



# THE ANTIOXIDANT ACTIVITY OF *TAMARINDUS INDICA* L. FRUITS AQUEOUS EXTRACT IN THE WHITE ALBINO MICE

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## Abstract

This study was designed to investigate the antioxidants activity of *Tamarindus indica* L. fruits aqueous extract in the white albino mice which has exposed to hydrogen peroxide ( $H_2O_2$ ) as oxidation agent. The period of the experiments was (30) days. The animals were divide in to four groups, each of it included (6) animals, the first group which has been considered as a control group was given the food and water as normal condition, the second group was given ( $H_2O_2$ ) (0.5%) for 10 days with the drinking water, the third was given ( $H_2O_2$ ) (0.5%) and 50 mg/kg *Tamarindus indica* L. fruits extract day after day consequently that is meaning one day was given the ( $H_2O_2$ ) and the next day was given the extract for 30 days, the fourth group was given the ( $H_2O_2$ ) (0.5%) and 100mg/kg *Tamarindus indica* extract for 30 days. The results of the current study showed significant increase ( $p \leq 0.05$ ). in liver function tests (ALT, AST, and ALP) in the second group how treats with hydrogen peroxide  $H_2O_2$  comparison with others tree groups. Also there was a significant increase ( $p \leq 0.05$ ). of MAD in  $H_2O_2$  treatment group in comparative with the others groups, while there was a significant decrease in glutathione level ( $p \leq 0.05$ ) in the second ,and third groups according to oxidation damage of  $H_2O_2$ , the current research showed a significant increase ( $p \leq 0.05$ ) in the serum glucose in the second group, comparative with the control and other groups.

**Key words:** *Tamarindus indica* , antioxidants, Albino mice,  $H_2O_2$

## Introduction

*Tamarindus indica* is large evergreen tree consist of fruit, leaves, and seeds which are embedded in a sticky edible pulp (Rao *et al.*, 1999). Leaves opposite, compound, with 10-18 pairs of opposite leaflets; Fruit is a pod, indehiscent, subcylindrical, 10-18×4 cm, straight or curved. Flowers attractive pale yellow or pinkish, in small, lax spikes about 2.5 cm in width (Jain *et al.*, 2007). *T. indica* is used as a Traditional medicine in many countries, all parts of the tree have others uses like nutritional use, chemical martials, pharmaceutical compounds, and textile industries, and as fodder, timber, and fuel (Warda *et al.*, 2007). *T. indica* is rich in nutrients and plays an important role in human nutrition, mainly in the developing countries, also contains a high level of protein which consist of many important amino acids

(Ugwuona and Onweluzo, 2013). The most important compound in this plant is the phenolic compounds, cardiac glycosides, tartaric acid, the mucilage, also contain the pectin, arabinose, xylose, galactose, glucose, and uronic acid, carbohydrates (Ugwuona, and Onweluzo, 2013; Al-Fatimi *et al.*, 2007). Some studies showed the presence of fatty acids and some trace elements in phenolic extract of *T. indica* (Siddhuraju, 2007).

The tamarind is most commonly evaluated for its fruits, especially pulp, which is used for a wide range of household and industrial purposes and as raw material for the manufacture of many industrial products, such as tamarind juice, tamarind powder, tartaric acid, pectin, tartrate and alcohol (Siddhuraju, 2007; Osawa *et al.*, 1994).

Oxidative stress plays an important role in the pathogenesis of many diseases. Oxidation is a normal

and necessary process that takes place in the body. Oxidative stress, on the other side, take place when there's an imbalance between free radical activity and antioxidant effect. When It works correctly, free radicals can help fight off pathogens (Ganie *et al.*, 2011). Pathogens result to infections, Recent years have seen interest in oxidants and Antioxidant agents where the use of antioxidants is common in many treatment or prevention diseases resulting from active classes of oxygen such as diabetes mellitus and liver disease, geriatric diseases, arthritis and retinal diseases (Rao *et al.*, 1999).

## Materials and Methods

### Experiment design

The experiment included (24) albino rat, were divided to 4 groups (6) animals for each. as following:

- 1: was given food and water as normal conditions, considered as control group.
- 2: was given (H<sub>2</sub>O<sub>2</sub>) (0.5%) for 10 days
- 3: was given (H<sub>2</sub>O<sub>2</sub>) (0.5%) and *Tamarindus indica* extract (50 mg/kg ). For 30 days
- 4: was given (H<sub>2</sub>O<sub>2</sub>) (0.5%) and *Tamarindus indica* (100 mg/kg) for 30 days

### Preparation of *Tamarindus indica* fruit aqueous extract

The fruits were obtained from local markets where the seeds were removed and the weight of (100g) of fruit added to (300ml) of distilled water to obtain aqueous extract and well mixed and then left to soak for twenty - four hours, then mixed in the blender to break the cells and exit of active substances in the mixture, The mixture was filtered with sterile gauze twice, the filter was then completed with the filter paper to obtain the precipitate which was placed in clean flask and transported to the oven at 45°C to evaporation for 10 days until we obtained the powder form, which kept in clean containers until the using in the experiment.

### Preparation of hydrogen peroxide

Hydrogen peroxide was preparation by the dilution methods with distilled water to obtain 50% concentration, the preparation was don daily at all period of experiment.

### Blood sample collection:

The samples of blood were collected from the jugular vein of the animals, the samples were set for half hour at the room temperature then put at the centrifuge to get the serum for the biochemical tests, which was put in sterile tubes and set at freezing under 20- c until the time of tests.

## Biochemical tests

Included (ALT, AST, ALP, and serum glucose) which were estimated as the direction of the producer company, by measuring the samples in the spectrophotometer to obtain the results.

### Glutathione estimation

Glutathione was estimated using the modified method used by (Burtis and Ashwood, 1999). The method is based on the use of the Elman's reagent solution containing the DTNB [5, 5-dithiosbis (2-Nitrobenzoic acid) reagent. The reagent reacts rapidly with glutathione and is reduced by the sulfadhlil group (S-group).

### Serum Malondialdehyde

Serum Malondialdehyde as a final product of oxidized lipid as described by (Wysocka, 1995).

### Statistical analysis

The variables were analyzed as means  $\pm$  Standard Deviation, and estimated by the one way ANOVA, followed by t-test. Differences were considered to be statistically significant if  $p \leq 0.05$ .

## Results and discussion

The results in Table 1 showed there was a significant increase ( $p \leq 0.05$ ). in liver function tests (ALT, AST, ALP) in the second group how treats with hydrogen peroxide H<sub>2</sub>O<sub>2</sub> comparison with others tree groups, and this finding corresponding with recent studies (Tayade *et al.*, 2009; Pimple *et al.*, 2007) how they found a significant increase in liver function test by the toxicity of chemicals

**Table 1:** Mseans  $\pm$ SD of e concentration the liver function tests.

variables	Group 1	Group 2	Group 3	Group 4
ALT (U/L)	18.22 $\pm$ 0.31a	65.12 $\pm$ 1.7b	45.24 $\pm$ 8.9b	28.17 $\pm$ 2.52c
AST(U/L)	22.51 $\pm$ 1.22a	42.58 $\pm$ 0.29b	40.63 $\pm$ 1.52b	29.24 $\pm$ 3.1c
ALP(U/L)	7.33 $\pm$ 0.66a	18.61 $\pm$ 0.74b	15.22 $\pm$ 1.2b	10.35 $\pm$ 0.89c

Horizontal Different liters refers to a significant differences at ( $p \leq 0.05$ )

**Table 2:** Mean + SD serum, malondialdehyde and glutathione ( $\mu$ mol/gm)

Variables	Group 1	Group 2	Group 3	Group 4
MAD ( $\mu$ mol/gm)	2.23 $\pm$ 0.71a	3.53 $\pm$ 0.49b	2.92 $\pm$ 0.39a	2.11 $\pm$ 0.42a
GSH ( $\mu$ mol/gm)	5.74 $\pm$ 0.34a	2.58 $\pm$ 0.25b	2.89 $\pm$ 0.52b	4.24 $\pm$ 0.11a

Horizontal Different liters refers to a significant differences at ( $p \leq 0.05$ )

**Table 3:** Mean + SD, serum glucose (mg/dl)

variables	Group 1	Group 2	Group 3	Group 4
Glucose (mg/dl)	140.58 $\pm$ 3.31a	290.88 $\pm$ 2.11b	222.49 $\pm$ 2.9b	164.35 $\pm$ 1.82a

Horizontal Different liters refers to a significant differences at ( $p \leq 0.05$ )

compounds. The results also showed a significant increase ( $p \leq 0.05$ ) in liver function test in group 3 and 4 comparison with control group. In general the level of these enzymes start to decrease in groups treated with the extract of *Tamarindus indica* this is lead to suggest that the extract act as antioxidant agents, the elevated of liver enzymes occur in case of oxidation resulting from hepatic disorder or hepatic lesion thus the disorder of producing these enzymes.

Liver is essential organ attacked by ROS (Koleva *et al.*, 2002; Inal *et al.*, 2001). Parenchymal cells of the liver are first cells subjected to oxidative stress induced injury in this organ, Kupffer cells, which are hepatic stellate cells and endothelial cells are more exposed or sensitive to oxidative stress-related molecules (Warda *et al.*, 2007; Ganie *et al.*, 2013). These disorders of the liver cells caused in disorder of liver enzymes secreted by it.

The results in table 2 showed a significant increase ( $p \leq 0.05$ ). of MAD in  $H_2O_2$  treatment group comparative with the others groups, this results corresponding with a recent study (Inal *et al.*, 2001; Ugwuona, *et al.*, 2013) since the value of MAD return to its normal rat in the group treated with *Tamarindus indica* fruit extract. The development of oxidative stress by hydrogen peroxide leads to occurrence oxidative effects acting on fatty acids of lipid peroxidation, in cellular membranes leading to the production of a number of cytotoxic compounds such as MAD (Al-Fatimi, *et al.*, 1994). Antioxidant characteristic, of *T. indica* seed, fruits, and leaves has been shown in recent studies (Khalid *et al.*, 2018; Ugwuona *et al.*, 2013) Not only phenolic characteristic, (tannins) of raw seeds; but also heat dried seeds.

There was a significant decrease in glutathione ( $p \leq 0.05$ ) in the second and third groups according to oxidation damage of  $H_2O_2$ , the level of (GSH) begin to return to its normal level in the fourth group.

GSH maintains levels of reduced glutaredoxin, and glutathione peroxidase. It is one of the main endogenous antioxidants agent produced in the cells, equal the free radicals and reactive oxygen compounds, all so maintaining exogenous antioxidants” such as vitamins C and E in their reduced (active) forms”. (Pinar, 2014). Oxidative damage is strongly associated with  $H_2O_2$  effect; polyphenol compounds of *T. indica* seed and fruits extract has antioxidant enzymes induction properties and cancer related signal pathway inhibition influence. (Osawa, 1994).

The current research showed a significant increase ( $p \leq 0.05$ ) in the serum glucose in  $H_2O_2$  treatment group, comparative with the control and other group, the level of serum glucose start to return to its normal value in the group treatment with the aqueous extract of *T. indica*

fruit pulps. Both types of diabetes mellitus (1 and 2), are caused by damage resulting from chronic inflammation of pancreatic  $\beta$ -cell island (Osawa *et al.*, 1994; Maiti *et al.*, 2004). *T. indica* seed and fruits extract shows pancreatic  $\beta$ -cell island protective effectiveness with its anti-inflammatory properties, BG regulation, and inversion of damage to pancreatic cell tissue. These effects are happened because of increase in pancreatic intracellular  $Ca^{2+}$  level, and “plasma insulin action rather than decrease in glucose absorption”. (Pimple *et al.*, 2007).

With the aid of these effects, polyphenol rich *T. indica* fruits extract can be used as nutritional complement, and can be linkage with hypoglycemic factors (Pinar, 2014).

## Conclusions

Tamarind is used as a functional nutrients, and as a medical plant for many purpose such as wound, analgesic, abdominal pain. Also the current research explained the role of *Tamarindus indica* as a antitoxic agent according to functional substance contain in like: phenols’, amino acids, proteins ...act.

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