EFFECT OF AQUEOUS EXTRACT OF Zingiber officinale RHIZOMES ON CCl_4 –INDUCED OXIDATIVE STRESS IN RATS

By

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ABSTRACT

Ginger (Zingiber officinale) is one of the essential dietary products that have been used for the treatment of numerous ailments such as hypertension, migraines, arthritis, nausea and colds. Recently, interest has increased in ginger and its active components as therapeutic agents. A study was carried out to evaluate the effect aqueous extract of Ginger on CCl_4 induced oxidative stress in albino rats. The Proximate, antinutrient, and Phytochemical contents of ginger extract were analyzed using standard AOAC methods while mineral contents were determined using atomic absorption spectrometry. Oxidative stress markers were also analyzed using colorimetric assay kit. The serum levels of oxidative stress markers were compared between the normal and test groups. Experimental rats were divided into five groups: Normal control group, negative control (CCl_4) group, standard drug (Vitamin C) group, ginger low and high dose group. At the end of the experiment, significant increase in malondialdehyde level and decrease in superoxide dismutase, catalase, reduced glutathione and glutathione Peroxidase activities were recorded in CCl_4-exposed rats as compared to normal control group. In the ginger supplemented groups, the level of MDA along with the activities of SOD, CAT, GSH and GPx were comparable with the normal control rats ($p>0.05$). Thus, it appears that ginger extract ameliorate the effect of CCl_4; suggesting that consumption of natural compounds with an antioxidant profile may be a preventive alternative to those diseases associated with oxidative stress.

Key Words: Ginger extract, carbon tetrachloride, Oxidative stress, ameliorate, antioxidant

Introduction

Ginger is a tropical plant, grows well in hot and humid climates. The plant is cultivated in China, Nepal, US, India, Bangladesh, Taiwan, Jamaica, Nigeria and Indonesia. Other than its anti-oxidative, anti-inflammatory, and anti-carcinogenic activities, it also has lifespan-extending property (Ippoushi *et al*., 2003; Lee *et al*., 2018). Zingiber officinale Roscoe, Zingiberaceae, is one of regularly eaten spice and also used as traditional herbal medicine for thousands of years (Han *et al*., 2013). Ginger root is the most commonly used for home remedies like headache, nausea, common cold and emesis. It possesses various properties, such as antioxidant, anti-microbial, anti-inflammatory, anti-cancer activities (Mao *et al*., 2019). Adding evidence to that ginger has also been reported to inhibit and regulate several diseases, such as neurodegenerative diseases, heart diseases, metabolic disorders (diabetes mellitus and obesity), and respiratory

disorders (Mao *et al*., 2019). Most abundant compounds in ginger are phenolic and terpene compounds (Prasad and Tyagi, 2015). The main phenolic compounds are gingerols, 6-shogaols, and 6-paradols that have been reported to have various biological activities. The major polyphenols identified in fresh ginger are gingerols (6-gingerol, 8-gingerol, and 10-gingerol) (Mao et al., 2019; Prasad and Tyagi, 2015).
Oxidative stress refers to the imbalance between free radicals and their stabilizing agent's antioxidant enzymes in the body. Reactive oxygen species or free radicals can be produced by normal cellular metabolism and react with biomolecules like protein, lipid, and DNA to cause cellular damage and responsible for degenerative changes. Many research groups have analyzed the antioxidant properties of natural products. These properties have been investigated through chemical and biological methods, or both. It has been suggested that the consumption of food rich in antioxidants can retard or avoid the occurrence of many diseases (White et al., 2014; Singh et al., 2015). Biologically active components in plant-based foods, such as redox-active antioxidants (polyphenols, carotenoids, tocopherols, vitamins C and E, glutathione) and enzymes (superoxide dismutase (SOD) and catalase (CAT)) with antioxidant activity have high potential for modulating many processes during the development of diseases (Hyson, 2011; Dumbravă et al., 2011). The present study aimed at evaluating the effects of aqueous extract of zingiber officinale rhizomes on some oxidative stress markers of CCl_4 induced rats.

Materials and Method

Chemicals: All chemicals and reagents used for the research were of analytical grade and purchased from reputable chemical manufacturers. The laboratory equipments used were also of standard quality.

Plant Materials: Fresh plant material (Ginger) was obtained from a farm at Kofar Kabuga, Gwale Local Government Area Kano State, Nigeria. The plant was taken to the Herbarium, Biological Sciences Department, Bayero University Kano for verification. It was then identified and given a voucher number of BUKHAN0070.

Ethical approval: All animals studies conducted were approved by the Animal Ethics Committee of the College of Health Science, Bayero University, Kano.

Experimental Animals: Wistar albino rats of either sex weighing between 150-200g were obtained from the Animal House, Department of Biological Sciences, Bayero University Kano. They were maintained under the standard condition and were given standard feeds with water available. They were acclimatized for one week before commencement of the study.

Preparation of Ginger Extract: Ginger rhizomes were peeled and thoroughly washed in clean water. The cleaned ginger rhizomes were cut into small pieces by using a clean stainless steel knife and allowed to shade dry. The rhizomes were ground to powder. 100g of the powder was weighed and soaked in 500ml of distilled water for 24hrs, the extract was filtered using Whatman No.1 filter paper and kept in a small bottle and ready for administration. The concentration of extract was calculated as follows;
Concentration of Extract = Weight of Extract / Volume of distilled water
Vitamin C as a standard drug was also prepared using the above relation and was administered using the relation in "**Extract Administration"**.

Extract Administration

The following formula was used in calculating the volume of extract to be administered.

Volume (ml) = Weight (kg) of rat × Dosage / Concentration of extract (mg/ml)

Acute toxicity Study

Acute toxicity studies were performed for Ginger Extracts according to the toxicity classification method as per guidelines 423 prescribed by OECD, (2001) using wistar albino rats. Thirteen experimental animals were used for the test. In the investigation, three groups containing three rats each were administered 10, 100, and 1000mg/kg respectively of the aqueous extracts intraperitoneally. They were observed closely for 24hr for lethality or any other behavioural response. Based on the result, further increased doses of 1500, 2000, 3000 and 5000 mg/kg were administered intraperitoneally to four other rats respectively. They were also observed for 24hr for any death or behavioural changes.

Experimental Design

A total of 30 rats were randomly distributed into 5 groups 6 per each group. They were treated for four (4) weeks.

Group 1: Control group were given standard food and water

Group 2: Negative control group were induced with oxidative stress using CCl_4 (150mg/kg)

Group 3: Were induced with oxidative stress and given standard drug (Vitamin C, 250mg/kg)

Group 4: were induced with oxidative stress and given low dose of ginger juice (1500mg/kg)

Group 5: were induced with oxidative stress and given high dose of ginger juice (4000mg/kg)

Sample Collection and Preparation

Rats from the various groups were sacrificed by decapitation treatment 24h after respective treatment period. The blood was collected into a plain container and allowed to stand for 30min to clot before being centrifuged at 2000rpm for 10min to separate the serum. Immediately, the serum was used to estimate the levels of oxidative stress markers (catalase, GSH, GPx, SOD) and MDA.

Preliminary Phytochemical Screening

The Extract was subjected to preliminary phytochemical test to detect the presence or absence of plant phytochemical constituents such as alkaloids, saponins, tannins, flavonoids, carbohydrate, protein and amino acids. All screening procedures were carried out using the method of Tiwari et al. (2011).

Quantitative Determination of Some Phytochemicals

Total phenolic compound was determined according to Ganapaty et al. (2013). Total flavonoids, total alkaloids and glycosides were all determined according to Soladoye and Chukwuma (2012).

Anti-nutrients Determination
Phytic acid and oxalate were determined using the AOAC method (2005).

Proximate Analysis
The proximate composition of the Extract was determined using conventional standard methods of analysis of Association of Official Analytical Chemists, AOAC (1995).

Mineral Analysis
Ca, Mg, Fe, and Zn were determined using Atomic Absorption Spectrophotometer (AA6300 Shimadzu Model, England). Flame photometer (Model 400, Corning U.K.) was used for K and Na determination, while phosphorous was determined by the vanodo-molybdate method using spectrophotometer (optima sp-300 model) at 660 nm according to the method described by AOAC (2005).

Estimation of Oxidative stress markers
Lipid peroxidation was determined by measuring the levels of malondialdehyde produced during lipid peroxidation according to the method described by Varshney and Kale (1990), catalase activity was determined according to the method of Claiborne (1985), SOD activity was determined by the method of Misra and Fridovich (1972), the method of Beutler et al. (1963) was used in estimating the level of reduced glutathione, while GPx activity was determined by the method of Albrecht Wendel (1981).

Statistical Analysis: All quantitative variables were expressed as mean and standard deviation (SD). Analysis of variance (ANOVA) was used to analyze the data. Significant differences between means were assessed at 95% level of significance i.e. P-value less than 0.05 ($p<0.05$) was considered significant.

Results
Phytochemical Screening Result: The result for the qualitative and quantitative phytochemical analysis of ginger extract in Table 1, revealed the presence of alkaloids, carbohydrate, glycosides, saponins, tannins, phenols, flavonoids, protein and amino acids, Quantitative estimation shows the concentration of flavonoid, % alkaloid, total phenol, glycoside and total saponin.

Table 1: Qualitative and Quantitative Phytochemical Analysis Result of Aqueous Ginger Extract

Phytochemicals	Qualitative	Quantitative (%)
Alkaloids	+	52.07±0.45
Carbohydrates	+	-
Glycosides	+	27.13±0.47

Saponins	+	32.11±0.48
Phenols	+	20.11±0.47
Tannins	+	-
Flavonoids	+	45.04±0.46
Protein and amino acids	+	-

Key: (+) = present (-) = absent

Table 2: Anti-nutrients and Mineral Contents of Aqueous Ginger Extract in mg/100g

Parameters	Ginger Extract
Phytic acid	72.61±1.23
Oxalate	33.00±0.77
Calcium (Ca)	187.55±29.45
Magnesium (Mg)	195.20±4.52
Sodium (Na)	4.85±0.16
Potassium (K)	142.77±2.81
Iron (Fe)	3.23±1.24
Zinc (Zn)	3.76±0.50
Phosphorous (P)	5.24±0.10
Manganese (Mn)	4.57±0.59
Copper (Cu)	1.26±0.15

All values are means of triplet determinations ± standard deviation (SD)

Table 3: Proximate Contents of Aqueous Ginger Extracts (%)

Proximate Composition	**Ginger Extract**
Moisture content	9.98±2.94
Crude Fat	8.22±0.58
Ash content	6.67±0.60

Crude fibre	10.33±0.26
Crude protein	10.85±0.79
Carbohydrates	35.66±0.55

All values are means of triplet determinations ± standard deviation (SD)

The results of the effect of treatment with graded concentrations (4000 and 1500 mg/Kg) of aqueous ginger extract on some antioxidant indices (Catalase activity, Reduced Glutathione, superoxide dismutase, glutathione peroxidase and malondialdehyde levels) are presented in Table 4.

Table 4: Serum Catalase, Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Glutathione peroxidase (GPx) Activities and MDA Levels in Rats Induced with Ccl_4 and Treated with Aqueous Ginger Extract For Four Weeks.

Group	MDA(nmol/mL)	SOD (µ/mL)	GSH(µmol/L)	CAT(µ/mL)	GPx(µ/mL)
Normal Control	10.57±4.36^{a}	11.96±1.62^{a}	34.86±9.19^{b}	12.20±4.23^{a}	36.23±7.07^{b}
Negative Control	18.52±1.69^{f}	2.77±0.69^{c}	14.06±3.03^{a}	3.840±4.36^{c}	8.95±5.23^{d}
Standard Drug (Vitamin C) (250mg/kg)	9.917±5.42^{d}	11.13±2.25^{a}	23.61±2.95^{e}	9.883±6.51^{d}	29.77±19.84^{e}
Ginger Extract (1500mg/kg)	14.04±0.15^{a}	9.233±5.10^{d}	17.55±8.54^{f}	7.687±6.30^{d}	21.38±2.44^{e}
Ginger Extract (4000mg/kg)	11.00±3.86^{a}	10.30±3.67^{a}	18.47±8.68^{f}	8.587±4.41^{d}	24.20±5.48^{e}

Values are expressed as mean ± SD., Mean values having different superscript letter in the same column are significantly different at ($p<0.05$).

Discussion

The qualitative phytochemicals screening of aqueous extract of ginger revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, tannins, protein and amino acids. This finding agrees with that of Osabor et al., (2015). The anti-nutrients such as oxalate and phytic acid in this study were found similar to those obtained by Olubunmi et al., (2013). The level at which they occur are safe for consumption by man and animals.

The Proximate compositions of ginger extract were presented in Table 4. Ginger extract have low moisture content which is similar to the findings of Olubunmi et al., (2013).

The protein content determination showed that ginger extract has high protein content (10.85 ± 0.79) but this value was higher when compared with those reported by (Akpata and Miachi, 2001). In this study, ginger extracts contained appreciable amount of carbohydrates. It also contains high fat, ash and crude fiber contents.

The mineral analysis of ginger extract indicated richness in Calcium, Magnesium, Potassium, Phosphorous and Manganese. The trace elements Copper and Zinc varied in content among the extract within much narrower limits. Potassium was found to be higher. High amount of potassium in the body was reported to increase iron utilization, and it is beneficial to people taking diuretics to control hypertension and suffering from excessive excretion of potassium through the body fluid (Arinathan et al., 2003). The levels of Manganese, Potassium and magnesium were found to be low but had a higher Calcium content which is similar to the findings of (Olubunmi et al., 2013). Most of these mineral elements are essential activators for enzyme-catalyzing reactions.

The present study investigated the propensity of CCl_4 to induce oxidative stress and its possible attenuation by ginger in liver of rats. Oxidative stress was induced by intraperitoneal administration of 150 mg/kg body weight of CCl_4 to the Wistar albino rats. Liver diseases are mostly mediated by reactive oxygen species (ROS) which play a significant role in the development of tissue injury and pathological conditions in the living system (Mada et al., 2014).

In the current study, it was observed that there was decrease in the level of oxidative stress markers in the CCl_4-induced oxidative stress control rats. The results indicated that the CCl_4-induced oxidative stress control group have lower levels of serum reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase activity but have higher level of serum MDA, (a marker of lipid peroxidation). The increase in lipid peroxidation as revealed by the high level of MDA formed in the CCl_4-induced oxidative rats compared to the normal control rat suggests that the natural antioxidant defense mechanism to scavenge excessive free radical has been compromised in rats induced with oxidative stress. This finding agrees with that of Babandi et al. (2017) who found that the increased levels of lipid peroxidation products (MDA) generally induces compensatory changes expressed by enhanced production and activity of serum antioxidative vitamins and serum redox metals.

Results of the effect of treatments with different doses (1500 and 4000mg/Kg) of aqueous ginger extract after four weeks of treatment showed elevation in the serum levels of reduced GSH, superoxide dismutase, glutathione peroxidase and CAT activity in comparison with that of the negative control (CCl_4) group. It is known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals.

Singh et al., (2010) reported that the activities of antioxidant enzymes such as SOD, CAT, glutathione peroxidase, and GST were found to increase significantly in the erythrocytes accompanied by a decrease in the activity of the glucose-6-phosphate dehydrogenase, following atrazine exposure.

Similarly, Siddaraju and Dharmesh (2007) reported that ginger–free phenolic and ginger hydrolysed phenolic fractions exhibited free radical scavenging, inhibition of lipid peroxidation, DNA protection and reducing power abilities indicating strong antioxidant properties. Ansari et al., (2006) showed that the ethanolic Z. officinale extract pre-treatment for 20 days in isoproternol treated rats induced oxidative

myocardial necrosis in rats, enhances the antioxidant defense (CAT, SOD and GSH) and exhibits cardioprotection property. These results indicate the possible involvement of free radicals in CCl_4-induced toxicity and highlight the protective action of ginger, an indigenous medicinal plant product. This makes it a very effective agent for prevention of reactive oxygen species production.

Conclusion

In conclusion, results of the study demonstrated that CCl_4 induced oxidative stress in rats, in terms of increase in malondialdehyde level and decrease in the activities of GSH, SOD, CAT and GPx. However, ginger administration ameliorated the effects of CCl_4 as levels of MDA, GSH, SOD, CAT and GPx were comparable with those of apparently healthy rats, suggesting that ginger have potential antioxidants against CCl_4-induced oxidative stress and may be of benefits in many of oxidative stress associated diseases.

Recommendation

Base on the findings of this studies, the following recommendations are made;

1. Further studies should be performed to determine whether treatment with aqueous ginger extract is dose dependent or not.
2. This study should be extended to human subjects on the use of ginger extract in the treatment of oxidative stress.

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