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Hypoglycemic and Antioxidant Activity of the Residual Aqueous Extract of *Tamarindus Indica*

Yerima M.[1*] and Samaila A.[2]

[1]*Department of Pharmacology and Toxicology Usmanu Danfodiyo University Sokoto, Nigeria*
[2]*Department of Pharmacology Umaru Musa Yaradua University Katsina, Nigeria*

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***Correspondence Info:**
Yerima M.
Department of Pharmacology and Toxicology,
Usmanu Danfodiyo University,
Sokoto, Nigeria

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Abstract

Introduction: The harmful effect of oxidative stress to cells of all aerobic organisms due to the high reactivity of the species can no longer be overemphasized. Reactive Oxygen Species are free radicals because the molecules have a lone electron in the outer shell. *Tamarindus indica* Linn, belongs to the Dicotyledonous family Leguminosae Sub Family Caesalpiniaceae, which is the third largest family of flowering plants.
Aim: The current study is aimed at determining the hypoglycemic effect and the free radical scavenging activity of residual aqueous extract of *T. indica.*
Methodology: The oral median lethal dose (LD_{50}) of the RAF in rats was conducted according to the method of Lorke (1983). Hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight of alloxan to 12 hours fasted rats. Hydrogen peroxide scavenging activity, reducing power assay and Free radical scavenging activity of the fraction was investigated.
Results: The residual aqueous fraction of the extract at doses used significantly ($p < 0.02$) lowered the Blood Glucose Level (BGL) with the 500 and 250 mg/kg doses in the first hour. At the eighth hour 250 mg/kg of the extract significantly ($p < 0.02$) lowered the BGL. Graded concentrations of the extract used showed a dose depended increase in the antioxidant activity using the hydrogen peroxide scavenging activity and reducing power assay. The DPPH antioxidant activity of the fraction was estimated from the absorbance of the free radical.
Conclusion: The results showed a concentration dependent percent scavenging antioxidant activity.
Keywords: Alloxan, antioxidant, hyperglycemia, *Tamarindus indica*.

1. Introduction

The harmful effect of oxidative stress to cells of all aerobic organisms due to the high reactivity of the species can no longer be overemphasized. Reactive Oxygen Species are free radicals because the molecules have a lone electron in the outer shell. The lone electron makes the radicals reactive as they seek stability by abstracting an electron from nearby molecules. Oxidative stress is one of the major factors in the induction of many chronic and degenerative diseases such as diabetes.

The body is able to offset these harmful effects of free radicals via the antioxidant defense system; should the body fail in getting rid of the free radicals, there will be need for exogenous supply of antioxidants. Kedare and Singh in 2011 showed that antioxidants can be freely found in food [1]. Most of the antioxidants naturally found in food are in the form of flavonoids. Researchers have thus focused their studies on plant-derived antioxidants [2].

Tamarindus indica Linn, belongs to the Dicotyledonous family Leguminosae Sub Family Caesalpiniaceae, which is the third largest family of flowering plants with a total of 727 genera and 19, 327 species [3]. Tamarind tree is slow growing tree that is resistant to strong winds and perennial. Leaves are 7.5 -15 cm long, alternate, stipulate, petiolate, paripinnantely compound, petiole up to 1.5 cm long, leaving a prominent

scar after falling, blade sub-oblong in outline, up to 13 cm X 15 cm with 8 -16 pairs of leaflets narrowly oblong, 1 -3.5 cm X 0.5 – 1 cm, entire oblique, rounded at base, and asymmetric, rounded to slightly emarginated at apex.

The current study is aimed at determining the hypoglycemic effect and the free radical scavenging activity of residual aqueous extract of *T. indica.*

2. Materials and Methods

2.1 Materials

The materials used for the study include the following:

- Stem-bark of *Tamarindus indica* Linn

Solvents

- Methanol 90% BDH Chemicals Ltd England

Drugs

- Alloxan Sigma-Aldrich Germany
- Metformin Sigma-Aldrich Germany
- The stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and sodium carbonate (Na_2CO_3) were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany).

2.2 Methods

2.2.1 Plant collection

A sample of the plant (stem-bark of *Tamarindus indica* L.) was collected by scrapping the trunk. The plant was collected in the month of March from Namaye in Bunkure Local Government Area of Kano state Nigeria. Botanical identification was done at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria. Mallam U. S. Gallah of the herbarium unit compared the sample with voucher specimen 00026.

The stem-bark was cleaned, and air-dried under shade for 26 days. This was done until a constant weight was obtained for three consecutive days. It was then pulverized using a pestle and mortar and then sieved to obtain the fine powder. The powder was weighed and kept in an airtight container.

2.2.2 Animals used in the study

Male and female Wistar albino rats (weighing 150-200 g) obtained from the animal house facilities of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used. The rats were housed in polypropylene cages at room temperature and maintained on standard laboratory animal feed obtained from the Department and water *ad libitum*, throughout the study. These studies were carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

2.2.3 Preparation of the extracts

To 500 g of the powder 1L of 90% methanol was added and allowed to soak for 48 hours in a separating funnel. The filtrate was then collected in a conical flask and transferred to an evaporating dish where it was evaporated to dryness on a water bath at a temperature of 42^{o}C. The extract was collected in an air tight container and labeled as methanol stem-bark extract of *Tamarindus indica,* and it was kept in a desiccator until ready for use.

2.2.4 Acute Toxicity Studies

The oral median lethal dose (LD_{50}) of the RAF in rats was conducted according to the method of Lorke (1983) with modifications [4]. The method was divided into two phases. In the initial phase, 3 groups of three rats each were treated with the methanol stem-bark extract at doses of 10, 100 and 1000 mg/kg body weight orally and the rats were observed for clinical signs and symptoms of toxicity within 24 h and death within 72 h.

In the second phase, 3 groups each containing one fresh rat was administered with three more specific doses of the extract based on the result of the initial phase. The animals were also observed for clinical signs and symptoms of toxic effects and mortality for 14 days.

The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1).

2.2.5 Alloxan-Induced Hyperglycaemia

Hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight of alloxan to 12 hours fasted rats [5, 6]. Six hours after the alloxan administration, the rats were maintained on 5 % glucose solution for the next 24h to prevent hypoglycaemia that may result from acute massive pancreatic release of insulin [7]. Seventy-two hours after drug administration, the rats were examined for hyperglycaemia by cutting the tail tip and using a one touch glucometer with compatible strips [Lifescan, Milpitas, CA]. Animals with fasting blood glucose of 180 mg/dL and less than 550 mg/dL were used in the study. Blood samples for blood glucose determination were collected from the tail at intervals of 0, 1, 4, 8, 16 and 24 hours. Determination of blood glucose level was done by the glucose-oxidase principle using the one touch Basic [8].

The alloxan-induced hyperglycaemic Wistar rats were randomly divided into five groups of six rats each as shown below,

Group I : Received normal saline orally
Group II : Received 250 mg/kg body weight of residual aqueous fraction of *T. indica* orally
Group III : Received 500 mg/kg body weight of residual aqueous fraction of *T. indica* orally
Group IV : Received 1000 mg/kg body weight of residual aqueous fraction of *T. indica* orally
Group V : Received metformin 250mg/kg body weight orally [9, 10]

2.2.6 Antioxidant Study

Hydrogen peroxide scavenging activity

A 40mM hydrogen peroxide solution in phosphate buffer (pH 7.4) was prepared. Five different concentrations of RAF (250, 500, 1000, 1500, and 2000ug/ml) were added to hydrogen peroxide solution (0.6ml, 40mM). Absorbance of hydrogen peroxide solution at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. A quartz cuvette was used for the assay. Percentage scavenging of hydrogen peroxide of the extract and the standard (ascorbic acid) was calculated using the formular:

% scavenging (H_2O_2) = $A_0 – A_1/A_0$ x 100

Where

A_0 = absorbance, A_1 = absorbance of sample

Reducing power assay

The reducing power of the extract was determined according to the method described by Oyaizu 1986 [11]. Five different concentrations of RAF (250, 500, 1000, 1500, and 2000ug/ml) in 1ml methanol were mixed with phosphate buffer (2.5ml, 0.2mM, pH 6.6) and potassium ferrocyanide (2.5ml 1%). The mixture was incubated in the refrigerator for 20 minutes. A portion (2.5ml) of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance of the sample was measured at 710nm and compared with the standard (ascorbic acid). Increased absorbance of the reaction mixture indicates increased reducing power.

Free radical scavenging activity

The method described by Shimada (1992) was used, three different concentrations (50, 100 and 200ug/mL) of the residual aqueous fraction was prepared and DPPH was added to the solutions and standard antioxidant substance and stirred. This is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The resultant mixture was allowed to remain in the dark for 30 minutes and the absorbance was measure at 517 nm against a blank [12].

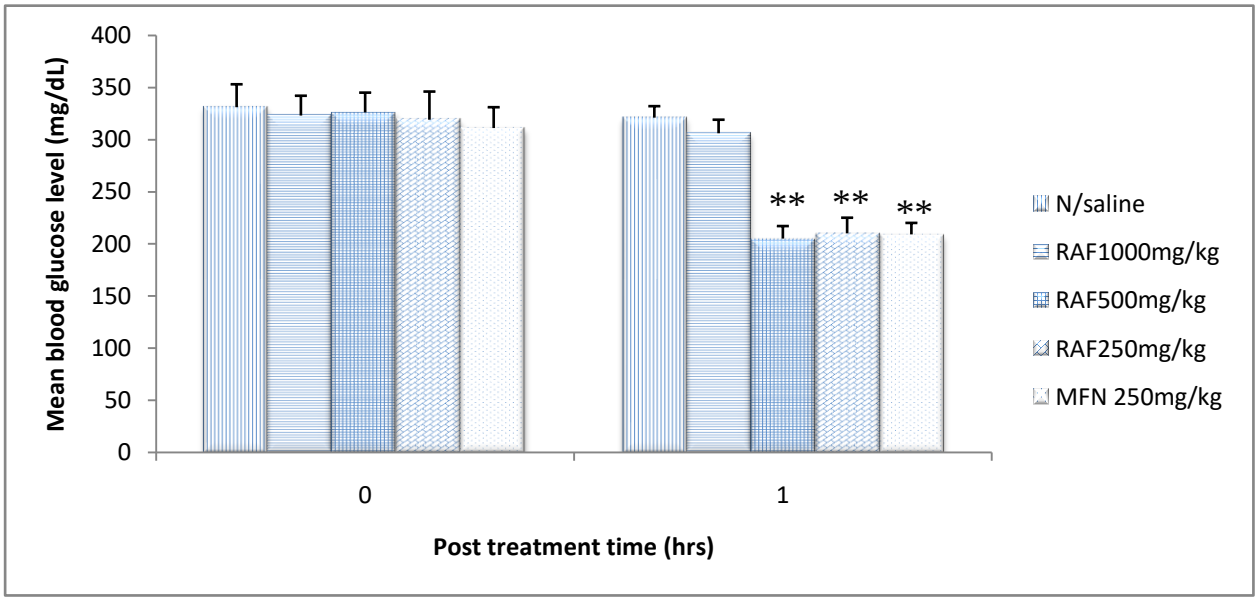
3. Results

The fraction gave a percentage yield of 57.3. The oral LD_{50} in rats for the residual aqueous fraction of *T. indica* was found to be greater than 5,000 mg/kg.

3.1 Residual Aqueous Fraction

The residual aqueous fraction of the extract at doses used significantly ($p < 0.02$) lowered the Blood Glucose Level (BGL) with the 500 and 250 mg/kg doses in the first hour. At the eighth hour 250 mg/kg of the extract significantly ($p < 0.02$) lowered the BGL. The 500 mg/kg dose at the 16th and 24th hour also significantly ($p < 0.02$) lowered elevated BGL when compared to normal saline group.

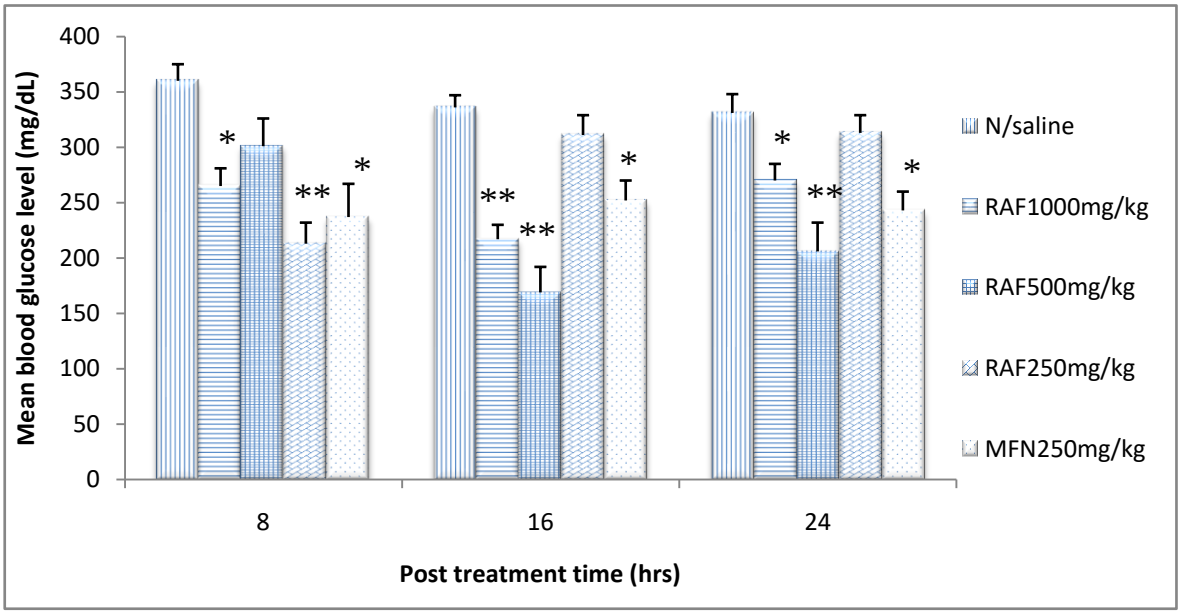
Figure 1a: The effect of the residual aqueous fraction of *T. indica* on blood glucose levels of alloxan-induced hyperglyceamia



n = 5 ** = sig. at $p < 0.02$ Vs Normal saline group *Student's T-test*

RAF = Residual aqueous fraction MFN = Metformin

Figure 1b: The effect of the residual aqueous fraction of *T. indica* on blood glucose levels of alloxan-induced hyperglycaemia



n = 5 * = sig. at $p < 0.05$ Vs Normal saline group *Student's T-test*

** = sig. at $p < 0.02$ Vs Normal saline group

RAF = Residual aqueous fraction MFN = Metformin

3.2 Antioxidant Studies

Graded concentrations of the extract used showed a dose depended increase in the antioxidant activity using the hydrogen peroxide scavenging activity Figure 2, and reducing power assay Figure 3.

Figure 2: Hydrogen peroxide scavenging activity of different concentrations of the extract and ascorbic acid

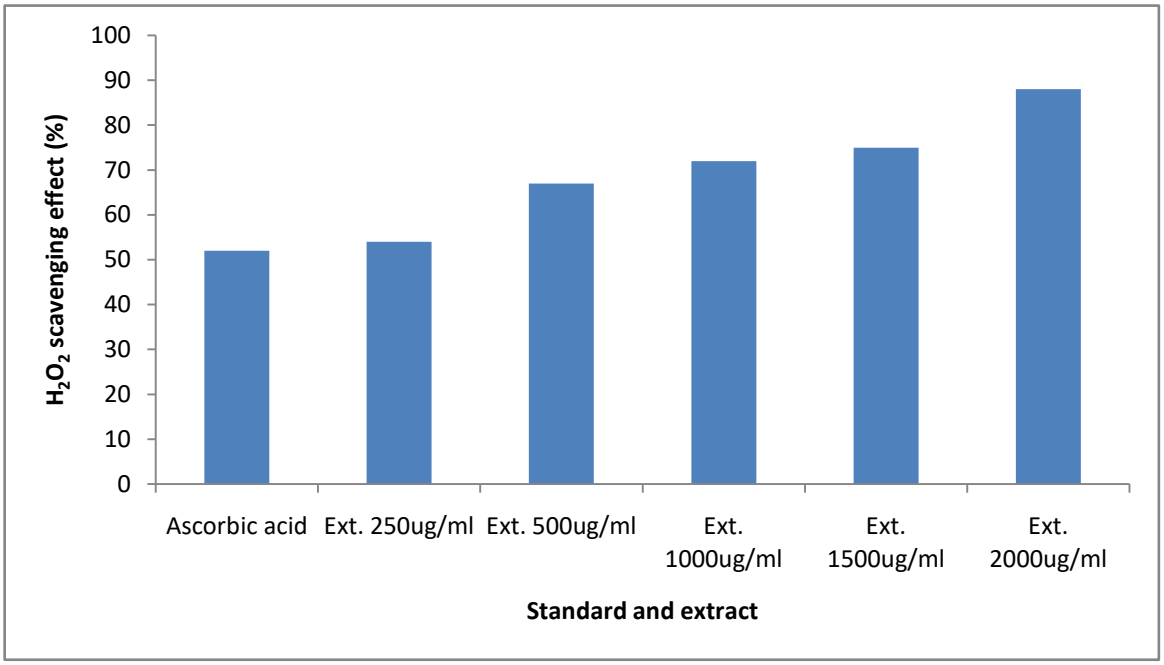
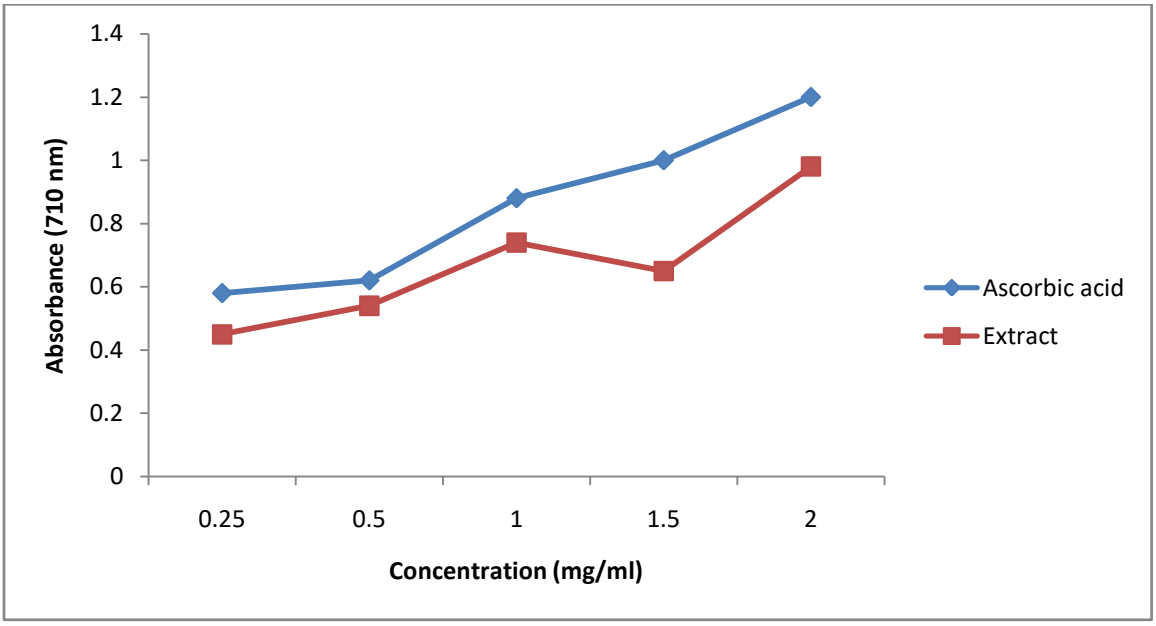
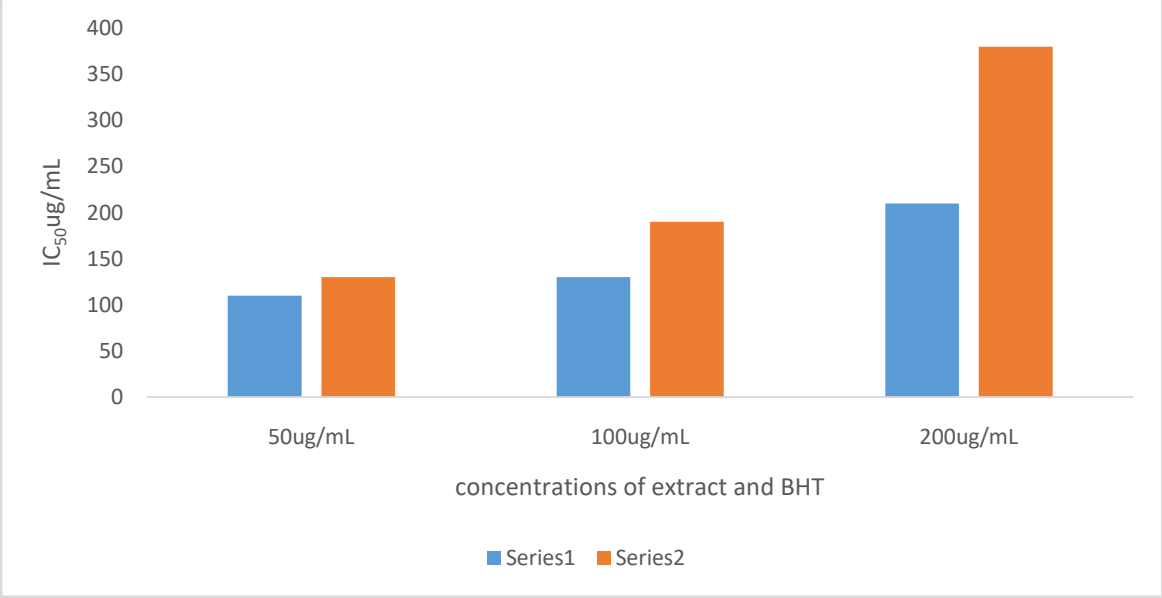


Figure 3: Reducing power of ascorbic acid and different concentrations of the extract



The DPPH antioxidant activity of the fraction was estimated from the absorbance of the free radical. The results showed a concentration dependent percent scavenging antioxidant activity. It was observed that antioxidant values of the extract are generally higher with no significant difference from that of BHT ($p \leq 0.05$) except at 200 µg/ml.

Figure 4: Free radical scavenging effects of RAF and butylated hydroxytoluene (BHT) at various concentrations



4. Discussion

An acute toxicity study in animals is important to drug development. In most cases the study tries to establish a precise median lethal dose (LD_{50}) in laboratory animals. The oral median lethal dose of residual aqueous fraction of *Tamarindus indica* L. in rat was found to be greater than 5,000 mg/kg. This suggests that the stem-bark extract is non-toxic when administered orally. A scale proposed by Lorke, roughly classifies substance as; very toxic (LD_{50} < 1.0 mg/kg), toxic (LD_{50} up to 10 mg/kg), less toxic (LD_{50} up to 100 mg/kg), only slightly toxic (LD_{50} up to 1000 mg/kg), LD_{50} values greater than 1000 mg/kg are considered safe and values greater than 5,000 mg/kg are of no practical interest in toxicological studies.

Alloxan is one the various chemical methods of inducing experimental diabetes; the diabetogenic properties of alloxan were reported later by Dunn, *et al*, who studied the effect of its administration in rabbits and reported a specific necrosis of pancreatic islets. Graded doses of the extract and metformin lowered the glucose concentration significantly ($p < 0.05$) within the first 24 hours with the 500 mg/kg dose of the extract being the most effective. The 500 mg/kg dose lowered the BGL throughout the hours of study. The greatest change in glycaemic concentration (40%) was seen with the 1000 mg/kg dose after 4 hours followed by metformin (38%) also at 4 hours. The 250 mg/kg dose increased the BGL at the first and the eighth hours. The animals in the first group were administered normal saline and the BGL increased from the first to the 16^{th} hour. It can be suggested that *T. indica* lowers the elevated glucose level by increasing peripheral glucose uptake. This is supported by the fact that metformin also lowered glucose level meaning that there is still some residual function of the β-cells. The primary action of biguanides is to increase sensitivity of peripheral tissues to endogenous insulin [13].

Oxidative stress is developed from an imbalance between free radical generating and radical scavenging system. In diabetes, oxidative stress has been found to be mainly due an increased production of oxygen free radicals and sharp reduction of antioxidant defenses [14]. The scavenging effect of the extract on hydrogen peroxide was observed with the highest concentration of the extract (2000ug/ml) showing the highest scavenging effect. The analysis of reducing power of a compound can serve as an indicator of its antioxidant effect. In this assay, ferric chloride (Fe^{3+}) was reduced to ferrous chloride (Fe^{2+}). Increased absorbance of the reaction mixture indicates increasing reducing power [15].

Tamarindus indica has effective reducing power using the potassium ferric cyanide reduction method when compared with the standard (ascorbic acid). The various concentrations of the extract showed powerful reducing ability, the result showed the electron donor properties of *T. indica* extract for neutralizing free radicals by forming stable products.

Antioxidants can protect the body from free radical and reactive oxygen species effect. They retard the progress of many chronic diseases [16]. The seed extract of *T. indica* has been reported to cause an elevation in the activities of SOD, CAT, GST and peroxidase in hepatic and renal tissues and muscles in Streptozotocin-induced diabetic rats [17]. Leaf extracts of *T. indica* also exhibit antioxidant activity in the liver [18]. The antioxidant activity of the leaves reported by Ramos *et al* [19] was similar to the activity observed by Al-Fatimi *et al* [20].

5. Conclusion

The fraction showed an interesting hypoglycemic activity and also the results of antioxidant evaluation based on hydrogen peroxide scavenging reducing power and DPPH scavenging used in this study revealed that the residual aqueous fraction of *T. indica* possess antioxidant activity.

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Competing of interest: None declared

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