Zingerone Ameliorates Free Radical Scavengers and Lipid Profile of Wistar Albino Rats

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Abstract - *Zingerone* the bioactive component of Ginger (*Zinziber officinale*) was prepared according to WHO protocol CG-06 (10). 5 g of *Zingerone* powder and 100 ml of double distilled water was stirred on a magnetic stirrer for 1.5 hour. The mixture was twice filtered through Whatmann filter paper no. 1. The filtrate was collected and allowed to dry. The aim of our study is to investigate the antioxidant potential of *Zingerone* (Z) the active component of Ginger, *Zingerone* was tried at two doses (250 mg and 500 mg per kg bw) for its effect on free radical scavengers and lipid profile of albino rats. Serum levels of Superoxide dismutase (SOD) and Catalase (CAT) were significantly ($P<0.05$) lower in the animals that received extract (both doses) than both controls. Also, GSH levels were lower in the animals that received the extract than both controls. However, Malondialdehyde (MDA) levels were significantly elevated ($P<0.05$) in the rats that received the extract when compared with the controls. Vitamins C and E levels did not display dose-dependence and did not differ significantly ($P>0.05$) from those of the controls. The mean HDL and LDL levels in both groups of rats did not differ significantly ($P<0.05$) at 250 mg but at 500 mg, HDL level was significantly lowered and LDL remained constant. Irrespective of dosage, the total cholesterol and triacylglycerol levels of the experimental rats did not differ from those of the controls. The *Zingerone* extract displayed antioxidant effect and little elevation of HDL and other bad lipids.

Key words: *Zingerone*, *Zinziber officinale*, SOD, CAT, MDA, GSH, LDL, HDL, Vitamin C & E.

Introduction

Oxidative Stress

The term oxidative stress refers to a condition in which cells are subjected to excessive levels of molecular oxygen or its chemical derivatives called reactive oxygen species (ROS).Under physiological conditions, the molecular oxygen undergoes a series of reactions that ultimately lead to the generation of superoxide anion ($O2^-$), hydrogen peroxide (H_2O_2) and H_2O. Peroxynitrite ($OONO^-$), hypochlorus acid (HOCl), the hydroxyl radical ($OH^.$), reactive aldehydes, lipid peroxides and nitrogen oxides are considered among the other oxidants that have relevance to vascular biology.

Free Radicals

Free radicals are reactive compounds that are naturally produced in the human body. They can exert positive effects (e.g. on the immune system) or negative effects (e.g. lipids, proteins or DNA oxidation). Free radicals are normally present in the body in minute concentrations. Free radicals are toxic molecules, may be derived from oxygen, which are persistently produced and incessantly attack and damage molecules within cells, most frequently, this damage is measured as peroxidized lipid products, protein carbonyl, and DNA breakage or fragmentation. Collectively, the process of free radical damage to molecules is referred to as oxidative stress. To limit this harmful effect, an organism requires complex protection – the antioxidant system. This system consists of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic antioxidants (e.g. vitamin E [tocopherol], vitamin A [retinol], vitamin C [ascorbic acid], glutathione and uric acid). An imbalance between free radical production and antioxidant defense leads to an oxidative stress state, which may be involved in aging processes and even in some pathology (e.g. cancer and Parkinson’s disease).

Oxidative Damage to Lipids (Lipid Peroxidation)

The peroxidation of lipids involves three distinct steps: initiation, propagation and termination. The initiation reaction between an unsaturated fatty acid and the hydroxyl radical involves the abstraction of an H atom from the methyl vinyl group on the fatty acid. The remaining carbon-centred radical, forms a resonance structure sharing this unpaired electron among carbons 9 to 13. The basis for the hydroxyl radical's extreme reactivity in lipid systems is that at very low concentrations it initiates a chain reaction involving triplet oxygen,

the most abundant form of oxygen in the cell [1]. The lipid hydroperoxide (ROOH) is unstable in the presence of iron or other metal catalysts because ROOH will participate in a Fenton reaction leading to the formation of reactive alkoxy radicals. Therefore, in the presence of iron, the chain reactions are not only propagated but amplified. Among the degradation products of ROOH are aldehydes, such as malondialdehyde, and hydrocarbons, such as ethane and ethylene, which are commonly measured end products of lipid peroxidation [2]. MDA can react with amino and thiol groups, the aldehydes are more diffusible than free radical which means damage is exported to distance sites. Aldehydes are quickly removed from cells as several enzymes control their metabolism [3].

Antioxidants

Antioxidants are thought to protect the body against the destructive effects of free radicals. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron- gain reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. They act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and disease [4]. The body produces several enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPX), that neutralize many types of free radicals. Supplements of these enzymes are available for oral administration. Melatonin is a hormone secreted by pineal gland and proves to be powerful antioxidant and free radical scavenger [5,6].

Liver

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. Weighing in at around 3 pounds, the liver is the body's second largest organ. It has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The liver performs many essential functions related to digestion, metabolism, immunity, and the storage of nutrients within the body. These functions make the liver a vital organ without which the tissues of the body would quickly die from lack of energy and nutrients. The major functions of the liver are carbohydrate, protein and fat metabolism detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But when drug habits, alcohol infections and autoimmune disorders, prescribed (antibiotics, chemotherapeutic agents) cum over-the-counter drugs can eventually lead to various liver ailments like hepatitis cirrhosis and alcoholic liver disease. Liver has an incredible capacity for regeneration of dead or damaged tissues it is capable of growing as quickly as a cancerous tumor to restore its normal size and function.

Ginger (*Zingiber Officinale Roscoe*) is one of the world's best known spices, and it has also been universally used throughout history for its health benefits. Ginger extract possesses antioxidative characteristic, since it can scavenge superoxide anion and hydroxyl radicals [7]. Ginger acts as a hypolipidemic agent in cholesterol-fed rabbits [8,9]. Ginger has dominative protective effect on DNA damage induced by H_2O_2 [10]. Feeding rats ginger significantly elevated the activity of hepatic cholesterol-7α-hydroxylase, the rate-limiting enzyme in bile acids biosynthesis, thereby stimulating cholesterol conversion to bile acids, resulting in elimination of cholesterol from the body [11]. In addition, a pure constituent from ginger [E-8 beta, 17-epoxylabd-12-ene-15, 16-dial (ZT)], was shown to inhibit cholesterol biosynthesis in homogenated rat liver [12]. All the indigenous materials with antitubercular potential of ginger are also good source of antioxidants and therefore may be capable of preventing tissue damage by ROS [13]. Decrease in ROS production will reduce the protein deprivation in all the animals. Ginger (*Zingiber officinale Roscoe*, Zingiberaceae) has been widely used as a dietary spice, as well as in traditional oriental medicine and possesses potential in chemopreventive activities. *Zingerone* is also called as Vanillylacetone, is a key component of the pungency of ginger. *Zingerone* is a crystalline solid that is sparingly soluble in water, but soluble in ether. *Zingerone* is similar in chemical structure to other flavor chemicals such as vanillin and eugenol. It is used as a flavor additive in spice oils and in perfumery to introduce spicy aromas. Fresh ginger does not contain *zingerone*, cooking the ginger transforms gingerol, which is present, into *zingerone* through a retro-aldol reaction (reversal of aldol addition).Ginger compounds have been shown to be active against entero-toxigenic *Escherichia coli* heat-labile enterotoxin-induced diarrhea. This type of diarrhea is the leading cause of infant death in developing countries. *Zingerone* is likely the active constituent responsible for the antidiarrheal efficacy of ginger.

Ginger extracts showed selective anticancer activity. It has been implicated as one of the promising chemopreventive agents against colon and skin cancer. Ginger is also known to possess antioxidant activity and significantly lowered the lipid peroxidation by maintaining the activities of superoxide dismutase, catalase and glutathione peroxidase. The present investigation is an attempt to evaluate the ameliorative effect of an aqueous extract of *Zingerone* (ginger), on Hexane-Chloroform induced Hepatocytes damage in the ratio of 4:6 at 2.5ml per rat.

Materials and Methods

Chemicals and Reagents

Zingerone was procured as a gift from the Department of Chemistry, My friend, Research Scholar from University of Madras, Chennai. All other chemicals were purchased from Sigma-Aldrich, Chemicals Pvt. Ltd, India. All other chemicals used were of good quality and analytical grade.

Animal Model

Male albino rats of Wistar strain (100±160g), (*Rattus novergcus*) were procured from Tamil Nadu University for Veterinary and Animal Sciences, (TANUVAS) Chennai, India were used for the study. Animals were fed with commercially available standard rat pelleted feed (M/s Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and water was provided *ad libitum*. The rats were housed under conditions of controlled temperature (25±2°C) and acclimatized to 12-h light, 12-h dark cycle. Animal experiments were conducted according to the guidelines of institutional animal ethical committee. All the drugs (standard and test as well as vehicle were administered per-orally using insulin syringe.

Experimental Design

Male Wistar albino rats were used for the study. The *Zingerone* was dissolved in double distilled water before the treatment. The male Wistar albino rats were divided into four groups of four rats each. The first two groups being the test groups (A and B) received 250 mg/kg and 500 mg/kg aqueous extract of *Zingerone* orally respectively, and the last two groups (C and D) being the normal and negative control groups that received water and feed only. The animals were kept for 10 days. All the animals used for this study were initially subjected to hepatocyte damage using hexane and chloroform in the ratio of 4:6 at 2.5 ml per rat.

Experimental Design

Seggregation of Groups

Experimental animals were divided into four groups of four rats each as follows.

Group A : Served as Test Groups received 250mg/kg aqueous extract of *Zingerone* orally treated for 10 days.

Group B : Served as Test Groups received 500mg/kg aqueous extract of *Zingerone* orally intra gastric tube gavage for 10 days

Group C : Normal Groups received water and feed only

Group D : Negative Control Groups received water and feed only.

All the animals used for this study were initially subjected to hepatocyte damage using hexane and chloroform in the ratio of 4:6 at 2.5 ml per rat.

Collection of Samples for Biochemical Analysis

After the experimental period, the animals were anaesthetized by intra-peritoneal injection of phenobarbital sodium (30mg/kg body weight) and were sacrificed 24hr after the 10 days of administration by cervical dislocation.

Protein concentration of the liver tissue fraction was determined by the Lowry's method using Bovine Serum Albumin (BSA) as standard.

Serum Separation

The blood was taken by heart puncture and centrifuged at 3000 rpm for 10 min to obtain the serum. The resultant clear supernatant was pipetted out and preserved in small vials in the freezer for the purpose of biochemical investigations.

Preparation of Tissue Homogenate

The liver was removed, washed in ice-cold 1.15% KCl to remove blood and other extraneous substances, dried in a filter paper and weighed. It was later homogenized in four parts of ice cold buffer containing 50 mM tris-HCl and 1.15% KCl, pH 7.4. The homogenate was centrifuged at 10,000 rpm for 15min at 4°C. The supernatant obtained was kept under refrigeration until further biochemical analysis.

Histopathological Study

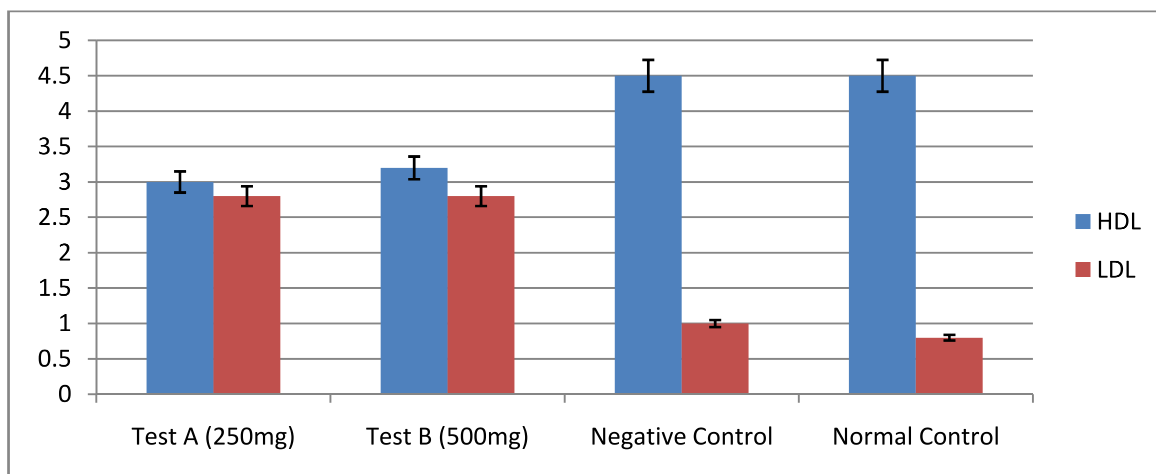
Haematoxylin and Eosin Staining

A portion of hepatic (liver) tissue was fixed in 10 % formalin. The washed tissue was dehydrated in descending grades of isopropanol and cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5-µm thickness and stained with haematoxylin and eosin (H&E) .

The sections were then viewed under light microscope for histopathological changes in the Liver. The assessment of liver injury was carried out in a blinded fashion. Liver section with histo-pathological alterations like vacuolation, hydropic degenerative changes or apoptosis was recorded.

Statistical Analysis

Data obtained were analyzed statistically using statistical package for social science (SPSS) (version 17.0). The data are expressed as mean ± standard deviation using bar charts comparisons were made between the control animals that received the *Zingerone* extract of 250mg/kg and 500mg/kg of *Zingerone* using paired T-test. The significance difference was accepted at ($p<0.05$).

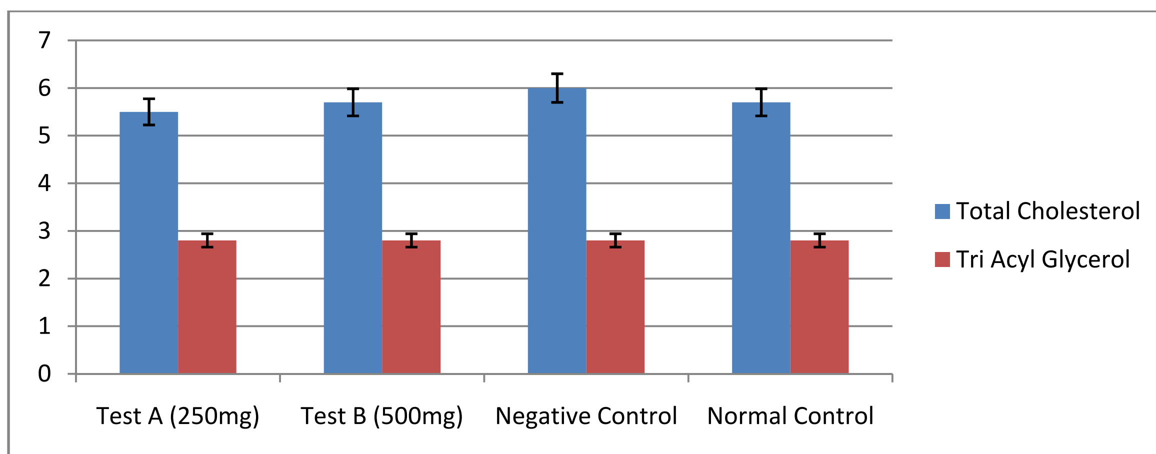


Graph 1. The effect of *Zingerone* on Test and Control

From the above Graph, the mean value result of the 250 mg *Zingerone* extract (2.9±1.95) and 500 mg *Zingerone* extract (3.14±1.73) on HDL has a non- significant (P>0.05) increase and increase respectively when compared with the negative control (4.20±0.91) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (4.16±0.16).

Also the mean value of 250 mg *Zingerone* extract (2.93±0.64) and 500 mg *Zingerone* extract

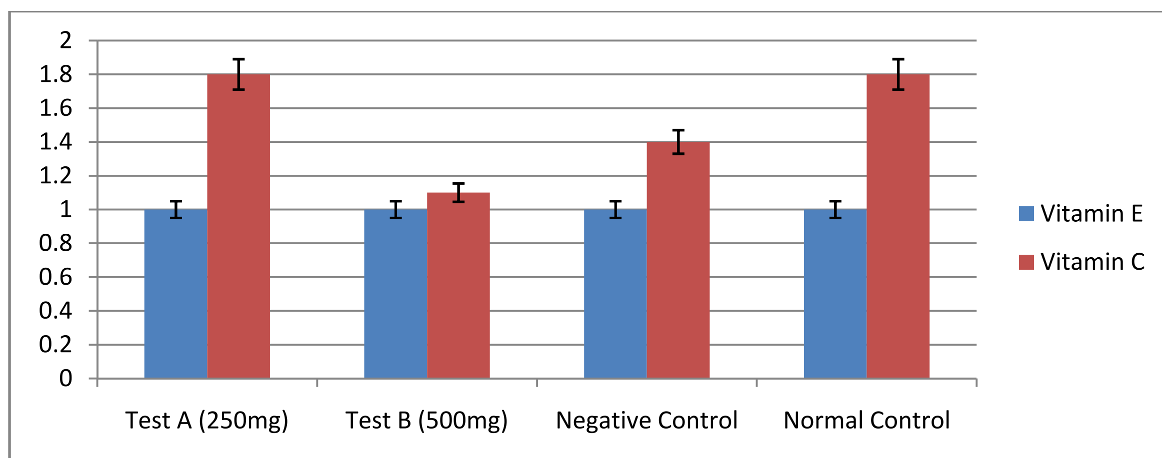
(2.68±1.36) on LDL has a non-significant (P>0.05) increase and decrease when compared with the negative control (1.18±0.82) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (0.99±1.06).



Graph 2. The effect *Zingerone* on test and control (Total Cholesterol and TAG)

From the above Graph, the mean value result of the 250 mg *Zingerone* extract (5.19± 0.77) and 500 mg *Zingerone* extract (5.43±0.80) on Total Cholesterol has a non- significant (P>0.05) increase and decrease respectively when compared with the negative control (5.86± 0.58) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (5.54±1.157).

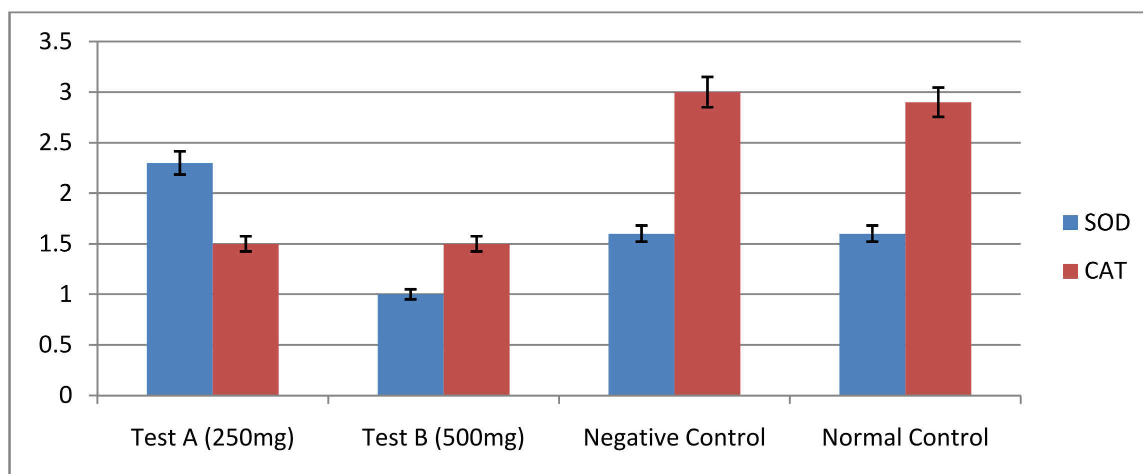
Also the mean value of 250 mg *Zingerone* extract (2.64±0.34) and 500 mg *Zingerone* extract (2.60±0.31) on TAG has a non-significant (P>0.05) increase and decrease when compared with the negative control (2.48±0.43) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (2.67±0.63).



Graph 3. The effect of *Zingerone* on test and control (Vitamin E&C)

From the above Graph, the mean value result of the 250 mg *Zingerone* extract (1.11±0.03) and 500 mg *Zingerone* extract (1.09±0.03) on Vitamin-E has a non- significant (P>0.05) decrease and increase respectively when compared with the negative control (1.13±0.06) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (1.12±0.24).

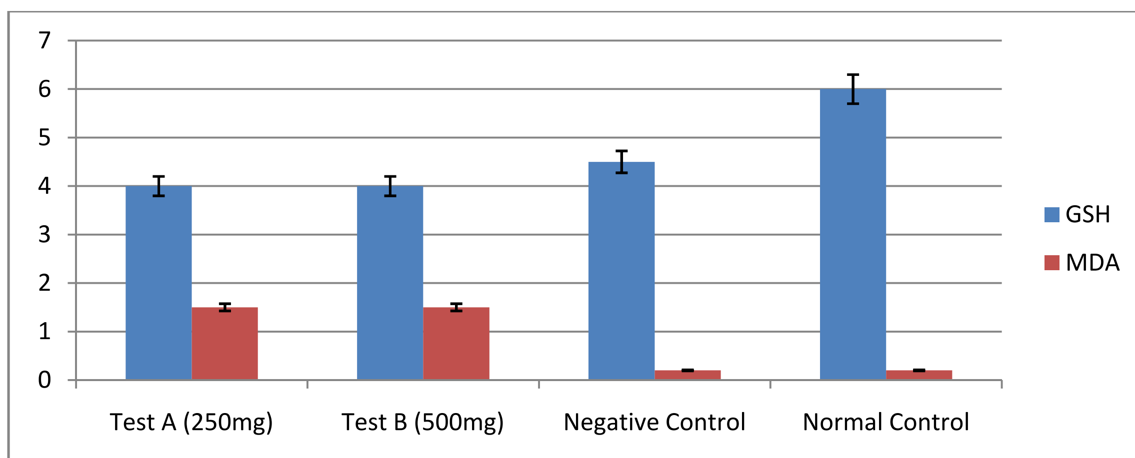
Also the mean value of 250 mg *Zingerone* extract (1.67 ±2.39) and 500 mg *Zingerone* extract (1.13±1.48) on Vitamin C has a non-significant (P>0.05) increase and decrease when compared with the negative control (1.35±0.13) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (1.72±0.69).



Graph 4. The effect of *Zingerone* on test and control (SOD and CAT)

From the above Graph, the mean value result of the 250 mg *Zingerone* extract (2.22±1.57) and 500 mg *Zingerone* extract (0.81±0.06) on SOD has a non- significant (P>0.05) increase and decrease respectively when compared with the negative control (0.91±0.05) as well as non-significant (P>0.05) decrease and increase respectively when compared with the normal control (0.99 ±1.11).

Also the mean value of 250 mg *Zingerone* extract (1.52±1.22) and 500 mg *Zingerone* extract (1.54±1.23) on Vitamin-C has a non-significant (P>0.05) decrease and increase when compared with the negative control (3.09±0.61) as well as non-significant (P>0.05) decrease and increase when compared with the normal control (2.95±2.17).



Graph 5. The effect of *Zingerone* on test and control (MDA and GSH)

From the above Graph, the mean value result of the 250 mg *Zingerone* extract (3.99±0.49) and 500 mg *Zingerone* extract (3.79± 0.74) on GSH has a non- significant (P>0.05) increase and decrease respectively when compared with the negative control (4.73±0.48) as well as non-significant (P>0.05) increase and decrease respectively when compared with the normal control (5.80 ± 3.64).

Also the mean value of 250 mg *Zingerone* extract (0.96 ±0.59) and 500 mg *Zingerone* extract (0.95±0.60) on MDA has a non-significant (P>0.05) increase and decrease when compared with the negative control (0.14±0.52) as well as non-significant (P>0.05) increase and decrease when compared with the normal control (0.10±0.03).

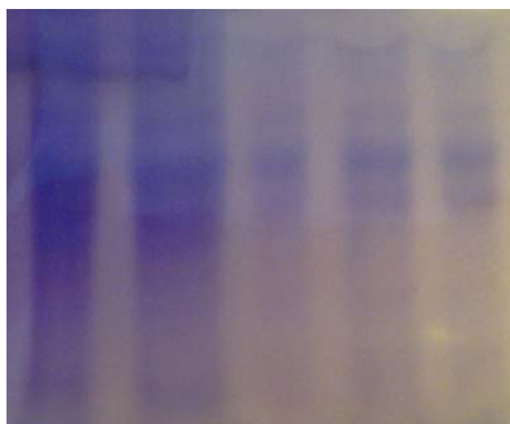


Fig 1. Effect of Hexane & Chloroform (4:6) and *Zingerone* on the SDS PAGE pattern of the blood plasma of control and experimental groups (Lane 1,2,3,4 & 5).

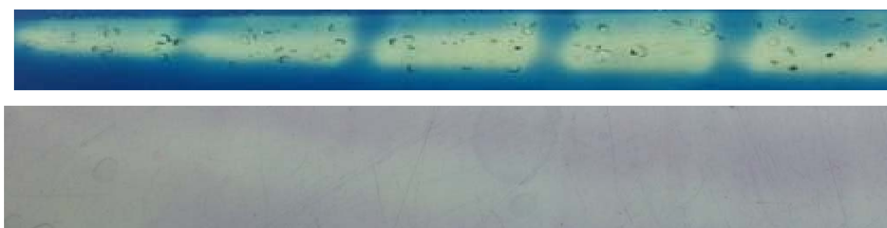


Fig 2. Effect of Hexane & Chloroform (4:6) and *Zingerone* on the Native gel electrophoresis (SOD & CAT) pattern of the blood control and experimental groups.

Results & Discussion

Free radical scavenger is a vitamin, mineral, or enzymes that is able to destroy free radicals. Mammalian cells possess elaborate detoxification. Key metabolite steps are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which destroy toxic perioxides. In addition to antioxidant enzymes, non-ezymatic molecules including thiols, thioredoxin, and disulfide-bonding play important roles in

antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α-tocopherol, β-carotene and ascorbic acid, and such micronutrient elements as zinc and selenium [14].

If cellular constituents do not effectively scavenge free radicals, they lead to disease conditions to the cell membranes and DNA, including oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutation leading to cancer degeneratives, and other diseases [15]. The results of this study demonstrate that *Zingerone* is a rich source of potential antioxidant and such effect may be related to the negative control and normal control. The total cholesterol, HDL, LDL, TAG, MDA, GSH, Vitamin E, SOD and catalase shows no significant ($P < 0.05$) difference between these parameters in the wistar albino rats as compared to the negative control and the normal control.

A number of research studies have however shown statistically significant ($P < 0.05$) that the aqueous extract of *Zingerone* hepatoprotective activity against chloroform and hexane induced hepatoxicity in wistar albino rats. Also, *Zingerone* has been reported to have antioxidant, anti-arthritic, anti-ulcer and anti-inflammatory activity. Histopathological studies revealed alterations of the hepatic tissue caused by hexane:chloroform exposure were prevented by *Zingerone* extract administration (Fig 6).

The electrophoretic pattern of proteins by SDS-PAGE also showed the protective role of *Zingerone* on Hexane and Chloroform induced protein fragmentation (Fig 1&2).

Conclusion

The results of the present study demonstrate that *Zingerone* is a rich source of potential antioxidants and such effects may be related to their biochemical. The study was carried out to investigate the hepatoprotective effect of aqueous extract of *Zingerone* on hexane/chloroform induced hepatocellular damage on male wistar rats. The *Zingerone* extract showed Serum levels of Superoxide dismutase (SOD) and Catalase (CAT) were significantly ($P<0.05$) lower in the animals that received extract (both doses) than both controls. Also, GSH levels were lower in the animals that received the extract than both controls. However, Malondialdehyde (MDA) levels were significantly elevated ($P<0.05$) in the rats that received the extract when compared with the controls. Vitamins C and E levels did not display dose-dependence and did not differ significantly ($P>0.05$) from those of the controls. The mean HDL and LDL levels in both groups of rats did not differ significantly ($P<0.05$) at 250 mg but at 500 mg, HDL level was significantly lowered and LDL remained constant. Irrespective of dosage, the total cholesterol and triacylglycerol levels of the experimental rats did not differ from those of the controls. The electrophoretic pattern of proteins by SDS-PAGE also showed the protective role of *Zingerone* on Hexane and Chloroform induced protein fragmentation (Fig 4&5). The *Zingerone* extract displayed antioxidant effect and little elevation of HDL and other bad lipids. Histopathological studies revealed alterations of the hepatic tissue caused by hexane:chloroform exposure were prevented by *Zingerone* extract administration (Fig 6).

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