Full Length Article

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

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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 as food 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_2O_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 (Trenerry, 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 chemo-prevention 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 deciduas* (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, 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 (Khairunnuur *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; Khairunnuur *et al.*, 2009). Osawa *et al.* (1994) found that an ethanol extract prepared from the seed coat exhibited anti-oxidative 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 (CCl$_4$) 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 + CCl$_4$, CCl$_4$ 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 Kamiyama *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:

$$\text{Haemoglobin concentration} = \frac{1}{3} x PCV \text{ concentration}$$

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_2O_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_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 min on a spectrophotometer. Catalase activity was calculated using the molar extinction coefficient, Є (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 uL 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 autoxidation 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 CCl4 intoxication showed statistically significant reduced levels of MDA when compared to that treated on CCl4 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 CCl4 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
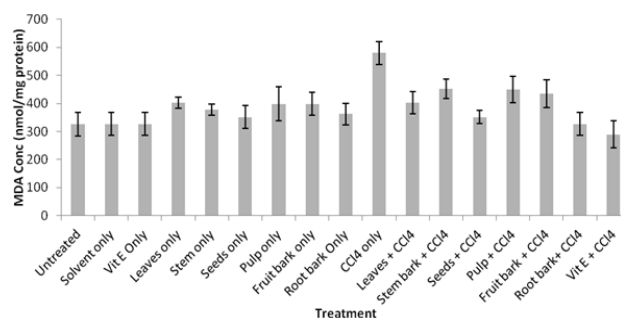
Fig. 1: Mean malondialdehyde (MDA) concentration in liver of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)
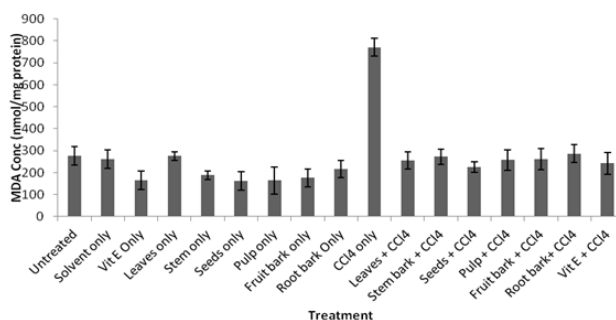

Fig. 2: Mean malondialdehyde (MDA) concentration in liver of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days
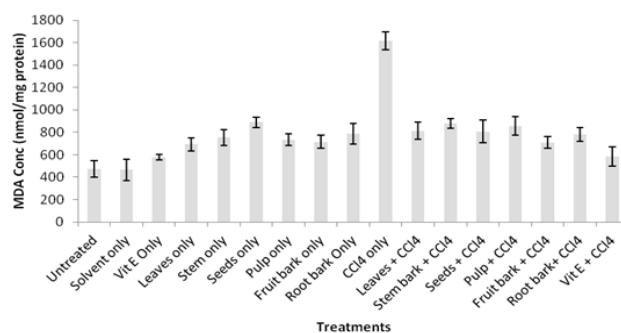

Fig. 3: Mean malondialdehyde (MDA) concentration in kidney of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)

Fig. 4: Mean malondialdehyde (MDA) concentration in kidney 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. 5: Mean malondialdehyde (MDA) concentration in heart of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)

Fig. 6: Mean malondialdehyde (MDA) concentration in heart 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

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 CCl_4 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
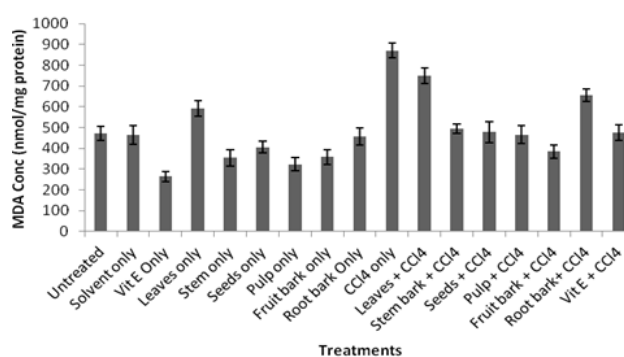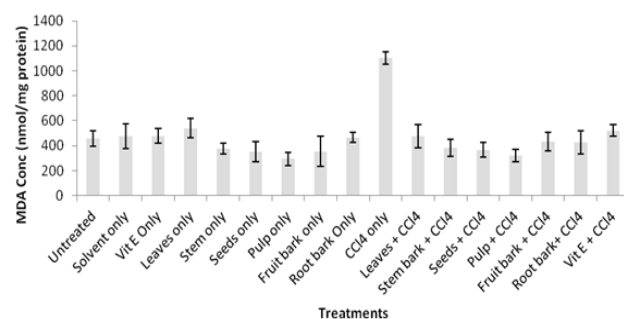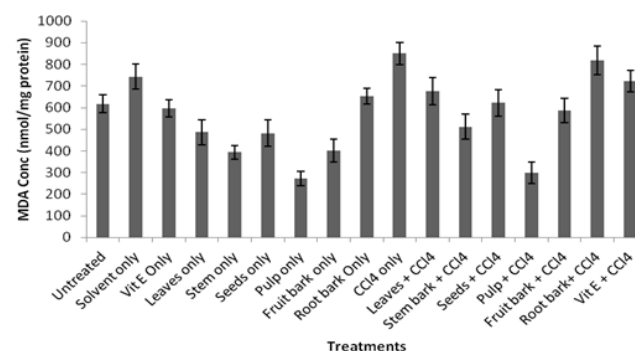
significantly ($p < 0.05$) enhanced catalase activity when compared with the CCl_4 control in most cases. Furthermore groups on concomitant treatment with both CCl_4 and extracts also showed significantly ($p < 0.05$) elevated levels of catalase activities. This was observable in both the acute and chronic experiments with most of the extracts; except
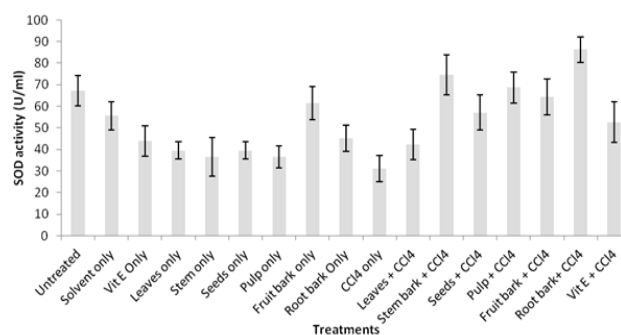
Fig. 7: Mean superoxide dismutase (SOD) activity in serum of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)
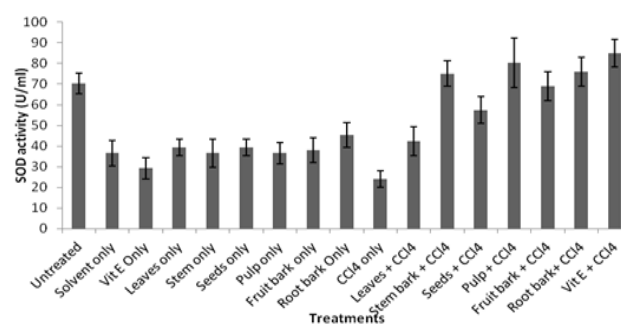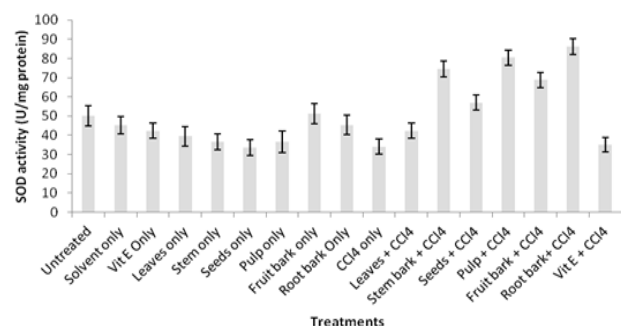

Fig. 8: Mean superoxide dismutase (SOD) activity in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

Fig. 9: Mean superoxide dismutase (SOD) activity in kidney of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)

the leaves (Figs. 14, 16 and 19) demonstrating good potential to reverse the depletion of catalase activity caused by the CCl$_4$ 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
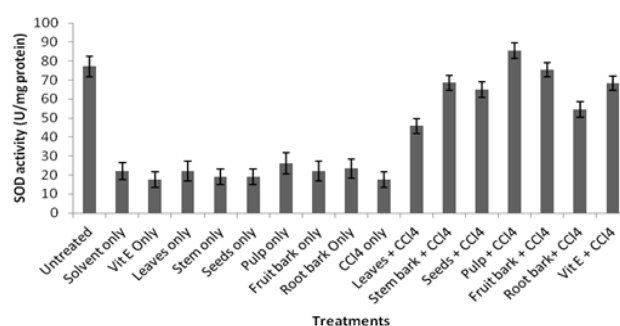

Fig. 10: Mean superoxide dismutase (SOD) activity in kidney of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days
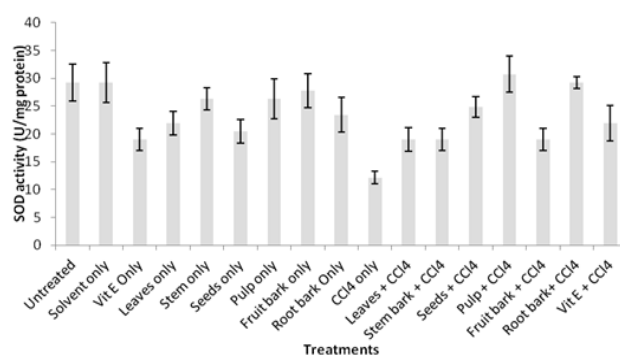

Fig. 11: Mean superoxide dismutase (SOD) activity in heart of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)
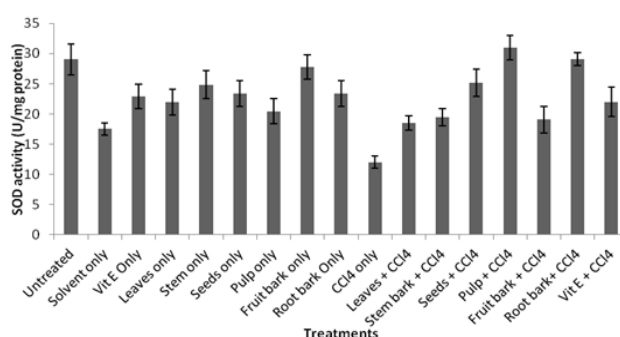

Fig. 12: Mean superoxide dismutase (SOD) activity in heart of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

(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.
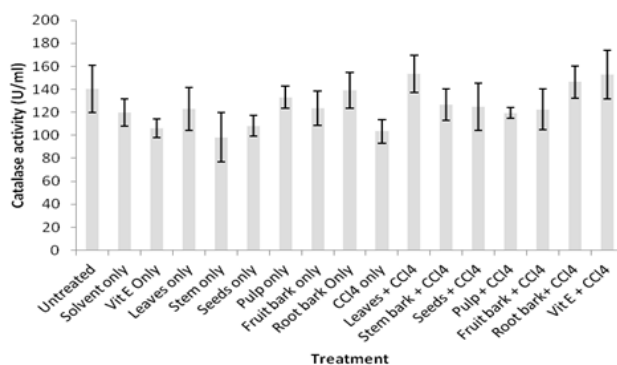
Fig. 13: Mean catalase activity in serum of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)
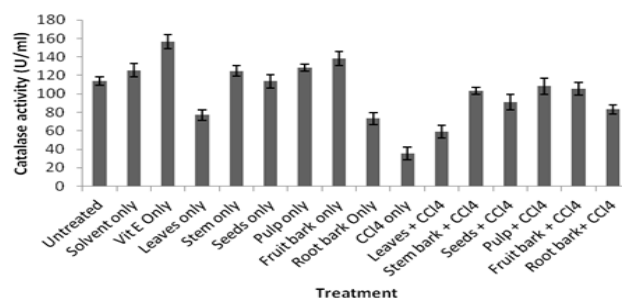

Fig. 14: Mean catalase activity in serum of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

Fig. 15: Mean catalase activity in kidney of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)

Fig. 16: Mean catalase activity in kidney 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
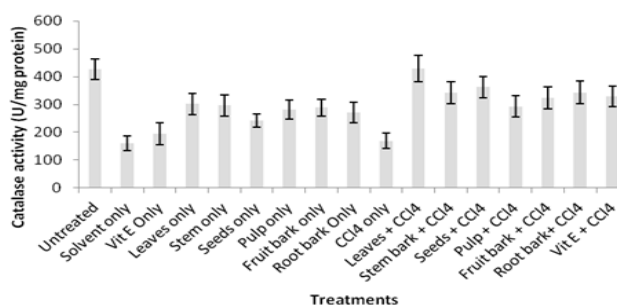

Fig. 17: Mean catalase activity in heart of rats intoxicated with carbon tetrachloride (0.6 mL/kg) following three days pre-treatment with methanol extracts of *Tamarindus indica* (5.0 mg/kg)

Fig. 18: Mean catalase activity in heart 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

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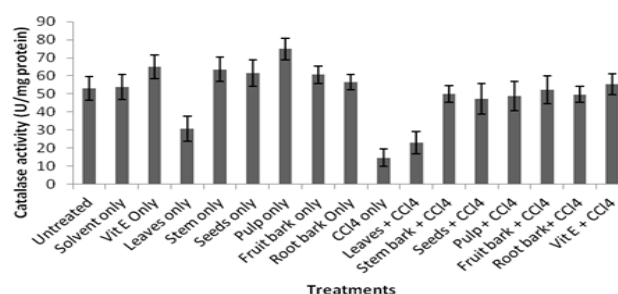 and decrease packed cell volume (PCV) due to destruction of erythrocytes (Fakurazi *et al.*, 2008). At the hallmark of oxidative stress, antioxidant enzymes defense systems like catalase, superoxide dismutase (SOD) etc., get overwhelmed and subsequently their activities lowered (Luengthanaphol *et al.*, 2004).

From the results obtained (Figs. 1-6), there was an

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
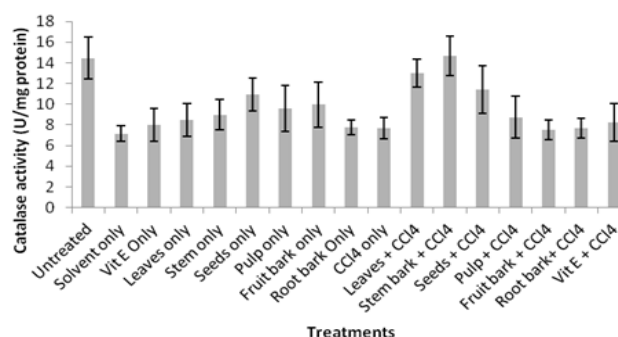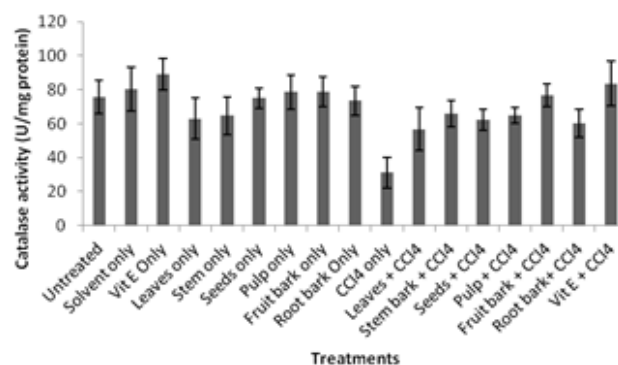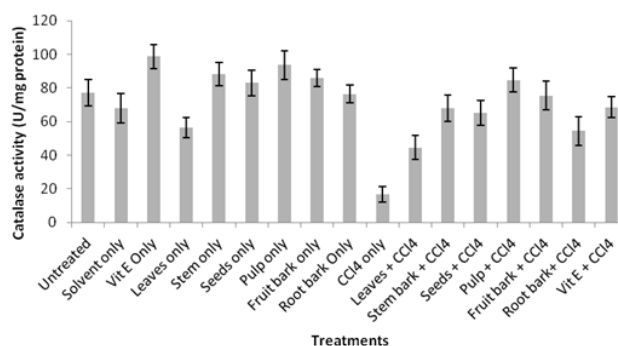

Fig. 20: Mean superoxide dismutase (SOD) activity in liver of rats following daily intraperitoneal administration of *Tamarindus indica* extracts (2.5 mg/kg) with 72 hourly injection of carbon tetrachloride (0.3 mL/kg) for 12 days

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
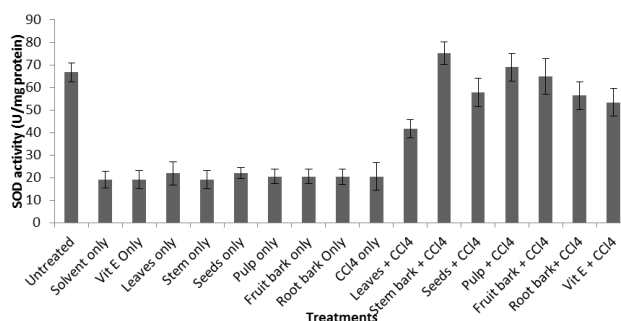
elevated level of MDA, with decreased levels of erythrocytes and antioxidant enzymes (catalase and SOD) activities in the control group administered carbon tetrachloride only. This is in line with the reported potentials of CCl4 to cause oxidative stress (Kamiyama *et al.*, 1993) with possible cellular 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
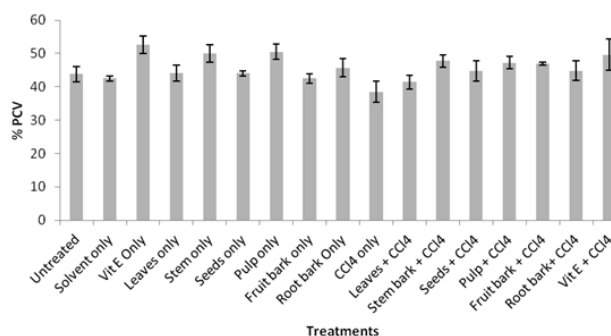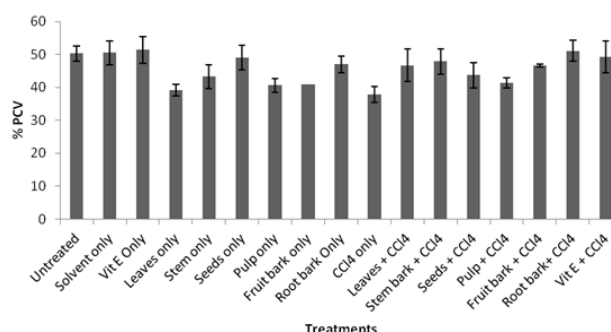
in all the three organs were generally elevated significantly ($p<0.05$) in CCl4 control group as compared to the untreated control. On the other hand, rats administered *T. indica* extracts with CCl4 showed significantly decreased ($p<0.05$) level of the MDA, thus suggesting the inhibition of CCl4-induced 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. 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 CCl_4 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 CCl_4 control decreased significantly ($p<0.05$), indicating that there might have been possible destruction of erythrocytes by CCl_4. This observation agrees with known phenomenon that CCl_4 toxicity can lead to destruction of hepatocytes and erythrocytes (Usha *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, procyanindin 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

Abei, H., 1974. Catalase In: *Method of Enzymatic Analysis*, pp: 673–684. Academic Press, New York, USA

Atawodi, S.E., 2005. Antioxidant potentials of African medicinal plants. *Afr. J. Biotech.*,4: 128–133

Atawodi, S.E., J.C. Atawodi, G.A. Idakwo, B. Pfundstein, R. Haubner, G. Wurtele, B. Spiegelhalder, H. Bartsch and R.W. Owen, 2009a. Polyphenol composition and antioxidant potential of *Hibiscus esculentus* L. fruit cultivated in Nigeria. *J. Med. Food*, 12: 1316–1320

Atawodi, S.E., J.C. Atawodi, Y. Pala and P. Idakwo, 2009b. Assessment of polyphenol profile and antioxidant properties of leaves, stem and root barks of *Khaya senegalensis* (Desv.) A. Juss. *Elect. J. Biol.*, 5: 80–84

Atawodi, S.E., J.C. Atawodi, G.A. Idakwo, B. Pfundstein, R. Haubner, G. Wurtele, H. Bartsch and R.W. Owen, 2010a. Evaluation of the Polyphenols contents and antioxidants properties of methanol extracts of leaves, stem and root barks of *Moringa oleifera* Lam. *J. Med. Food.*, 13: 710–716

Atawodi, S.E. and G.S. Onaolapo, 2010b. Comparative *in vitro* antioxidant potential of different parts of *Ipomoea asarifolia*, Roemer and Schultes, *Guiera senegalensis* J.F. Gmel and *Anisopus mannii* N.E. Brown. *Braz. J. Pharm. Res.*, 46: 245–250

Atawodi, S.E., O.O. Adekunle and I. Bala, 2011a. Antioxidant, organ protective and ameliorative properties of methanol extract of *Anogeissus leiocarpus* stem bark against carbon tetrachloride-induced liver Injury. *Int. J. Pharm. Sci. Res.*, 2: 748–753

Atawodi, S.E., J.C. Atawodi, B. Pfundstein, B. Spiegelhalder, H. Bartsch and R. Owen, 2011b. Assessment of the polyphenol components and *in vitro* antioxidant properties of *Syzygium aromaticum* (L.) merr. and perry. *Elect. J. Environ. Agric. Food Chem.*, 10: 1970–1978

Atawodi, S.E., 2012. Antimutagenic polyphenols in African foods: Sixth Annual Conference on Environmental Mutagens in Human Population *Q. Sci. Proc.*, 3: 96

Asuku, O., S.E. Atawodi and E. Onyike, 2012. Antioxidant, hepatoprotective and ameliorative effects of methanolic extract of leaves of *Grewia mollis* Juss. on carbon tetrachloride - treated albino Rats. *J. Med. Food*, 15: 83–88

Chun, K.O., J.C. Sang and O.S. Won, 2007. Estimated dietary flavonoid intake and major food sources of U.S. adults. *J. Nutr.*, 7: 22–31

Fakurazi, S., U. Nanthini and I. Hairuszah, 2008. Hepatoprotective and antioxidant action of *Moringa oleifera* Lam. against acetaminophen induced hepatotoxicity in rats. *Int. J. Pharmacol.*, 4: 270–275

Garry, G.D., 1999. Parseley, polyphenols and antioxidant antioxidants. *Brit. J. Nutr.*, 81: 425–426

Ghosh, J., T.K. Mishra, Y.N. Rao and S.K. Aggarwal, 2006. Oxidised LDL, HDL- cholesterol levels in patients of coronary artery disease. *Ind. J. Clin. Biochem.*, 21: 181–184

Hanaa, H., E. Abd, K. Farouk, E. Baz1 and S.E. Gamal, 2009. Phenolics from *Spirulina maxima*: Over-production and *in vitro* protective effect of its phenolics on CCl_4-induced hepatotoxicity. *J. Med. Plants Res.*, 3: 024–030

Hussain, S., M.Z. Khan, A. Khan, I. Javed and M.R. Asi, 2009. Toxico-pathological effects in rats induced by concurrent exposure to aflatoxin and cypermethrin. *Toxicon*, 53: 33–41

Kamiyama, T., C. Sato, J. Liu, K. Tajiri, H. Miyakawa and F. Marumo, 1993. Role of peroxidation in acetaminophen-induced hepatotoxicity; comparison with carbon tetrachloride. *Toxicol. Lett.*, 66: 7–12

Khairunnuur, F.A., A. Zulkhairi, A. Azrina, M.A.M. Moklas, S.1. Khairullizam, M.S. Zamree and M.A. Shahidan, 2009. Nutritional composition, *in vitro* antioxidant activity and *Artemia salina L.* lethality of Pulp and Seed of *Tamarindus indica* L. extracts. *Mal. J. Nutr.,* 15: 65–75

Komutarin, T., S. Azadi, L. Butterworth, D. Keil, B. Chitsomboon, B. Sattujit and J. Meade, 2003. Extracts of the seed coat of *Tamarindus indica* inhibits nitric oxide production by murine macrophages *in vitro* and *in vivo*. *J. Food Chem. Toxicol.,* 42: 649–658

Luengthanaphol, S., D. Mongkholkhajornsilp, S. Douglas, P.L. Douglas, L. Pengsopa and S. Pongamphai, 2004. Extraction of antioxidants from sweet Thai tamarind seed coat: Preliminary experiments. *J. Food Eng.,* 63: 247–252

Martin, J.P., M. Dialey and E. Sugarmann, 1987. Negative and positive assay of superoxide dismutase based on hematoxylin autoxidation. *Arch. Biochem. Biophys.,* 255: 329–336

Mazaih, M., M. Norhaiza and M. Hakiman, 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila. J. Med. Plants Res.,* 3: 217–223

Nisar, M., M. Qayum, S. Ćavar, M.R. Shah, M. Zia-Ul-Haq, I. Khan, K.W. Ahmad and Z.A. Qayum, 2012. Chemical constituents and antioxidant activity of n-hexane extract of Impatiens bicolor Royle. *Chem. Nat. Comp*., 48: 143–146

Osawa, T., T. Tsuda, M. Watanabe, K. Oshima and A. Yamamoto, 1994. Antioxidative components isolated from the seeds of tamarind (*Tamarindus indica* L.). *J. Agric. Food Chem.,* 42: 2671–2674

Rizwan, K., M. Zubair, N. Rasool, M. Riaz, M. Zia-Ul-Haq and V. De Feo, 2012. Chemical and biological study of *Agave attenuata. Int. J. Mol. Sci.*, 13: 6440–6451

Trenerry, V.C., J. Lako and S. Rochfort, 2008. Routine analytical methods for use in South Pacific regional laboratories for determining naturally occurring antioxidants in food. *Int. Food Res. J.,* 15: 154–160

Usha, K., K.G. Mary and P. Hemalatha, 2007. Hepatoprotective effect of *Hygrophila spinosa* and *Cassia occidentalis* on carbon tetrachloride induced liver damage in experimental rats. *Ind. J. Clin. Biochem.,* 22: 132–135

Wiam, I.M., T.W. Jacks and Y.A. Zongoma, 2005. Acute toxiciy and phytochemical studies of *Cassia simea* extracts in rats. *Pak. J. Biol. Sci.,* 8: 318–320

Zia-Ul-Haq, M., S. Ćavar, M. Qayum, I. Imran and V. DeFeo, 2011. Compositional studies: antioxidant and antidiabetic activities of *Capparis decidua* (Forsk.) Edgew. *Int. J. Mol. Sci.,* 12: 8846–8861

Zia-Ul-Haq, M., S. Ahmad, L. Calani, T. Mazzeo, D.D. Rio, N. Pellegrini and V. DeFeo, 2012a. Compositional study and antioxidant potential of *Ipomoea hederacea* Jacq. and *Lepidium sativum* L. *Seeds. Mol*., 17: 10306–10321

Zia-Ul-Haq, M., S.A. Shahid, S. Ahmad, M. Qayum and I. Khan, 2012b. Antioxidant potential of various parts of *Ferula assafoetida* L. *J. Med. Plant Res.*, 6: 3254–3258