Antioxidant Effects of *Tamarindus indica* following Acute and Chronic Carbon Tetrachloride Induced Liver Injury

S.E. Atawodi\(^1\), M.L. Liman\(^1\) and E.O. Onyike\(^1\)

\(^1\)Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna state, Nigeria

*For correspondence: atawodi_se@yahoo.com*

**Abstract**

This study aimed at evaluation of *in vivo* antioxidant effects of the methanolic extracts of different parts of *Tamarindus indica* Linn. in rats. To this end, rats were administered (pre-treatment) with plant extracts (5 mg/kg) for 3 days and intoxication with carbon tetrachloride (0.6 mL/kg) for acute liver injury experiment. The chronic liver injury experiment on the other hand involved intoxication with 0.3 mL/kg of carbon tetrachloride at every 72 h interval with concomitant 24 h administrations of the extracts (5 mg/kg) for twelve days. Malondialdehyde (MDA), catalase and superoxide dismutase (SOD) were determined from liver, kidney and heart homogenates as indicators of oxidative stress. Serum catalase and SOD as well as packed cell volume (PCV) of the blood were also determined. The results obtained showed a statistically significant (p<0.05) enhancement in the levels PCV, catalase and SOD activities in the extract-treated groups relative to the controls while the MDA was significantly lowered (p<0.05) in the extract-treated groups when compared to the CCl\(_4\) control. The extracts showed potent antioxidant potential in the following order: fruit pulp > stem bark > fruit bark > seeds > root bark > leaves. These results suggest that *T. indica* possesses strong antioxidant properties to justify its usage in traditional medicine and culinary purposes leading to extra health benefits on its use in many tropical countries. © 2013 Friends Science Publishers

**Keywords:** *Tamarindus indica*; Antioxidant; *In vivo*; Liver injury; Chemoprevention

**Introduction**

The reactive species superoxide (O\(_2^-\)), hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl radical (HO\(^•\)), nitrogen oxide (NO\(^•\)), peroxynitrite (ONOO\(^•\)) and hypochlorous acid (HOCl), are all products of normal metabolic pathways of the human organs (Mazaih, 2009), but under certain conditions, when in excess they can exert harmful effects (Trenerrey, 2008; Zia-Ul-Haq *et al.*, 2012a, b). While these reactive species have some beneficial uses, including protecting the cellular environment against invasion by pathogens, their presence in excess can be harmful to cell since they are capable of causing peroxidation of the cell membrane phospholipids. However, in normal biological systems, their action is usually opposed by a balanced system of antioxidant defenses including antioxidant compounds and enzymes (Atawodi, 2005). Upsetting this balance causes oxidative stress, leading to cell injury and death. Such activated oxygen species were now increasingly recognized to be the mediator of the cell injury in diseases and have been implicated in the pathogenesis of certain human diseases such as, cancer, atherosclerosis, neurodegenerative disorders, digestive system disorders, autoimmune pathologies and diabetes (Atawodi, 2005). Other processes that generate ROS include inflammation, strenuous exercise, detoxification processes, exposure to certain chemicals and radiation, cigarette smoke and alcohol etc. Current researches into free radicals have confirmed that foods and medicinal plants that are rich in antioxidants could play essential roles in the chemoprevention of cardiovascular diseases, cancer and neurodegenerative disorders (Atawodi *et al.*, 2009a, b; Atawodi *et al.*, 2010a, b; Atawodi *et al.*, 2011a, b; Asuku *et al.*, 2012). Therefore, much attention has been focused on the use of natural antioxidants to inhibit lipid peroxidation, or to protect the damage caused by free radicals (Atawodi *et al.*, 2011a, b; Asuku *et al.*, 2012).

Phenolic antioxidants from plants secondary metabolism are good sources of natural antioxidants agents. The use of plant foods with pharmaceutical properties have received increased interest in recent times. For instance, it has been observed recently that plant foods and medicinal plants including *Agave attenuate* (Rizwan *et al.*, 2012), *Impatiens bicolor* Royle (Nisar *et al.*, 2012), *Capparis decidua* (Zia-Ul-Haq *et al.*, 2011) *Moringa oleifera* (Atawodi *et al.*, 2010a), Palm oil (Atawodi *et al.*, 2011a, b), *Anisopus mannii* (Atawodi *et al.*, 2010b), *Canarium schweinfurthii* oil (Atawodi *et al.*, 2010), *Syzygium aromaticum* (Atawodi *et al.*, 2010b) and *Hibiscus esculentum* (Atawodi *et al.*, 2009a) etc., contained a host of antioxidant polyphenols with demonstrated capacity to chemoprevent oxidative stress-related diseases. These plant foods and medicinal plants play important roles in public health.
health, especially in developing countries.

Tamarindus indica L. (Leguminosae) is an important food resource especially for the African and Asian population. The fruit plays the most important role in nutrition. Tamarind pulp is widely used in beverage preparation and consumed in many countries around the world. T. indica is also widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders (Khairunnur et al., 2009). Numerous studies on aqueous extracts of tamarind seeds and pulp have shown strong antioxidant potential with demonstrated antidiabetic effect in rats (Osawa et al., 1994; Komutarin et al., 2003; Khairunnur et al., 2009). Osawa et al. (1994) found that an ethanol extract prepared from the seed coat exhibited antioxidative activity as measured by the thiocyanate and thiobarbituric (TBA) method. Ethyl acetate extracts prepared from the seed coat also possess a strong antioxidant activity and was confirmed by Luengthanaphol et al. (2004). Komutarin et al. (2003) reported antioxidant abilities of both the pulp and seed extract both in vitro and in vivo by modulation of nitric oxide production. It is not however clear whether the antioxidant potentials of this plant is confined only to the fruit and seed or is diffused through all the plant parts. Also it is not known if the antioxidant activities demonstrated by the fruit and seed in vitro will still show viable impact in vivo. Therefore, this work attempted a detail comparative evaluation of the in vivo antioxidant potential of the leaf, fruit pulp, fruit bark, seed, stem bark and the root bark of T. indica Linn.

Materials and Methods

Chemicals and Reagents

Thiobarbituric acid, trichloroacetic acid, hydrogen peroxide and hematoxylin were purchased from Sigma Chemical Co. Ltd (USA). All other chemicals and solvents used in this study were of analytical grades obtained from BDH (Poole, UK).

Plant Material Collection and Extraction

Various parts of T. indica (leaves, stem bark, root bark and whole fruits) were carefully collected from a tree in Zaria Local Government area of Kaduna State, Nigeria. The plant was authenticated at the Herbarium Section of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria where a voucher number 900265 was assigned.

The whole fruit was then carefully peeled off to obtain the fruit bark and subsequently the seeds separated from the pulp. Samples of leaves, stem bark, root bark, fruit bark and seeds were separately dried at room temperature and pulverized using mortar and pestle. They were then first defatted with petroleum ether for 6 h and then extracted with methanol (4 h × 2 times) using soxhlex extractor. The combined methanol portion was taken to dryness in vacuo in a desiccator and kept at -4°C until required.

The fruit pulp extracts were obtained by gentle warm maceration over a bath at 50°C. A weighed amount of the fruits were put into a beaker with 300 mL methanol and allowed to stand for 2 h. The mixture was then thoroughly macerated, and the seeds and debris picked out with the aid of laboratory tongs. Petroleum ether (300 mL) was then added and the entire mixture was shaken intermittently for 2 h. The liquid suspension was then carefully decanted into a separating funnel, while the remaining debris was discarded. The mixture in the separating funnel was then allowed to stand for clear separation of the two immiscible layers. The individual layers were then carefully run out and collected in a pre-weighed bottle. The process was repeated and the methanolic layer was combined and then dried in vacuo to obtain the dried extracts, which were then stored in air tight dark glass bottles in a refrigerator at -4°C until required.

Experimental Animals

Male albino rats weighing 150-200 g (7-8 weeks old) were obtained from the animal house of the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria, Nigeria. They were acclimatized in a well ventilated room within the animal facility of the Department of Biochemistry, Ahmadu Bello University Zaria for two weeks before the commencement of the study. They were allowed free access to rat feeds (obtained from Vital Feed Ltd., Bukuru, Jos, Nigeria) and tap water ad libitum through the course of experiment. Animals were weighed and randomly assigned to each of 17 treatment groups (n=5). Permission was obtained from the University’s Ethical Committee for laboratory use of the animals.

Animal Treatments

The study was carried out in two phases. The first phase involved the assessment of antioxidant potential of the extracts of different parts of T. indica in acute liver injury model. In this acute experimental model, the effects of the extracts were investigated in rats first by pre-treatment intraperitoneally with 5 mg/kg body weight of the extract for two days followed by intoxication with carbon tetrachloride (CCl₄) at 0.6 mL/Kg on the third day.

In the second phase, the possible therapeutic potential of the extract in chronic liver injury was evaluated. This was carried out by repeated intoxication of rats with carbon tetrachloride (0.3 mL/Kg) at every 72 h intervals with concomitant daily administration of the extracts for twelve days. In all cases control groups treated with vitamin E alone, vitamin E + CCl₄, CCl₄ only or solvent alone were also included.
**Animal Sacrifice and Tissue Collection**

Twenty four hours (24 h) after the last treatment all animals were sacrificed under mild chloroform anaesthesia. Blood was collected directly at sacrifice; serum was separated after coagulating and centrifuging at 3000 rpm for 15 min. Major organs; liver, kidney and heart were also collected, rinsed with 0.87% ice-cold saline to remove all the red blood cells. The required quantity of each organ was sliced and suspended in ice cold 0.1 M phosphate buffer (pH 7.4) to provide 10% (w/v), and then homogenized using a tissue homogenizer. Serum and homogenates were kept frozen at -20°C until required for analysis.

**Determination of Lipid Peroxidation**

Lipid peroxidation was determined as thiobarbituric acid reactive substance as earlier described by Kamijama et al. (1993) using trichloroacetic acid (TCA) and thiobarbituric acid (TBA). This method is based on the fact that lipid peroxidation generates peroxide intermediates which upon cleavage release malondialdehyde (MDA), a product, which react with thiobarbituric acid to form a coloured complex that is measured at 535 nm. Briefly, the method involves; 1 mL of 14% trichloroacetic acid, 1 mL thiobarbituric acid and 50 μL of the tissue homogenate were added in a test tube. The mixture was incubated at 80°C for 30 min in a water bath and then allowed to cool rapidly in ice-cold water for 3 min, followed by centrifugation at 3000 rpm for 10 mins. The absorbance of the clear supernatant was read spectrophotometrically at 535 nm. MDA concentration was calculated using the molar extinction coefficient of pure MDA, which was 1.56 and concentrations were expressed in nmol/mg protein.

**Determination of Packed Cell Volume (PCV) and Haemoglobin**

During sacrifice whole blood samples were collected into heparinized capillary tubes, filled up to 2/3 the length, sealed with plasticine and centrifuged at 3,000 rpm in a haematocrit centrifuge for 10 mins. Packed cell volume was determined using a haematocrit reader and PCV was expressed as percentage erythrocytes blood contained while the haemoglobin concentration was estimated from the PCV values using the formula:

\[
\text{Haemoglobin concentration} = \frac{1}{3} \times \text{PCV concentration}
\]

**Determination of Catalase activity**

Catalase (CAT) activity was measured using Abei (1979) method, which is based on the principle that catalase can convert hydrogen peroxide (H$_2$O$_2$) to water and oxygen. Briefly, 10 uL of serum or homogenate were added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of freshly prepared 30 mM H$_2$O$_2$ and the decomposition rate of H$_2$O$_2$ was measured at 240 nm for 5 min on a spectrophotometer. Catalase activity was calculated using the molar extinction coefficient, $\varepsilon$ (0.041).

**Determination of Superoxide Dismutase (SOD) Activity**

Superoxide dismutase activity was assayed according to the method of Martin et al. (1987) based on the principle that SOD-mediated decrease in the rate of auto-oxidation of haematoxylin in aqueous alkaline solution, yields a chromophore with maximum absorbance at 560 nm. Briefly, the method involves addition of 920 μL of phosphate buffer pH 7.8 to test tube containing 40 μL of serum or homogenate; mixing and incubating for 2 min at 25°C following, which 40 μL of hematoxylin was added, quickly mixed and the absorbance measured at 560 nm after one minute. The SOD activity was calculated from percentage inhibition of the rate of autooxidation of hematoxylin.

**Statistical Analysis**

The results obtained were statistically analyzed using analysis of variance (ANOVA) to get the grouped mean and Duncan multiple range test was used to test for significant difference between the grouped means at 95% confidence level ($p = 0.05$).

**Results**

The MDA levels in the liver, kidney and heart were significantly ($p<0.05$) elevated in the carbon tetrachloride group compared to the extract treated or pre-treated groups (Figs. 1-6). The levels, however in the extracts treated groups were not significantly ($p>0.05$) different with that of the untreated controls. In the acute experiment, groups pre-administered with extract prior to CCl$_4$ intoxication showed statistically significant reduced levels of MDA when compared to that treated on CCl$_4$ only (Figs. 1, 3 and 5). This was more clearly observed in the chronic liver injury experiment, especially in the MDA levels of the liver (Fig. 2), where a statistically significant ($p<0.05$) decrease was observed with the extract treated and Vitamin E treated groups. The activity levels of serum antioxidant enzyme superoxide dismutase (SOD) are shown in Fig. 7 and Fig. 8 for the acute-injury and chronic-injury experiment respectively. Likewise, the SOD activities in the major organs were shown in Fig. 9-12. Generally group administered CCl$_4$ alone showed significant decrease ($p<0.05$) in the SOD activity when compared with the extracts or vitamin E only treated groups and the untreated control. This was also true in both the acute-injury experiments and the chronic-injury experiments. However, comparatively, the effects were more appreciable in the latter as clearly demonstrated in the case of the serum...

and heart SOD activities (Figs. 8 and 12), where significant (*p*<0.05) difference were observed between the Vitamin E- treated group, extracts treated (especially the stem bark, fruit pulp and fruit bark) and the CCl₄ only treated control.

Similarly, the catalase activity levels in the serum and organs are shown in Fig. 13-19 for the acute injury (Figs. 13, 15 and 17) and chronic injury experiments (Figs. 14, 16, 18 and 19), respectively. The extract-treated groups, vitamin E-treated group and the untreated control showed significantly (*p*<0.05) enhanced catalase activity when compared with the CCl₄ control in most cases. Furthermore groups on concomitant treatment with both CCl₄ and extracts also showed significantly (*p*<0.05) elevated levels of catalase activities. This was observable in both the acute and chronic experiments with most of the extracts; except
the leaves (Figs. 14, 16 and 19) demonstrating good potential to reverse the depletion of catalase activity caused by the CCl₄ administration.

Similarly, the result in Figs. 21 and 22 show that there was no significant difference (p>0.05) in the percentage levels of erythrocytes (PCV) and haemoglobin between the groups administered only extracts and the untreated controls. However, the carbon tetrachloride control (group 1) showed significant decrease in the percentage levels of erythrocyte and haemoglobin. Nevertheless, treatment with the extracts was able to ameliorate the condition to near normal level in both the acute-injury (Fig. 21) and the chronic liver injury (Fig. 22) experiments.
Discussion

Carbon tetrachloride intoxication is reported to produce free radicals, which are capable of inducing oxidative stress that has been implicated in diverse pathological issues. Generally oxidative stress associated with cellular damages is correlated with increased level of thiobarbituric reactive substances and decrease packed cell volume (PCV) due to destruction of erythrocytes (Fakurazi et al., 2008). At the hallmark of oxidative stress, antioxidant enzymes defense systems like catalase, superoxide dismutase (SOD) etc., get overwhelmed and subsequently their activities lowered (Luengthanaphol et al., 2004).

From the results obtained (Figs. 1-6), there was an
elevated level of MDA, with decreased levels of erythrocytes and antioxidant enzymes (catalase and SOD) activities in the control group administered carbon tetrachloride only. This is in line with the reported potentials of CCl₄ to cause oxidative stress (Kamiyama et al., 1993) with possible cellular damages and expectedly this impact was more profound in the chronic experiment than in the acute experiment. In most of the instances pre- or post-treatment of the animals with Vitamin E, a well known antioxidant vitamin alleviates the situation, confirming the abilities of tocopherols to counteract CCl₄-induced oxidative stress as suggested by earlier studies (Wiam et al., 2005; Usha et al., 2007; Mazaih et al., 2009).

Oxidant radicals like trichloromethyl resulting from carbon tetrachloride metabolism are capable of causing peroxidative degradation of cellular membranes and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to formation of lipid peroxides, which in turn produce thiobarbituric (TBA) reactive substances like malondialdehyde (MDA) (Hanaa et al., 2009; Hussain et al., 2009) that consequently interacts to form the TBA-MDA complex, which was measured as oxidative marker in the rat’s liver, kidney and heart homogenates. The MDA levels in all the three organs were generally elevated significantly (p<0.05) in CCl₄ control group as compared to the untreated control. On the other hand, rats administered T. indica extracts with CCl₄ showed significantly decreased (p<0.05) level of the MDA, thus suggesting the inhibition of CCl₄-induced lipid peroxidation in rat organs with concomitant enhanced resistance to oxidation (Figs. 1-6). In addition, no significant differences were observed, particularly for the liver, between the group administered only T. indica extracts or vitamin E and the untreated. Interestingly, the groups administered the extracts with CCl₄ showed in most instances no significant difference (p<0.05) with those administered vitamin E with CCl₄ (Figs. 2, 4 and 5). This suggests the ability of most of these extracts to counteract lipid peroxidation in a similar fashion with vitamin E (Ghosh et al., 2006; Trenerry et al., 2008). These inhibitory patterns were shown in the acute (Figs. 1, 3 and 5) and chronic experiment model (Fig. 2, 4 and 6), suggesting that different parts of T. indica contain substance capable of both protecting and ameliorating major organs against acute and chronic oxidative stress-related injuries. Comparatively, the pulp, stem, fruit bark and seed extracts exhibited the highest potential (Fig. 1-6), while the root (Fig. 6) and the leaves

**Fig. 19:** Mean catalase activity in liver of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

**Fig. 20:** Mean superoxide dismutase (SOD) activity in liver of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

**Fig. 21:** Mean percentage packed cell volume (PCV) in rats intoxicated with carbon tetrachloride (0.6ml/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)

**Fig. 22:** Mean percentage packed cell volume (PCV) in rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

(Fig. 4) showed the least potential to inhibit lipid peroxidation. These observations seem to justify the fact that fruit and stem are the more widely used parts of T. indica in traditional medicine. Cellular antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase normally challenges oxidative stress. Catalase and SOD are ubiquitous and as such their activities in the serum and organs are important indices for evaluating oxidative stress in animals (Fakurazi et al., 2008). With respect to catalase and SOD, it was observed that there was generally a significant decrease (p<0.05) in these enzymes activities in the CCl4 control when compared to the T. indica extracts treated groups and no significant difference (p>0.05) existed between the extract treated, vitamin E-treated and the untreated control groups (Figs. 7, 9, 15, 16 and 18). These findings clearly demonstrated extracts of T. indica particularly the stem bark, fruit pulp, seeds and the fruit bark exerted strong antioxidant potential while the roots and the leaves showed low capabilities in counteracting the acute and chronic oxidative challenge.

The packed cell volume (PCV) and haemoglobin concentration of CCl4 control decreased significantly (p<0.05), indicating that there might have been possible destruction of erythrocytes by CCl4. This observation agrees with known phenomenon that CCl4 toxicity can lead to destruction of hepatocytes and erythrocytes (Usah et al., 2007). Treatments with T. indica extracts, however alleviated this situation and restored the erythrocytes levels to near normal values in most instances, suggesting that under both acute and chronic conditions T. indica consumption could protect against oxidative damage.

Methanolic extracts of plants have been reported to have phenolics as a major constituent (Atawodi et al., 2009a, b). Many researchers including: Osawa et al. (1994), Luengthanaphol et al. (2004), and Komutarin et al. (2003) independently reported the in vitro antioxidant capabilities of both the pulp and seed extract. The findings in this research confirm further, that various parts of T. indica particularly the fruit, the seed and the stem possessed polyphenolic compounds with antioxidant capabilities. These capabilities were demonstrated in vivo in rats and correlates the earlier reported in vitro antioxidant potentials.

Several studies have revealed the presence of various phytochemical in T. indica with possible antioxidant capabilities. T. indica seeds was reported to contain various polyphenolic compounds dominated by proanthocyanidins in the form of catechin, epicatechin, procyanidin dimers, procyanidin tetramers, procyanidin hexamers and flavonoids (taxifolin, apigenin, eriodictyol, luteolin, and naringenin) (Osawa et al., 1994; Atawodi, 2012). Phenolic compounds commonly found in plants have been reported to have several biological activities, including antioxidant roles, and hence health benefits (Garry, 1999; Komutarin et al., 2003; Chun et al., 2007; Atawodi et al., 2010a; Atawodi et al., 2011b).

Thus, to conclude, T. indica Linn, widely consumed in many African and Asian countries confer several health benefits on these population through amelioration and protection against acute and chronic oxidative assault that could initiate development of diseases like cancer, hypertension, diabetes, cardiovascular dysfunction and neurodegenerative disorders.

References


(Received 30 August 2012; Accepted 10 September 2012)