



In-Vitro Antioxidant Activity of Tamarind 'Ichekwu' Fruits Extract' and its Protective effect on Oxidative Damage in Animal Model

Umerah, N. N.^{1*}

^{*1}Department of Food Science and Technology,
Enugu State University of Science and Technology, Nigeria.
nkemumerah@yahoo.com

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Abstract

Oxidative stress is the major driving factor responsible for the initiation and progression of sudden death, cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes. This study was carried out to investigate the In-vitro Antioxidant activity of *Tamarindus indica* 'ichekwu' fruits and its protective effect on oxidative damage in Animal model. *Tamarindus indica* (Tamarind) fruits extract (TFE) were analyzed for antioxidant, and phytochemicals using standard methods. The animals were induced oxidative stress using 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) for 14 weeks at 1.5ng/kg/day and treated for 8 weeks and were euthanized at the end of the experiment. Antioxidant assays such as LPO, SOD, CAT, and GPx were used to evaluate the extract's free radical scavenging ability. The results showed that the SOD, CAT, LPO and GPX ranged from 11.62-23.57U/mg, 250.22-375.30µmol/mg, 1.72-3.48nmol/mL and 2.03-4.73nmol/mL respectively. The findings suggest that *Tamarindus indica* fruits extract could be used as a natural, low-cost antioxidant as potential therapeutic applications in the treatment of oxidative stress-related diseases.

Keywords: Oxidative stress, *Tamarindus indica* fruits, antioxidant, phytochemicals, animal model.

Background

Oxidative stress is characterized by the excessive production of reactive oxygen species (ROS)/reactive nitrogen species (RNS) in the body, including superoxide anion, hydrogen peroxide, hydroxyl radicals, nitric oxide and nitrogen dioxide (Schieber and Chandel, 2014). Oxidative stress refers to the imbalance between the production of free radicals in the body and the capability of cells and tissues to clear them (Pizzino et al., 2017). Oxidative stress is caused by excessive oxidants and a lack of antioxidants. Antioxidants refer to compounds able to impede or retard the oxidation of a substrate, acting at a lower concentration compared with that of the protected substrate (Pisoschi et al., 2021). Numerous studies have shown that ROS could induce cell apoptosis by DNA damage and cell ferroptosis by triggering lipid peroxidation (Ye et al., 2016). Until now, diverse studies have demonstrated that oxidative stress causes aging and stress-provoked diseases (Dossena and Marino, 202; Singh, 2006). Therefore, eliminating excessive ROS is of great significance for homeostasis recovery. The major antioxidant defense system of body includes enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and nonenzymatic antioxidants, such as glutathione (GSH), which can protect against oxidative

stress (Shim and Kim, 2013). In addition, exogenous antioxidant supplementation is an important strategy to eliminate free radicals and prevent or treat related diseases (Pisoschi and Pop, 2015).

Tamarind (*T. indica* L.) belongs to sub family Caesalpinioideae. In fruit, the pulp constitutes 30–50 %, the shell and fibre account for 11–30 % and the seed about 25–40 %. Tamarind is not only a fruit but also poses significant medicinal and industrial value (Dal et al., 2015). The tamarind fruit is palatable due to its sweet and sour taste. Tamarind fruit is abundant in nutrients, such as fructose, glucose, cellulose (Adeola and Aworh, 2010) and amino acids, which can promote saliva secretion, stagnate digestion and whet the appetite (Shao et al., 2010; Sadik, 2010). Tamarind fruits can be consumed directly or processed into tamarind fruit juice beverages, jelly, pastries and other foods (Razali et al., 2015). *T. indica* seed coat has been shown to possess epidermal wound healing property (Mohd Yusof et al. 2012). *T. indica* is used as valuable ingredient in medical practice and for culinary purposes, however, not much work has been reported on the evaluation of biological potential, particularly antioxidant and hepatoprotective potential of its fruits.

Studies have reported that TFE is rich in flavonoids and could scavenge free radicals in vitro (Li H and Cui, 2014). However, the antioxidative effects in vivo have not been confirmed and the bioactive components in TFE remain unclear. In the present study, the antioxidant and phytochemical content of TFE were quantified by ultra-performance liquid chromatography coupled to electrospray ionization quadrupole orbitrap mass spectrometry (UPLC-MS/MS). The objective of the present study was to evaluate antioxidant profile of various extracts of tamarind fruits by various *in-vitro* methods and to determine the effect of the fruit extract on carbon tetrachloride (CCl₄) induced oxidative damage in animal models.

Materials and methods

Raw material

Tamarind indica L. fruits were purchased from a local market (New Market) Enugu, Enugu State of Nigeria from April–May 2024 and were separated by removing the coat. The fruits were ground into flour after separated from the seed kernel. It was ground into fine powder (particle size 0.75 mm) and stored at room temperature till further use.

Extraction

100 g fruit powder was extracted separately with 500 ml MeOH, water and acetone in Soxhlet extractor for 8 h and was filtered through Whatman No. 4 filter paper. The extracts were evaporated under reduced pressure (34–36 kPa) using a rotary vacuum-evaporator at 45 °C and the contents were dried on hot water bath. The extracts were used for the study.

Determination of Total Phenolics

The concentration of phenolic compounds in the extracts was determined, as described by Jayaprakasha et al. (2001) and results were expressed as tannic acids equivalents (TAE). The extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of ten fold diluted Folin–Ciocalteu reagents and 0.8 mL of 7.5 % sodium carbonate solution. After 30 min at room temperature, the absorbance was measured at 765 nm using Spectronic 20 spectrophotometer. The estimation was carried out in triplicate and the results were averaged.

Bioassay

Male albino rats of Wistar strain (170–200 g) were used for the studies. The required permission from competent authorities was taken for the use of animals in the experiment. The animals were placed into five groups containing five animals in each group. The first group served as control, the second group to the last group were administered carbon tetrachloride (CCl₄) to induce oxidative damage. The extract was suspended in 0.5 % sodium carboxymethylcellulose and was fed to rats in group 2-5 rats via oral route at 50 mg (tannic acid equivalents)/kg body weight for 14 days. The doses were selected on the basis of the LD₅₀ value of polyphenols (Bombardelli and Morazzoni, 1995). The animals of the first groups were simultaneously administered normal saline until 14th day. The animals of the second, third, fourth and fifth

groups were given a single oral dose of CCl₄ (1:1 in liquid paraffin) at 1.25 ml/kg of body weight after acclimatization. After 14 weeks of trial study, animals were sacrificed, and the liver was isolated to prepare the homogenate (5 %, w/v with 0.15 M KCl) and centrifuged at 800 *g* for 10 min. The cell-free supernatant was used for the estimation of lipid peroxidation (LPO), glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD).

Radical Scavenging Assay (RSA)

Radical scavenging activity of the extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was determined, as described by Murthy et al. (2002). Different concentrations (25, 50 and 100 ppm) of TFCE (*Tamarind indica* L fruit concentrate extract) and butylated hydroxy anisole (BHA) were taken in 100 µl with MeOH. About 5.0 ml of 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were kept for 20 min in dark. The control was prepared as above without any extract. The absorbance of the samples was measured at 517 nm. RSA was expressed as the inhibition percentage and was calculated using the following formula,

$$\% \text{ RSA} = (\text{Control OD} - \text{sample OD} / \text{Control OD}) \times 100.$$

Superoxide dismutase (SOD)

The assay of SOD was based on the reduction of nitro blue tetrazolium (NBT) to water insoluble blue formazan (Beauchamp and Fridovich, 1971). Approximately 0.5 mL homogenate, 1 mL of 50 mM sodium carbonate, 0.4 mL of 24 µM NBT and 0.2 mL of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 mL of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25 °C. The control was simultaneously run without homogenate. Units of SOD were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50 %. The specific activity was expressed as units per mg proteins.

Peroxidase

This assay was carried out using the method of Nicholos (1962). In 0.5 mL liver homogenate, 1 mL of 10 mM KI and 1 mL of 40 mM sodium acetate were added. Approximately 20 µL of H₂O₂ (15 mM) was added and the change in the absorbance at 353 nm in 5 min was recorded, which indicates the amount of peroxidase. Units of peroxidase were expressed as the amount of enzyme required to change the OD by 1 unit per minute. The specific activity was expressed as units per mg proteins.

Lipid Peroxidation

Lipid peroxidation was carried out as described by Buege and Aust (1978). Liver homogenate (0.5 mL) and 1 mL of 0.15 M KCl were taken and peroxidation was initiated by adding 100 µL of 0.2 mM ferric chloride. The reaction was run at 37 °C for 30 min and was stopped by adding 2 mL of ice-cold mixture of 0.25 N HCl containing 15 % TCA, 0.3 % TBA and 0.05 % butylated hydroxyl toluene (BHT) and was heated at 80 °C for 60 min. The samples were cooled, centrifuged, and the absorbance of the supernatant was measured. The results were expressed as MDA equivalents in nanomoles per mg protein of homogenate, which were calculated as describe in materials and methods section. The specific activity was expressed as units per milligram of protein.

Statistical Analysis

The data were expressed as mean±SEM. The data were analyzed by one way analysis of variance (ANOVA) and mean separated using Duncan New significant different at *p*< 0.05.

Results

Table 1: Phytochemical Composition of Tamarind Leaves

Nutrient	Value
Flavonoids	0.23±0.03
Saponin	0.06±0.01
Lycopene	1.21±0.23
Alkaloid	0.42±0.02
Tannin	1.62±0.06
Phenol	1.86 ±0.28
Glycoside	0.72±0.44

Phytochemical Composition

Table 2 reveals the phytochemical composition of the tamarind fruits analyzed.

Flavonoids: The flavonoid content in the sample is 0.23%. Flavonoid possess several medicinal benefits including anti-cancer, antioxidant, anti-inflammatory and antiviral properties. They also have neuro protective and cardio protective effects. These biological activities depend on the type of flavonoid. Ngwa and Nnam (2019) observed that flavonoid extract from *Solanum macranthum* reduces the lipid profile of diet induced obese rat. Ngwa and Nnam (2019) found that obese rats fed rat chow and flavonoid extract had higher levels of high density lipoprotein and lower levels of low density lipoprotein, triglycerides, and cholesterol. According to Ngwa and Nnam (2019), a high dietary intake of flavonoids is linked to a lower risk of cardiovascular disease, where obesity and atherosclerosis are risk factors.

Saponin: The saponin content was 0.06%. Saponin have been reported to help with lowering blood cholesterol, scavenging free radicals, and stimulating the immune system (Akubor and Nwawi 2019). Saponin are bitter and reduces palatability of food and increases the excretion of cholesterol concentration by free radical that are bond with cholesterol and other pathogen in the body (Umerah and Nnam, 2019). High saponin levels have been linked to gastroenteritis, which is characterized by diarrhea and dysentery, according to Igile et al. (2013). One of the most abundant and varied classes of natural plant compounds are saponins. They have a variety of ecological functions, such as protecting plants against disease and herbivores and potentially acting as allelopathic agents in plant-to-plant competition (Arawande and Borokini, 2010).

Alkaloid: The alkaloid content of the sample is 0.42%. Alkaloids have diverse physiological effects; anti-inflammatory, hypnotic, psychotropic and anti tumor activity. Alkanoids are used as medicinal agent for anagelsic, antispasmodic and bactericidal effect (Umerah and Nnam, 2019). Food rich in alkanoids are good for lactating mothers because of its medicinal value.

Tannin: The tannin content of the sample is 1.62% .Tanins protect against cancer and degenerative diseases. Tannins are phenolic compounds that are water soluble with the ability to precipitate protein. Schiavone et al. (2008) found that the safe range for tannins is 0.15–0.20%, where they can act as phytochemicals and aid in the prevention and treatment of cancer (Umerah and Nnam, 2019).

Result

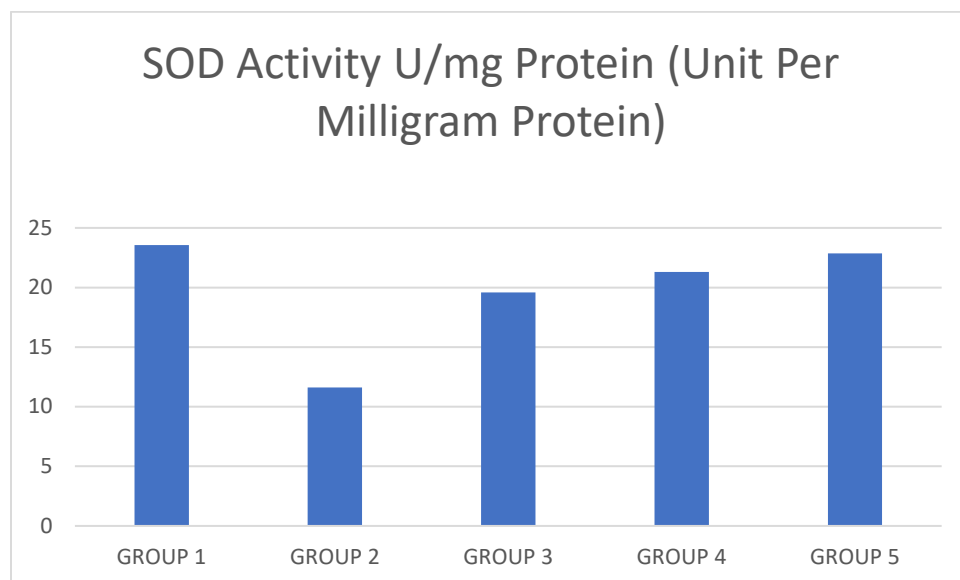


Fig. 1: SOD Activity U/mg Protein (Unit Per Milligram Protein)

Group 1- rat chow alone without inducing oxidative stress, Group 2- rat chow alone, Group 3- rat chow with 10% extract, Group 4- rat chow with 30% extract, Group 5-rat chow with drug.

The SOD activity was assessed after inducing oxidative stress and at the end of the trial period, group one (1) which had the rat chow alone (positive control) without inducing oxidative stress had 23.57U/mg which is the highest, 11.62U/mg in group two (2) which had rat chow alone (negative control), while group three (3) which had 10% had 19.58U/mg, 21.30U/mg in group four (4) which had 30% extract and group five (5) that had drug had 22.86U/mg.

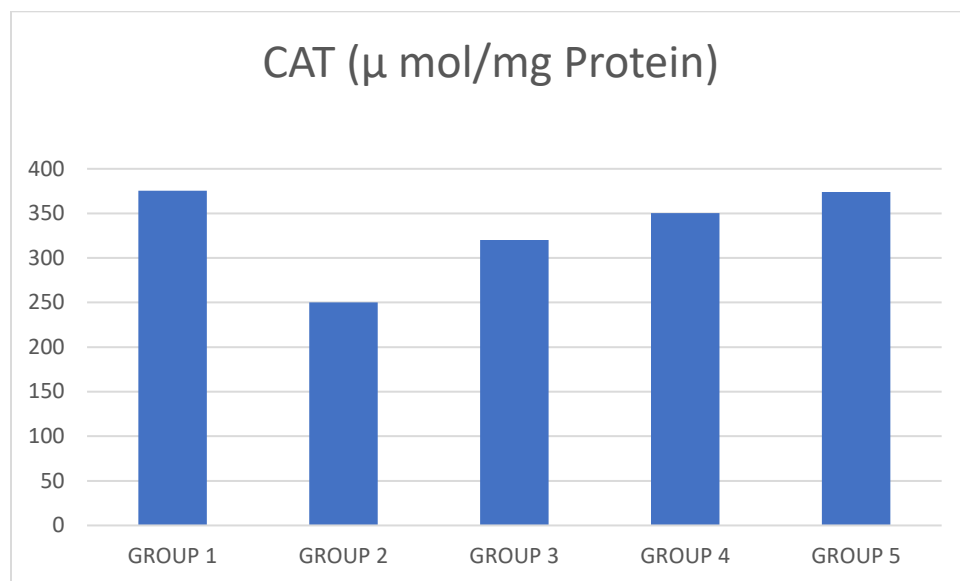


Fig. 2: CAT (μ mol/mg Protein)

Group 1- rat chow alone without inducing oxidative stress, Group 2- rat chow alone, Group 3- rat chow with 10% extract, Group 4- rat chow with 30% extract, Group 5-rat chow with drug.

The result of CAT was shown in figure 2. Group one (1) which had the diet alone had 372.40μmol/mg, 250.05μmol/mg in group two (2) which is the lowest, while group three (3) had 320.15μmol/mg, 350.26μmol/mg in group four (4) and group five (5) had 373.82μmol/mg which is the highest.

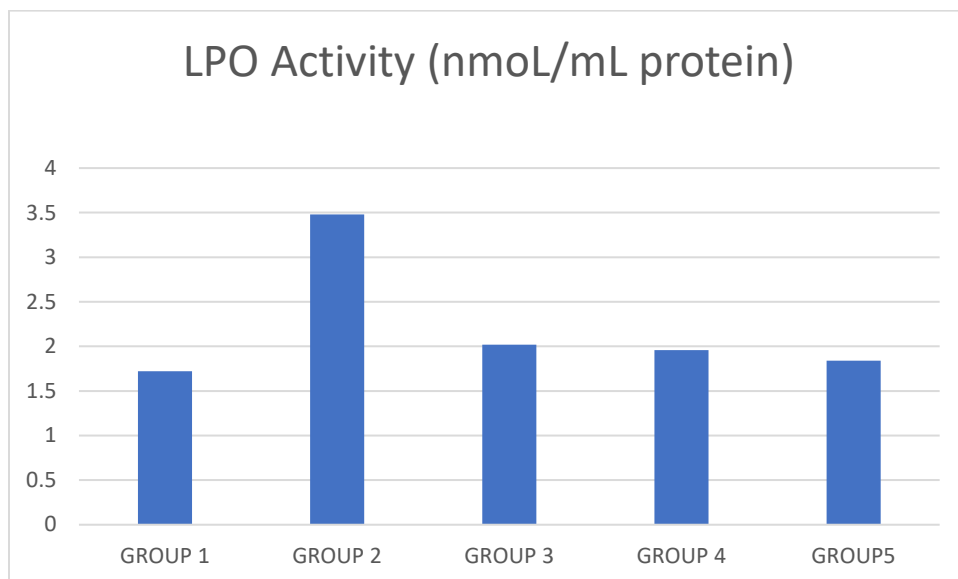


Fig. 3: LPO Activity (nmol/mL protein)

Group 1- rat chow alone without inducing oxidative stress, Group 2- rat chow alone, Group 3- rat chow with 10% extract, Group 4- rat chow with 30% extract, Group 5- rat chow with drug.

The result of LPO activity was shown in figure 3. Group one (1) had 1.78nmol/mL which is the lowest, 3.58nmol/mL in group two (2) which is the highest, while group three (3) had 2.02nmol/mL, 1.96nmol/mL in group four (4) and group five (5) had 1.84nmol/mL.

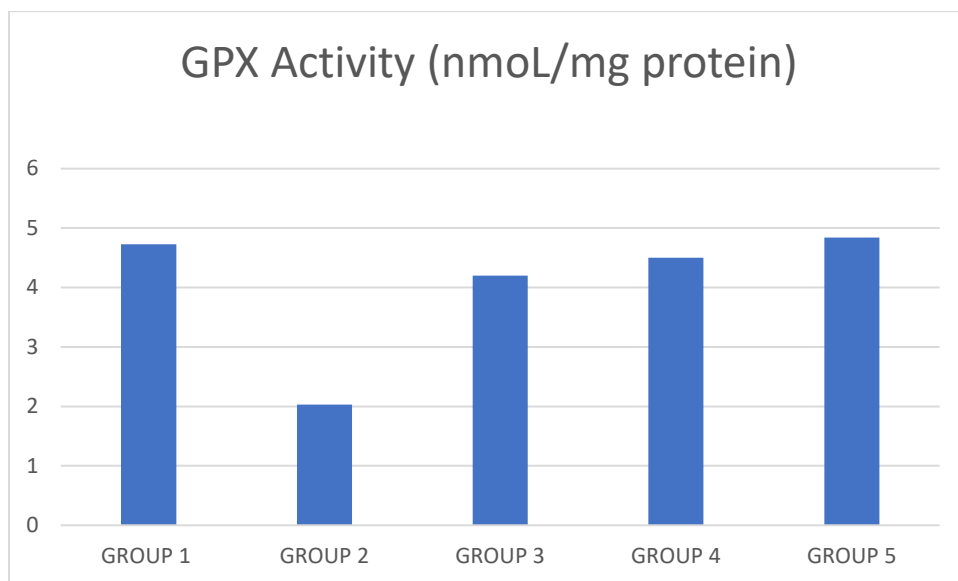


Fig. 4: GPX Activity (nmol/mg protein)

Group 1- rat chow alone without inducing oxidative stress, Group 2- rat chow alone, Group 3- rat chow with 10% extract, Group 4- rat chow with 30% extract, Group 5- rat chow with drug.

The result of GPX are shown in figure 4. At the end of the trial study, group one (1) had 4.73nmol/mL, 4.20nmol/mL in group two (2), while group three (3) had 4.20nmol/mL, 4.50nmol/mL in group four (4) and group five (5) had 4.84nmol/mL which is the highest.

Discussion

Research shows that free radicals interact with various cells components (e.g. DNA, protein, lipids), resulting in modifications and loss of function (Mansour and Mossa, 2009; Banerjee et al., 1999). It can disturb the biochemical and physiological functions of RBC and cause LPO (Mansour and Mossa, 2009). RBCs are highly susceptible to oxidative damage due to the high cell concentration of polyunsaturated fatty acid, hemoglobin and oxygen, which may produce oxidative changes in RBC. To protect itself, RBCs possess effective antioxidative enzyme systems e.g. SOD, CAT and GPx, which neutralize the reactive oxidants into none or less reactive species (Kyle et al., 1987). In fact, SOD, CAT and GPx are antioxidant enzymes that function as blockers of free radical process (Dormandy, 1978). SOD destroys the free radical superoxide (O_2^-) by converting it to hydrogen peroxide (H_2O_2) that can in turn be destroyed by CAT or GPx reactions to water and molecular oxygen. The results of the present work have shown that consumption of 10%, 30% tamarind fruits extract and antioxytic drug could increase the SOD activity in stress induced rat while the negative control had a decrease in SOD. The decrease in SOD activity of the rat that was induced stress without treatment was not a surprise as the rat chow does not contain antioxidant. The result of the CAT showed an increase in 10%, 30% tamarind fruit extract and the positive control group that was on antioxytic drug. Even though CAT is not essential for some cells type under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (Hunt, 1998). It is found as a soluble protein in erythrocytes, where it may protect hemoglobin from peroxidation (Banerjee et al., 1999). In the present study, rats treated with 10%, 30% tamarind fruits extract and drug showed significant increases in CAT activity and the most influence and increases were observed in 30%-treatments and antioxytic drug showing that it is dose dependent. In Comparison with the negative control administration there is significant decrease in CAT activity in group that was induced stress but were not treated. Some studies have indicated that superoxide radicals are potent inhibitors of CAT (Kono and Fridovich, 1982), and the increased H_2O_2 resulting from CAT inhibition could finally inhibit SOD activity. The increase of CAT activity following tamarind extract treatment may be due to the decrease of superoxide radicals as the result of the phytochemical and antioxidant content of the extract which are capable of protecting animals cells against oxidative damage.

Although GPx shares the substrate, H_2O_2 with CAT, it can react effectively with lipid and other organic hydroperoxides alone (Yan and Harding, 1997). Results of the present study showed that 10%, 30% tamarind fruits extract and drug caused significant increase in GPx activity in rat and administration of rat chow alone decrease the GPx of the rats. The increase in GPx activity of stress induced rats administered 10%, and 30% tamarind fruits extract may be attributable to the direct inhibitory oxidative effect of the phytochemical and antioxidant in the extract. In fact, the GSH redox cycle is a major source of protection against low levels of oxidant stress, whereas CAT becomes more significant in protecting against severe oxidant stress (Yan and Harding, 1997). In animals cells, especially in human erythrocytes, the principal antioxidant enzyme for the detoxification of H_2O_2 has been considered to be GPx for a long time, as CAT has much lower affinity for H_2O_2 than GPx (Izawa, 1996). However, reduction of antioxidant enzyme activity could be caused by a direct effect on the enzyme by stress induced reactive oxygen species (El-Shenawy et al., 2010).

The result of the LPO showed that 10%, 30% tamarind fruits extract and drug caused significant decrease in LPO level in the rat. This was in line with a previous study, where ASA (anti stress drug, 40 mg/kg) was reported to cause change in LPO and antioxidant enzyme activities in erythrocytes and liver samples of rats (Kirkova et al., 1994). Nair et al. (2006) reported that ASA caused significant decrease in the activity of reduced GSH, SOD, glutathione-s-transferase and CAT activities in intestine and colon of female rats.

Conclusion

The current study sought to assess the in vitro antioxidant activity of *Tamarindus indica* fruits extract, and its protective effects against oxidative damage in animal models. The results shows that *Tamarindus indica* fruits contain high concentration of phytochemical compound such as phenols, flavonoids, etc. Furthermore, the extract demonstrated protective effects against lipid peroxidation (LPO) and increased

antioxidant enzyme activity SOD, CAT, and GPx, confirming its role as a natural antioxidant source. In conclusion, *Tamarindus indica* fruits extract shows promise as a natural, affordable, and accessible antioxidant with therapeutic potential for managing oxidative stress-related diseases. Its application could be particularly beneficial in developing alternative antioxidant therapies and functional foods that contribute to overall health and disease prevention.

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