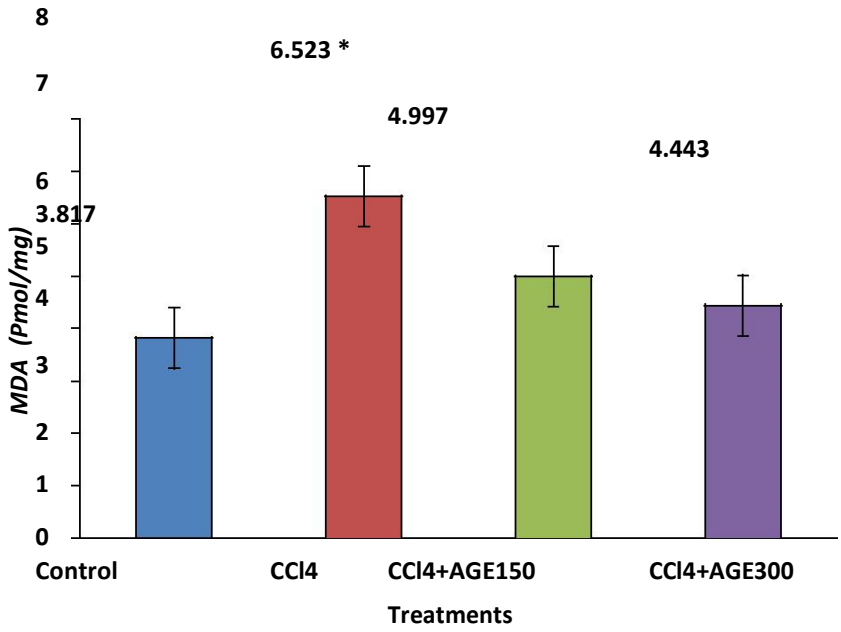
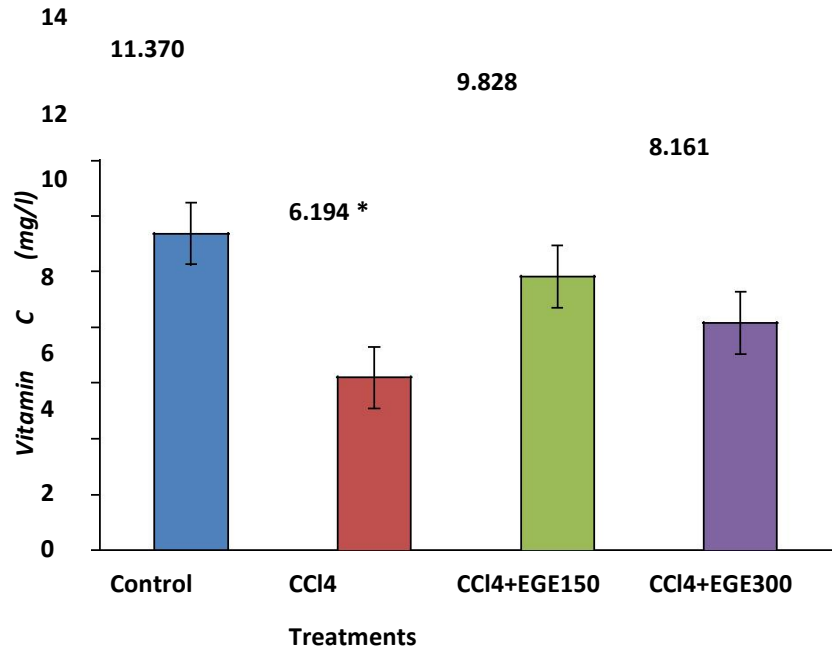


Figure (4): effect of aqueous ginger extract on MDA levels in the rats treated with CCl4 . *p<0.05 significant different to control group, ss= significant different to CCl4 group. Each value represents mean ±SD.



ISSN: 0975-8585

Figure (5): effect of ethanolic ginger extract on the vitamin C levels in the rats treated with CCl4 . *p<0.05 significant different to control group, ss= significant different to CCl4 group. Each value represents mean ±SD.

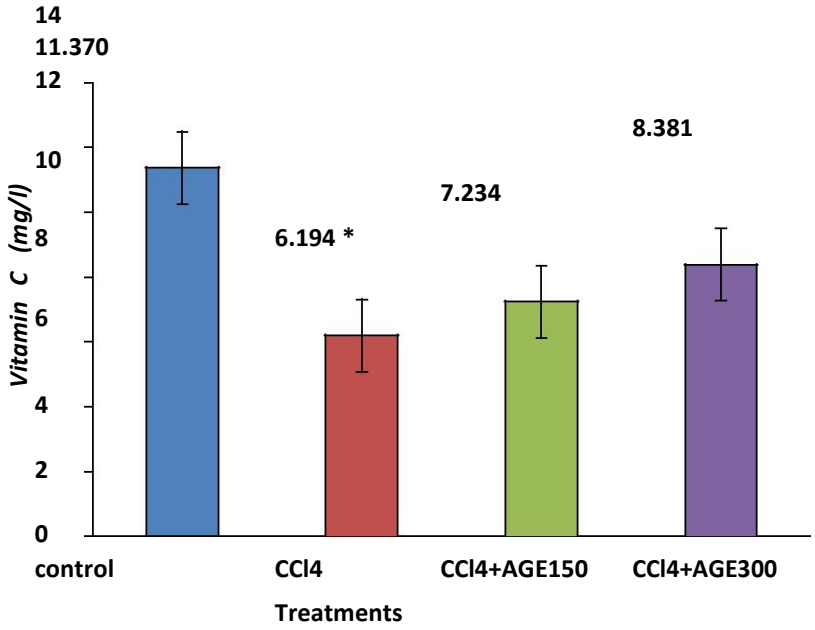


Figure (6): effect of aqueous ginger extract on the vitamin C levels in the rats treated with CCl4 . *p<0.05 significant different to control group, ss= significant different to CCl4 group. Each value represents mean ±SD.

The results in **figure5** shows a significant decrease in non- enzymatic antioxidant ascorbic acid (vitamin C) with the group treated with CCl4 for 8 weeks compared to control (p<0.05).Treatment the animals with EGE at concentration 150, 300 mg/kg of body weight) along with CCl4 for 8 weeks shows a significant increase in level of vitamin C compared with CCl4 group. Treatment of the animals with AGE at concentration 150, 300 mg/kg of body weight along with CCl4 for 8 weeks (p<0.05) showed a significant increase in vitamin C levels compared with CCl4 group (p<0.05), this is shown **figure6**.

Table (1): Effect of Zingiber officinale extracts on serum levels of urea, creatinine, and total protein in experimental animals model treated with CCl4-induced hepatotoxicity.

Treatment	Urea mg/dl	Total protein mg/dl	Creatinine mg/dl
Control	33.46±0.63	5.72±2.31	0.52±0.33
CCl4 group	54.24±2.65	0.73±0.91	1.16±0.99
CCl4+ 300 EGE	33.54±0.99	4.93±0.62	0.66±0.53
CCl4+ 150 EGE	34.93±1.62	3.55±0.41	0.73±0.33
CCl4+ 300 AGE	35.5±1.54	3.86±73	0.82±0.12
CCl4+ 150 AGE	35.67±1.60	3.93±1.30	0.94±0.23

Each value represents mean± SD. Number of animals = 7 for each treatment. p<0.05 in

respected to control.

DISCUSSION

Treatment with ginger extracts at concentration 150, 300 mg/kg for each extracts along with CCl4 for 8 weeks, the results in figure (1and 2), showed normalized the antioxidant levels through their rich of flavonoids that have the ability to scavenge free radicals (Mitra *et al;* 1998). These results agree with the results that were obtained by (Tarek *et al;* 2011). The GSH is a tripeptide composed of glycin, glutamic acid , and cystein. The important part of the molecules is the SH group of cysteine. Therefore, it exists both in oxidized and reduced forms (Sarker et al; 2006). CCl4 is one of extensively environmental toxicants. The reactive metabolite trichloromethyl radical (CCl3) formed by the metabolic conversion of CCl4 by cytochrome p-450. This reactive metabolite initiates the peroxidation of membrane poly-unsaturated fatty acids, generated PUFA radicals, covalently binds to membrane lipids and proteins and generates ROS (Gowri et al; 2008).

Evidence suggests that various enzymatic and non-enzymatic systems have been developed by the cell to attenuate ROS (Gowri et al; 2008). In agreement with these explanations, the observed decrease in GSH was recorded in CCl4 treated rats that may be due to inactivation of the antioxidative enzymes. This may cause an increased accumulation of superoxide radicals which could further stimulated lipid peroxidation. The result in figure (1) showed decrease in GSH activity which is probably due to decrease availability of GSH resulted during the enhanced lipid peroxidation processes.

The results in figure3 showed a significant increase in the level of lipid peroxidation (MDA) in group that were treated with CCl4 for 8-weeks compared to control. Treatment of the animals with ethanolicginger extracts at concentration 150, 300 mg/kg of body weight along with CCl4 for 8 weeks, figure (3), show a significant increase in the level of MDA compared to control, these results are due to ethanolic ginger extracts express antioxidant activity against free radicals that are formed from CCl4. Treatment of the animals with aqueous ginger extracts at concentration 150, 300 mg/kg of body weight along with CCl4 for 8-weeks, figure (4), show a significant decrease in the levels of MDA compared to control, The increase due to the ginger extract which acts as scavenge to free radicals that are formed from CCl4. Ginger has been reported to an increased glutathione and reduced lipid peroxidation in vivo and scavenging of various free radicals in vitro (Jagetia *et al;* 2003).

The results in figure 5 and 6 interpretion Besidesstudy shows that mobilization antioxidants in response to oxidative stress reflects a dynamic process through (Elsayed, 2001). All the above data may explain the increase in serum GSH of the group treated with CCl4 plus ginger extracts.

The results in the table (1) show increase in the urea and creatinine in rats that are treated with CCl4. This increase is due to CCl4 free radicals that cause liver cells damage (hepatocyte damage). But the total protein is decreased with rats that are treated with CCl4 that are caused by liver cell damaged by free radicals. This leads to decrease in protein synthesis or may be alter in the protein synthesis. The results which are obtained by (Araujo and Welch, 2006). The treatment with ginger extracts plus CCl4 leads to an increase in total protein but a decrease in urea and creatinine. These results agree with results that are obtained by (Sharma and Shukla, 2010).

REFERNCES

Amin, A. and Hamza, A. A., (2006). Effects of roselle and ginger on cisplatin induced reproductive toxicity in rats. Asian. J. Androl . 8: 607-612.

Araujo M, Welch WJ. (2006). Oxidative stress and nitric oxide in kidney function. Curr Opin Nephrol Hypertens. 15(1):72-7.

Bartley, J. and Jacobs, A., (2000). Effects of drying on flavor compounds in Australian-growth

ginger (*Zingiber officinale*). Journal of the science of Food and Agriculture. 80: 209-215.

Casarett and Doull's (2013). Toxicology; the basic science of poisons, 8th Edition. Mc-Graw Hill.557-576.

Dash, DK.; Yeligar, VC.; Nayak, SS.; Ghosh, T.; Rajalingam, D.; Sengupta, P.; Maiti,BC. and Maty, TK., (2007). Evalution of hepatoprotective and antioxidant activity of Ichnocarpusfrutescens (Linn.) R.Br. on paracetamol- induced hepatotoxicity in rats. Trop. J. Pharm. Res. 6(3): 755- 765.

David W. Held (2004). Relative Susceptibility Of Woody Landscape and Plants to Japanese Beetle (Coleoptera: Scarabaeidae). J. of. Arboricult 30(6): 328-335.

Elsayed, NM. (2001). Antioxidant mobilization in response to oxidative stress: adynamic environmental-nutritional ineraction. Nutrition. 17: 828-834.

Falch, J A., (1998). Low levels of serum ascorbic acid in elderly patient with hip Fracture. Scand.Clin. Lab. Invest. 58: 225-228.

Gowri Shankar, GNL.; Manavalan, R.; Venkappayya, D. and Raji, CD., (2008). Hepatoprotective and antioxidant effects of *Commiphoraberryi* (Arn) Engl bark extract against CCl4 -induced oxidative damage in rats. Food Chemtoxicol. 46: 3182- 3185.

Jagetia, G.C.; Baliga, MS.;Venkatesh , P. and Ulloor, JN., (2003). Influence of ginger rhizome (*Zingiber officinale Rosc)* on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. Radiat Res. 160: 584-592.

Matsumura, F., (1994). How important is the protein phosphorylation pathwayin the toxic expression of dioxin-type chemicals. Biochem. Pharmacol. 48: 215- 224.

Mitra , S.K.;Venkataraganna, M.V.;R. and Gopumadhavan, S., (1998). Effect of HD- 03, A Herbal Formulation on theAntioxidant Defense System in Rats. Phytother Res. 12 (2): 114-117

Morakinyo, AO.; Achema, PU. and Adegohe, OA., (2010). Effect of *Zingiber officinale* (Ginger) on sodium arsenite-indued reproductive toxicity in male rats. Afri. J. of Biomed. Res. 13: 39-45.

Sarker, A.; Ray, D.; Shrivastava, A. and Sarker, S., (2006). Molecular biomarkers: their significance and application in marine pollution monitoring. Ecotoxicolgy.15: 333-340.

Sharma, N. and Shukla, S., (2011). Hepatoprotective potential of aqueous extract of Butea monosperma against CCl(4) induced damage in rats. Exp Toxicol Pathol. 63(7-8):671-676.

Stoilova, I.; Krastanov, A.; Stoyanova, A.; Denev, P. and Gargova, S., (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem. 102: 764-770.

Tanas, S.; Odabasoglu, Z.; Halici, A.; Cakir, H.; Aygun, A.; Aslan, and Suleyman, H., (2010). Evaluation of anti-inflammatory and antioxidant activites of *Peltigera rufescens* lichen species in acute and chronic inflammation models. J Nat Med. 64: 42-49

Tarek, K.; Motawi;Manal, A.; Hamed; Manal, H.; Shabana; Reem, M .H. and Asmaa, F. A., (2011). *Zingibe rofficinale* acts as a nutraceutical agent against liver fibrosis. Nutr Metab. 8: 40-46.

Tewari S, Gupta V, Bhattacharya S (2000). Comparative study of antioxidant potential of tea with and without additives. Indian. J. physiol. Pharmacol. 44: 215-219.

Venukumar MR, Latha MS, (2002). Hepatoprotective effect of themethanolic extract of *Curculigoorchioides*in CCl4- treated rats. Indian J Pharmacol. 34: 269- 275.

ISSN: 0975-8585

Wang, Y.; Chen, J. and Zhang, J., (1998). 4-Aminobiphenyl DNA damagein liver tissue of hepatocellular carcinoma patients and control. AM. J. Epidemiol. 174: 315- 323.

Yemitan, O. K. and Izegbu, M. C., (2006). Protective effects of *Zingiber officinale* (Zingiberaceae) against carbon tetrachloride and acetaminophen induced hepatotoxicity in rats. Phytother Res.*20*: 997-1002.