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Annals of Biological Research, 2011, 2 (1) : 1-8
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library

ISSN 0976-1233
CODEN (USA): ABRNBW

Protective effect of ginger in normal and carbon-tetrachloride induced hepatotoxic rats

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ABSTRACT

The protective effect of ginger supplemented diet in normal and carbon-tetrachoride (CCl_4) induced hepatotoxic rats was investigated. The first set of animals received only ginger-supplemented diet while the other set were treated with CCl_4 before maintaining them on ginger-based diets. Consumption of the experimental diets by the normal rats produced significant elevation ($p<0.05$) in the activities of the hepatic aspartate aminotransferase and alanine amonotransferase as well as the concentration of protein and albumin. Kidney function analysis showed that the concentration of urea and potassium ion (K^+) were significantly affected ($p<0.05$) while other electrolytes were not ($p>0.05$). However, all the haematological parameters of the rats were not significantly affected ($p>0.05$) by the experiment. Administration of CCl_4 to the second set of rats resulted in the alteration of the liver function parameters and antioxidant enzymes. However, the significantly reduced ($p<0.05$) marker enzymes such as AST, ALT, GPx, CAT and SOD, due to CCl_4 treatment were restored towards normalization on consumption of ginger diet. The biochemical parameters like total protein and albumin were also restored towards normal levels. The results of this study suggests that the consumption of ginger-based diet maintain the integrity of the liver and protects it against damage.

Keywords: Spices, hepatotoxicity, *de novo* synthesis, aminotransferases.

INTRODUCTION

Spices are a group of esoteric food adjuncts, which have been in use for thousands of years. By virtue of their pleasing colour, flavour or pungency, they can transform our food into attractive and appetizing meal. In addition to these organoleptic properties, few spices are also known to possess several medicinal properties [1] and are effectively used in the

indigenous systems of medicine. In the past three decades, it has been experimentally documented that several common spices can also exert beneficial effects in health and diseases [2]. Examples of these spices include ginger, onion, pepper and tomato.

Ginger (*Zingiber officinale*) belongs to Zingiberaceae family. The part of the plant used is rhizome. The plant produces an orchid-like flower with petals that are greenish yellow streaked with purple colour. Ginger is cultivated in areas of abundant rainfall. Even though it is native to southern Asia, ginger is cultivated in tropical areas such as Jamaica, China, Nigeria and Haiti. It is an important spice crop in India. [3]. Ginger is an indispensable component of curry powder, sauces, ginger bread and ginger flavoured carbonated drinks. It is also used in some products like biscuits, pickles and confectionaries. It is extensively used in preparation of dietaries for its aroma and flavour. Dry ginger is used in the manufacture of oil, oleoresin, essence and processed meat [4][5].

The polyhalogenated compound $CC1_4$ is a well-known hepatotoxin and exposure to this chemical is known to result in hepatocellular necrosis in rodents. Depending on the dose of exposure to $CC1_4$ and prior exposure to other chemicals, extensive liver damage results in total hepatic failure and animal lethality [9].

The liver is an important organ which is actively involved in many metabolic functions and is the frequent target for a number of toxicants [6]. Hepatic damage is associated with distortion of these metabolic functions [7]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [8]. In view of severe undesirable side effects of synthetic agents, there is growing need to utilise abundant plant resources available and to evaluate scientific basis for the medicinal plants that are claimed to possess hepatoprotective activity.

Consequent upon the wide usage of this plant (ginger) in food preparations, the aim of this study is to evaluate the effect of consumption of ginger-supplemented diet on selected tissues in normal and CCl_4-induced hepatotoxic rats.

MATERIALS AND METHODS

Sample preparation

Ginger was grounded and sieved to a particle size of 250 μm. The rat chow – ginger concentrate (2.5%, 5% and 10%w/w of ginger in rat chow) was prepared by mixing normal chow and ginger which were stored in a dessicator.

Experimental animals

Adult male albino rats of Wistar strain (190 ± 10 g) were purchased from the animal house of College of Medicine of the University of Lagos, Idi-Araba, Lagos. They were housed (5 per cage) in animal cages under standard conditions of temperature, relative humidity 12 h light and 12 h dark cycle and given food and water *ad libitum*.

Protocol

The animals were divided into two groups of twenty animals each. Both groups were then subdivided into 4 groups consisting of five animals each. Animals in group 1 were treated as follows for 4 weeks:

Group 1A: received normal rat chow and water (control)
Group 1B: received normal rat chow supplemented with 2.5% ginger
Group 1C: received normal rat chow supplemented with 5% ginger
Group 1D: received normal rat chow supplemented with 10% ginger

Group 2 animals were also assigned into 4 groups and treated as follows for 7 days:
Group 2A: received olive oil and normal rat chow (control)
Group 2B: received CCl_4 as a 50% solution in olive oil (1ml/kg) on the first day and normal rat chow
Group 2C: received CCl_4 and fed on normal rat chow supplemented with 2.5% ginger.
Group 2D: received CCl_4 and fed on normal rat chow supplemented with 5% ginger

Tissue sample collection and preparation

At the end of the experiment, rats were anaesthetized in slight chloroform and blood samples collected into clean, dry heparinised centrifuge tubes by cardiac puncture. The liver and kidney were then excised, cleansed of superficial connective tissues and then transferred into ice-cold 0.25M sucrose solution. They were later blotted with clean tissue paper and weighed. The tissues were homogenized in ice-cold 0.25M sucrose solution [1:5w/v] using Teflon homogenizer [10]

Biochemical studies

The determination of albumin concentration was done using the method described by [11] and total protein concentration was estimated using the Biuret method [12]. The activities of aminotransferases (ALT and AST) were assayed basically by the method of [13]. Urea Concentration was measured using the diacetyl monoxime method of [14]. Sodium and potassium concentrations were determined using reagent set [15] and bicarbonate concentration was determined titrimetrically. Haematological parameters namely packed cell volume (PCV), haemoglobin concentration (Hb), white blood cell count (WBC), neutrophil and lymphocyte counts were determined using the method of [15]. Reduced glutathione (GSH) content was estimated according to the method of [16]. The superoxide dismutase (SOD) activity was measured based on the ability of the enzyme to inhibit the autoxidation of adrenaline and was assayed by using the method described by [17]. The Catalase (CAT) activity was estimated by the method of [18]. The glutathione peroxidase (GPx) activity was measured using the method of [19]. All measurements were done using Spectronic 21 digital spectrophotometer (Bausch and Lomb, N.Y.).

Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's't' test. P values <0.05 were considered as significant [20].

RESULTS

Hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of treated animals significantly ($p<0.05$) increased compared to the control (Table 1). The levels of proteins and albumin in the treated animals also increased significantly ($p<0.05$) compared to the control group.

The levels of sodium ion and bicarbonate ion were not significantly affected ($p \square 0.05$) by the presence of ginger in the experimental diets. However, potassium ion and urea concentration were significantly reduced ($p<0.05$) and increased respectively in the kidney.

Table I: Activities of aminotransferases and levels of albumin and protein in the liver of Rats fed ginger-supplemented diet

Parameters	Group 1A	Group 1B	Group 1C	Group 1D
AST (U/L)	28.27 ± 4.33	67.53 ± 5.42*	83.53 ± 2.89*	57.67 ± 4.39*
ALT (U/L)	30.40 ± 3.12	51.17 ± 5.53*	56.87 ± 7.06*	68.80 ± 5.13*
Albumin (g/L)	2.63 ± 0.19	3.70 ± 0.66	8.60 ± 0.30*	7.83 ± 0.27*
Protein (g/L)	2.90 ± 0.40	5.17 ± 0.27*	7.67 ± 0.91*	6.83 ± 0.20*

*Values are mean ± SEM of 5 determinations. Values with different superscripts are significantly different at P<0.05 (*p < 0.05).*

Table II: Status of Kidney function indices in the rats fed ginger-supplemented diet

Parameters	Group 1A	Group 1B	Group 1C	Group 1D
Na^+	13. 47 ± 2.03	13.00 ± 0.58	13.00 ± 1.53	12.27 ± 0.88
K^+	17.90 ± 1.47	10.30 ± 0.87*	11.40 ± 0.85*	8.40 ± 0.72*
HCO_3^-	17.00 ± 1.45	19.00 ± 1.63	16.33 ± 2.19	19.67 ± 1.20
Urea	23.33 ± 2.40	19.67 ± 0.88	43.67 ± 5.81*	62.15 ± 4.10*

*Values are mean ± SEM of 5 determinations. Values with different superscripts are significantly different at P<0.05 (*p < 0.05).*

Table 3 showed the effect of the ginger-diet on the haematological parameters of albino rats. Some fluctuations were noted in these parameters (PCV, Hb, WBC, neutrophil and lymphocyte) but there were no significant differences (p□0.05) between the control and the test groups.

Table III: Haematological parameters of rats fed ginger-supplemented diet

Parameters	Group 1A	Group 1B	Group 1C	Group 1D
PCV (%)	41.33 ± 4.06	41.67 ± 2.19	40.35 ± 1.86	36.33 ± 3.28
Hb (g/dl)	14.00 ± 1.16	13.67 ± 0.67	13.53 ± 0.77	12.00 ± 1.00
WBC (103/L)	6.60 ± 0.81	6.73 ± 0.64	5.87 ± 0.03	5.53 ± 0.32
Neut. (%)	62.00 ± 1.16	63.33 ± 0.88	60.67 ± 0.67	60.67 ± 0.33
Lym (%)	37.00 ± 1.73	35.00 ± 1.16	41.00 ± 1.00	39.67 ± 0.67

*Values are mean ± SEM of 5 determinations. Values with different superscripts are significantly different at P<0.05 (*p < 0.05).*

Table 4 shows that treatment of rats with single dose of CCl_4 (0.5 ml/kg body weight) after 7days led to the development of severe hepatic injury in the rats that were intoxicated with CCl_4 but not treated with ginger based diet (group 2B) compared to the control group and the groups (2C and 2D) that were treated with 2.5% and 5% ginger- based diet respectively.

There was a marked and highly significant (P<0.05) decrease in the concentration of the total protein (4.87) in the CCl_4-treated when compared to the control (7.80). However, ginger improved these values in group 2C (7.67) and 2D (8.10) respectively. Albumin concentration also witnessed significant reduction (p<0.05) in the CCl_4-treated rats (1.83) as against the control (3.67), group 2C (3.57) and group 2D (3.40)

The activity of AST decreased significantly (p<0.05) in group 2B animals (24.90) when compared to the control (51.77). Attempts were made by the ginger-based diet to restore this activity in group 2C and 2D but were not complete. However, the reduction (p<0.05) in the

activity of the ALT in the CCl_4-treated rats (35.00) was totally recovered in the group 2D animals.

Table IV: Effect of ginger-based diet on the hepatic total protein, albumin and some Transaminases in CCl_4 treated rats

Parameters	Group 2A	Group 2B	Group 2C	Group 2D
TP (g/L)	7.80 ± 0.79	4.87 ± 0.55*	7.67 ± 0.48	8.10 ± 0.50
ALB (g/L)	3.67 ± 0.12	1.83 ± 0.09*	3.57 ± 0.09	3.40 ± 0.21
AST (U/L)	51.77 ± 1.53	24.90 ± 0.96*	38.77 ± 1.24*	40.33 ± 1.26*
ALT (U/L)	77.33 ± 5.18	35.00 ± 2.31*	54.10 ± 2.77*	82.33 ± 2.53

*Values are mean ± SEM of 5 determinations. Values with different superscripts are significantly different at P<0.05 (*p < 0.05).*

A significant decrease ($p<0.05$) in the activities of hepatic CAT, SOD and GPx with a fall in GSH content in CCl_4-treated group was observed (Table 2). The consumption of ginger-based diet by CCl_4-treated rats showed relatively increased activities of these enzymes. The GSH content of the liver of the CCl_4-treated rats witnessed a reduction though not significantly different ($p \square 0.05$) from the control animals. This situation returned towards the control level in the ginger-supplemented groups.

Table V: Effect of ginger-based diet on the hepatic antioxidant parameters of the CCl_4 treated rats

Parameters	Group 2A	Group 2B	Group 2C	Group 2D
GPx	0.18 ± 0.00	0.13 ± 0.00*	0.20 ± 0.01	0.20 ± 0.01
GSH	25.31 ± 2.39	19.48 ± 6.67*	24.18 ± 1.82	26.72 ± 2.15
CAT	1.47 ± 0.11	0.08 ± 0.01*	0.20 ±0.02*	0.53 ± 0.04*
SOD	84.74 ± 7.99	59.14 ± 2.38*	65.35 ± 3.26*	83.16 ± 6.31

*Values are mean ± SEM of 5 determinations. Values with different superscripts are significantly different at P<0.05 (*p < 0.05).*

DISCUSSION

Study on normal rats

Measurement of the activities of marker enzymes, like AST and ALT can be used in the assessment of liver function [21][22]. Aspartate and alanine aminotransferases are normally localised within the cells of the liver, heart, gill, kidney, muscles and other organs. The enzymes are of major importance in assessing and monitoring liver cytolysis [23]. Their presence in the serum may give information on organ dysfunction [24]. The general increase in the activity of liver AST and ALT (Table 1) following the consumption of ginger diet could be due to *de novo* synthesis of the enzyme molecules or an adaptation by the liver to the presence of the ginger leading to activity higher than the control [25]. Due to the fact that the results obtained for the albumin and liver protein concentrations followed the same trend (Table 1), it thus implicates the same mechanism by which the extract exerts its effect on these three parameters. This shows that the consumption of the ginger diet by the rats may be increasing the rate of protein synthesis leading to the higher concentration of albumin and protein in the liver.

Concentration of Na^+ and HCO_3^- in the test groups and that of the control as shown in Table 2 indicated no significant difference between the four groups. The fact that these electrolytes

were not elevated in the serum showed that the osmotic regulatory function of the kidney was not affected upon consumption of the diet. One of the principal functions of the kidney is to maintain osmotic balance of the blood and this is done by reabsorption of ions among which are Na^+ and HCO_3^-. The significant decrease in Na^+ concentration may indicate excess destruction of cells [26]. Urea is the main end product of protein catabolism. It is one of the waste products of the body which is passed into the bloodstream to be removed by the kidney. Elevation of these waste products in the kidney as depicted in table 2 is an indication of maintenance of renal function [27][28].

Study on CCl_4-induced hepatotoxic rats

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function [29]. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($\bullet CCl_3$). Trichloromethyl free radical when combined with cellular lipids and proteins in the presence of oxygen form trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxyl free radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death [30].

The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents. Hypoalbuminemia and decline in total protein (TP) content can be deemed as a useful index of severity of hepatocellular damage. The lowered levels of TP and Albumin recorded in the liver of CCl_4-treated rats reveal the severity of hepatopathy [31]. In the present study, TP and Albumin concentrations were very low in rats treated with CCl_4. In groups III and IV these factors significantly increased when compared to the CCl_4-treated group and the values were closer to those of the control (Table 4).

The changes associated with CCl_4-induced liver damage are similar to those of acute viral hepatitis. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. The reduced activities of ALT and AST observed in CCl_4-treated rats in this study corresponded to the extensive liver damage induced by toxin. The tendency of these enzymes to return towards a near normal level in groups 2C and 2D is a clear manifestation of antihepatotoxic effect of ginger.

Carbon-tetrachloride induced adverse changes were evident from decreased hepatic antioxidant enzyme activities viz., catalase, SOD and GPX followed by GSH (table 5). In the present study, upon ginger supplementation the above enzymes were restored to normal in the liver. Moreover, as reported by [32], the possession of calcium, magnesium and phosphorus by ginger may have contributed largely to the observed elevation in the activities of antioxidant machinery as these minerals are capable of enhancing the concentrations of SOD, CAT and GPx and the content of GSH.

In conclusion, the ginger-supplemented diet afforded protection against CCl_4 induced liver damage. Possible mechanism that may be responsible for the protection of CCl_4 induced liver damage by ginger may be due to its free radical scavenging activity thereby intercepting those radicals involved in CCl_4 metabolism by microsomal enzymes. Thus, from the foregoing findings, it shows that ginger is safe for consumption at the doses tested and offers

protection to CCl_4-induced liver damage. This protective role may be due to the mineral and antioxidant chemicals present in it.

CONCLUSION

The results of this study suggest that the consumption of ginger-based diet maintains the integrity of the liver and protects it against damage caused by carbon-tetrachloride induced hepatotoxocity.

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