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EFFECT OF *Tamarindus indica* JUICE INTAKE ON SOME OXIDATIVE STRESS MARKERS IN CARBON TETRACHLORIDE INDUCED RATS

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ABSTRACT
Tamarind tree is a multipurpose tree of which almost every part finds at least some use, either nutritional or medicinal. Due to its pleasant acidic taste and rich aroma, the pulp is widely used for domestic and industrial purpose. A study was carried out to evaluate the effect of Tamarind juice intake in CCl_4 induced oxidative stress albino rats. The Proximate, antinutrient, and Phytochemical contents of tamarind juice 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, tamarind 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 tamarind 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 tamarind juice 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: Tamarind juice, carbon tetrachloride, Oxidative stress, ameliorate, Antioxidant

INTRODUCTION

Tamarind or *Tamarindus indica* L. of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds at least some use (Kumar and Bhattacharya, 2008), either nutritional or medicinal. Tamarind is scientifically known as *Tamarindus indica,* in Hausa as *Tsamiya*. *Tamarindus indica* (*T. indica*) is evergreen tree that can reach 24 m height and 7 m girth that has pale yellow and pink flowers (Bhadoriya *et al.,* 2011). It needs dry climate so the region it is commonly seen in Africa extends to Senegal in west, Sudan and Ethiopia in east, Mozambique and Madagascar in south (Havinga *et al.,* 2010). According to World Health Organization report, tamarind fruit is an ideal source of all essential amino acids except tryptophan (82%) (Glew *et al.,* 2005). According to phytochemical analysis results, T. indica contains phenolic compounds like catenin, procyanidin B2, epicatechin, tartaric acid, mucilage, pectin, arabinose, xylose, galactose, glucose, uronic acid and triterpen (Bhadoriya *et al.,* 2012).

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 or 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). An alternative way to consume proper amounts of fruits and vegetables is to choose beverages such as juices. During the last few years, the demand for these beverages has been increasing in several countries (Singh *et al.,* 2015). The present study aimed at evaluating the effects of tamarind juice consumption on some oxidative stress markers of CCl_4 induced rats.

MATERIALS AND METHODS

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 (Tamarind) was obtained from a farm at Kofar Kabuga, Gwale L.G.A Kano state, Nigeria. The plant was taken to a botanist in Biological Sciences Department, Bayero University Kano for authentication. It was then authenticated and given an accession 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.

Formulation of Tamarind juice

Tamarind Juice was prepared according to the method expressed by Sevcan *et al*. (2011). They were washed and drained. The skins were peeled and the pulp was separated from the seeds manually by scrubbing. The pulp was blended using a laboratory electrical blender (Model 32BL79, Waring, USA). The Tamarind juice obtained was vacuum-filtered through a nylon mesh and then transferred into a beaker. The juice was stored in a refrigerator at 4°C until use. The concentration of the juice was calculated as follows;

Concentration of *Tamarindus indica* = weight of *Tamarindus indica* / volume of distilled water

Vitamin C as a standard drug was also prepared using the above relation and was administered also following the relation below.

Juice Administration

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

Volume (ml) = weight of rat (kg) × dosage (mg/kg) / Conc. of extract (mg/ml)

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.

Acute toxicity Study

Acute toxic category method is a method for assessing acute oral toxicity that involves the identification of a dose level that causes mortality. An acute toxicity study was performed for Tamarind Juice according to the toxic classic method as per guidelines 423 prescribed by OECD, (2001) using wistar albino rats. The juice showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the juice at a dose of 5000mg/kg. From this, 4000mg/kg and 1500mg/kg were selected for the experimental study. Hence there is no LD_{50} and the juices tested are considered safe and nontoxic.

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 tamarind juice (1500mg/kg)

Group 5: were induced with oxidative stress and given high dose of tamarind 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 Juice 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 Juice 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 ± 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 tamarind juice in Table 1, revealed the presence of alkaloids, carbohydrate, glycosides, saponins, phenols, flavonoids, protein and amino acids, with the absent of tannin, Quantitative estimation shows the concentration of flavonoid, % alkaloid, total phenol, and total saponin.

Table 1: Qualitative Phytochemical Analysis Result of Tamarind Juice

Phytochemicals	**Qualitative**	**Quantitative (%)**
Alkaloids	**+**	38.18±0.54
Carbohydrates	**+**	**-**
Glycosides	**+**	**-**
Saponins	**+**	18.04±0.48
Phenols	**+**	13.39±0.25
Tannins	**-**	**-**
Flavonoids	**+**	37.15±0.49
Protein and amino acids	**+**	**-**

Key

(+) = present (-) = absent

Table 3: Anti-nutrients and Mineral Contents of Tamarind Juice in mg/100g

Parameters	Tamarind Juice
Phytic acid	79.20±1.54
Oxalate	37.41±0.98
Calcium (Ca)	94.25±20.55
Magnesium (Mg)	201.84±6.59
Sodium (Na)	5.32±0.20
Potassium (K)	144.54±2.81
Iron (Fe)	3.56±0.77
Zinc (Zn)	2.98±0.59
Phosphorous (P)	5.68±0.15
Manganese (Mn)	6.76±0.72
Copper (Cu)	1.58±0.25

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

Table 4: Proximate Contents of Tamarind Juice (%)

Proximate Composition	Tamarind Juice
Moisture content	9.15±1.37
Crude Fat	7.38±0.73
Ash content	9.57±2.75
Crude fibre	12.78±0.80
Crude protein	8.81±0.19
Carbohydrates	48.78±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 the tamarind juice on some antioxidant indices (Catalase activity, Reduced Glutathione, superoxide dismutase, glutathione peroxidase and malondialdehyde levels) are presented in Table 4.

Table 5: Serum Catalase, Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Glutathione peroxidase (GPx) activities and MDA levels in rats induced with CCl_4 and treated with Tamarind Juice 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}
Tamarind Juice (1500mg/kg)	16.03 ± 0.16^{f}	4.167 ± 3.88^{c}	14.67 ± 2.70^{a}	4.293 ± 2.18^{c}	12.34 ± 0.87^{a}
Tamarind Juice (4000mg/kg)	12.12 ± 6.05^{a}	4.787 ± 4.25^{c}	16.82 ± 2.27^{f}	4.970 ± 2.74^{c}	20.25 ± 4.48^{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 tamarind revealed the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, protein and amino acids with the absent of tannins. A comparative study on phytochemicals screening conducted by Sadiq *et al*. (2016) using the pulp and seed of Tamarindus *indica* shows the presence of tannins, volatile oils, saponins and steroids with absence of alkaloids and flavanoids respectively which the findings agrees with the present study on saponins and disagree on alkaloids, flavonoids and tannins. A study conducted by Gomathi *et al*. (2017) reported the presence of Quinines in addition to the phytochemicals detected in this study. The pharmacological significance of these secondary metabolites ranges from antibacterial effect to fungal effect against micro-organisms (Nkafamiya *et al*., 2006).

Quantitative screening indicated that *Tamarindus indica* has high level of flavonoids compared to other phytochemicals quantified. Flavonoids are found to be beneficial to human due to their biological properties. It is found in several parts of the plants, which include seeds, fruit, flower, herbs, and stem; they are classified as plant secondary metabolites and are known for their antioxidant property.

The knowledge of antinutritional factors of tamarind is of great significance because the nutritional values of legumes are limited by the presence of certain antinutritional components (Nowacki, 1980). The phytic acid content of tamarind pulp is similar to those of commonly consumed legumes like *P. tetragonolubus* and Lima Bean (Egbe and Akinyele 1990). Ishola *et al*. (1990) reported that tamarind pulps do not contain any detactable amount of phytic acid. Phytates could, however, be substantially eliminated by processing methods such as soaking and heat treatment, which is not consistent with the findings in the present study. Phytic acid is known to decrease the bioavailability of certain minerals and may interfere with the utilization of proteins due to the formation of phytate-protein and phytate-mineral-protein complexes, and also inhibits the digestive enzymes. The present study's findings are similar to that of Adeola and Aworh (2012).

The Proximate compositions of tamarind juice show high moisture content which is in contrast to the findings of Olununmi Ajayi *et al.* (2013). However, Krithika and Radhail Sri (2007) reported higher moisture content for tamarind pulp.

The difference in moisture content could be explained by time interval between harvest and analysis, method of drying and storage. The protein content determination is one of the most important and widely used analytical measurements in processing and testing quality of food sample. The results obtained showed tamarind juice has high protein content (8.81 ± 0.19) but this value was higher when compared with those reported by (Akpata and Miachi, 2001). In this study, tamarind juice contained appreciable amount of carbohydrates. The protein and carbohydrates contents of tamarind juice confirm the report of BAIF (2002) that tamarind fruits contain one of the highest levels of protein and carbohydrate of any fruit. Tamarind juice showed high fat, ash and crude fiber contents respectively.

Mineral analysis of tamarind juice indicated richness in Calcium, Magnesium, Potassium, Phosphorous and Manganese. The trace elements Copper and Zinc varied in content in the juice within much narrower limits while Potassium was found to be higher. High amount of potassium in the body was reported to increase iron utilization (Mada *et al*., 2014), 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 high in the juice, which is similar to the findings of (Olununmi *et al*., 2013). Most of these mineral elements are essential activators for enzyme-catalyzing reactions. Calcium, like Phosphorous plays a major role in teeth and development and its deficiency cause osteomalacia, poor fertility and subnormal growth (Ezeagu *et al*., 1997). Iron plays a major role in the synthesis of amino acids and protein and it is an essential activator for enzyme catalyzing reactions. Iron and Copper exist as Iron-Copper proteins (Ayaz *et al*., 2006).

The present study investigated the propensity of CCl_4 to induce oxidative stress and its possible attenuation by tamarind 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 glutathaione (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 previous study of Babandi *et al*. (2017) who suggested that the increased levels of lipid peroxidation products (MDA) observed, generally induces compensatory changes expressed by enhanced production and activity of serum antioxidative vitamins and serum redox metals.

The increased MDA observed may also support the hypothesis of Sengupta *et al*. (2001) who suggested that decrease in the levels of antioxidant accelerate the lipid peroxidation, thereby generating more MDA. It also causes inactivation of enzymes and receptors in membranes and thus changes membrane molecular properties. Increase levels of MDA were observed in this study, which represents an important finding to support the documented hypothesis, that depression of the antioxidant defence potential in the liver of experimental rats as a result of different doses of CCl_4 induction. Results of the effect of treatments with different doses (1500 and 4000mg/Kg) of tamarind juice 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. *In vitro* tamarind fruit pulp extract (70% ethanolic) exhibited significant radical scavenging activity and decreased lipid peroxidation in serum with improved antioxidant defence in terms of SOD, CAT and glutathione peroxidase activities (Martinello *et al*., 2006). The efficacy of tamarind fruit pulp extract in alleviating CCl_4- induced oxidative stress in rats may therefore be associated with the presence of these phyto-constituents.

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, tamarind 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 tamarind have potential antioxidants against CCl_4-induced oxidative stress and may be of benefits in many of oxidative stress associated diseases.

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