The Antioxidant Effects of Tamarind Fruit Extract Against Hepatic and Renal Function Disorders in Diabetic Rats

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Abstract:

Tamarind contains a good amount of bioactive components with potential health benefits. Therefore, the current study was conducted to analyze the antioxidant effects of tamarind fruit extracts in alloxan-induced diabetic rats. The number of experimental rats was 49, which were distributed into seven groups: Group 0 (G0) as a control group, Group 1 (G1) as a diabetic group, Groups 2 and 3 (G2 and G3) as diabetic treatment groups that received aqueous (G2) and ethanolic (G3) tamarind extracts at a dose of 500 mgkg-1 body weight, and Groups 4 and 5 (G4 and G5) as non-diabetic groups that received (aqueous tamarind extract G4, at 500 mgkg-1 body weight) and (ethanolic tamarind extract G5, at 500 mgkg-1 body weight). Group 6 (G6), the diabetic, was treated with insulin at a dose of 6 IU/kg body weight, with all groups having access to food and water ad libitum, and the study period was 30 days. At the study's conclusion, the rats were euthanized, and their serum samples were analyzed for biomarkers, including liver enzymes (ALT, AST, ALP), kidney markers (creatinine and urea), antioxidant enzymes (SOD, CAT and GPx), and an indicator of oxidative stress (MDA). The results showed that administration of tamarind extracts (500 mgkg-1 body weight) led to a significant reduction in the liver enzymes levels, creatinine, urea, and MDA in the serum of diabetic rats (G2 and G3), compared to the diabetic group (G1), which exhibited elevated levels of these biomarkers. The results indicate that tamarind extracts possess antioxidant and hepatic and renal-protective properties, mitigating alloxan-induced oxidative stress and organ damage.

1 INTRODUCTION

Diabetes mellitus is a prevalent and progressing metabolic disorder, and its metabolic disturbances are directly associated with increased mortality and morbidity. Therapeutic use of natural substances may be more effective and safer than synthetic hypoglycemic agents in the therapeutic management of diabetes, providing a more cost-effective solution for various diseases and conditions. This growing interest in natural phytochemicals as potential treatments for many diseases, including diabetes, is driven by the high costs of medications and the adverse effects associated with synthetic drugs. therapies Traditional derived from substances have demonstrated significant efficacy in controlling diabetes [1].

Some studies suggest that an ethanolic extract of tamarind (Tamarindus indica) may mitigate the hepatotoxic and nephrotoxic effects of diabetic medications, as daily oral administration of tamarind extract for 30 days demonstrated significant improvement in pancreatic histological abnormalities, as demonstrated by histopathological analysis. Significant improvements were also observed in pancreatic tissue, suggesting potential therapeutic benefits. The proposed mechanisms behind these effects are likely attributable to tamarind's antioxidant properties and tissue protective effects. Therefore, the use of this extract as a therapeutic agent may hold scientific interest [2].

Experimental research has revealed that tamarind possesses anti-inflammatory, antioxidant, and protective properties against heart, kidney, and liver damage [3]. It has also been shown to reduce blood pressure and cholesterol levels, in addition to exhibiting antibacterial activity [4]. This study assessed the antioxidant characteristics of both aqueous and ethanolic extracts of tamarind in diabetic rats, demonstrating their potential therapeutic efficacy.

2 MATERIALS AND METHODS

2.1 Chemical Materials and Reagents

Ghaia Al-marafain of Babylon, supplied the chemicals employed in the study. Biomerieux, France, supplied the analytical kits for glucose GOD, aspartate transaminase (AST) (GOP), alanine transaminase (ALT) (GPT), alkaline phosphatase (ALP) (RandoxLa. (France)), creatinine, and urea (Biomerieux. (France)), while the local market provided the rat feed components. Insulin was sourced from a local pharmacy in Iraq, while alloxan monohydrate was procured from CDH (India). Elabscience, (Korea), supplies commercially available kits used for the assessment of rat glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Tamarind was obtained from local markets in Al-Najaf for the study.

2.2 Tamarind Sampling and Extract Preparation

Tamarind fruit samples were collected to prepare the extract. The plant material was dried. An accurate amount of the dry sample was weighed and placed in a beaker, then soaked in distilled water, ensuring that the sample was completely submerged. The mixture was incubated overnight and then filtered through Whatman No. 1 filter paper. The filtrate was concentrated to be more clear, centrifugation was performed to enhance filtration, and the filtrate was then allowed to dry completely in an oven at 40°C. The extract was then skimmed and collected, and the process was repeated until the desired amount was obtained. This process was used to prepare the aqueous extract [5]. For the ethanolic extract, the same procedure was followed, replacing the ethanol with distilled water.

The dose used in the study was 500 mgkg-1 for the aqueous extract and the same for the ethanolic extract. Based on the animal's weight, the dose was calculated using the following equation and prepared by dissolving the extract in 1 ml of distilled water.

$$Dose(mg) = 500(mg) \times Rat \ weight(g)/1000g$$

2.3 Acquisition of Animal Habitation and Management

Forty-nine euglycemic male mature rats, each weighing 215 g and aged 10 weeks, were obtained

from the Kufa Institute of Sciences and adapted to a standard diet for 14 days in the institution's animal facility prior to the experiment. The plastic enclosures housed the animals, but the hardwood flooring was renewed regularly to uphold sanitation and avert infection. A temperature of 24 °C was sustained for 12 hours (12 a.m.-12 p.m.) in a climate-regulated environment for rats. Rats were given unlimited access to fresh water and a regular meal consisting of sunflower oil, dextrose, casein, starch, minerals, and vitamins. Body weight was assessed on the initial and concluding days of the experimental phase. The university's animal ethics board sanctioned all procedures and protocols necessary for the trial and analysis before the initiation of the investigation.

2.4 The Study Design

Forty-nine rats were allocated into into seven groups, each including seven , with the treatment groups, dosages, and administration methods as follows:

- **G0:** Control (Health rats (non-diabetic). Orally given 1 ml 0f 0.9% NaCl solution.
- **G1**: Diabetic control Rats administered a single dosage of alloxan (150 mgkg⁻¹, by intraperitoneal injection).
- **G2**: Diabetes + *T.indica* aqueous extract. Diabetic rats treated with (500 mgkg⁻¹/B.W./day) *T.indica* aqueous extract gavage: *T.indica* was dissolved in distilled water just before use.
- **G3**: Diabetes + *T.indica* ethanolic extract. Diabetic rats treated with (500 mgkg⁻¹/ B.W./day) *T.indica* ethanolic extract gavage: *T.indica* was dissolved in distilled water just before use.
- **G4**: *T.indica* aqueous extract. Non-diabetic rats Orally given (500 mgkg⁻¹/ B.W./day) *T.indica* aqueous extract gavage.
- **G5**: *T.indica* ethanolic extract. Non-diabetic rats Orally given(500 mgkg⁻¹/ B.W./day) *T.indica* ethanolic extract gavage.
- **G6**: Diabetes + insulin. Insulin-treated diabetic rats (6 IU/kg/day). Insulin was delivered subcutaneously on a daily basis.

2.5 Haematological Chemistry Assessment

After collecting the blood sample, the serum was centrifuged and then left undisturbed for evaluation using a blood chemistry analyzer (Siemens, Munich, Germany) to measure liver enzyme levels (AST, ALT, & ALP), creatinine, and urea.

2.6 Antioxidant Metrics and Malondialdehyde

Photometry was utilized to evaluate the activity of SOD, CAT, and GPx in rats, as specified by [6], using the Elabscience (Korea). The enzymatic activities of SOD, CAT, and GPx in the samples of blood animales were quantified in international units per milliliter (IU/L). A photometric method was utilized to determine the MDA concentration in erythrocytes, as cited in [7]. The MDA concentration was measured in millimoles per liter (mmol/L).

2.7 Induction of Type 1 Diabetes and Fasting Serum Glucose Levels

Rats were fasted for 12 hours overnight and then given an intraperitoneal injection of alloxan monohydrate (150 mg/kg/body weight dissolved in sterile normal saline) to induce diabetes [8]. Fasting blood glucose was initially measured during diabetes induction, and a subsequent assessment was performed at the end of the experiment (after 30 days). To assess blood glucose levels, the animals' tails were sterilized with 10% alcohol, amputated using scissors, and blood was allowed to drain into contact with the test strip according to the methods described in [9].

2.8 Statistical Analysis

Data were reported as mean \pm standard error (M \pm S. E.) and analyzed using SPSS. Data were also analyzed using one-way analysis of variance (ANOVA) to assess the presence of statistically significant differences between the studied groups. This type of ANOVA was chosen based on the study design, which involved comparing multiple independent groups. Following ANOVA, a post-hoc LSD (least significant difference) test was applied to determine which groups differed statistically from each other. The LSD test was chosen because there were no statistically significant violations of the assumptions of ANOVA (such as normal distribution and homogeneity of variances). A P-value < 0.05 was set for statistical significance.

3 THE RESULTS

3.1 Effect of Tamarind Fruit Extracts on Liver Enzymes in the Serum of Rats

Table 1 indicates that the induction of diabetes resulted in a remarkable elevation (P< 0.05) in the effectiveness of AST, ALT, and ALP in male rats of group G1, with values of (157.4 \pm 2.45, 172.4 \pm 1.77, & 453.5 \pm 2.97) IU/L in sequence, contrasted to the control group G0 (40.4 \pm 3.49, 37.0 \pm 2.79, & 108.8 \pm 2.79) IU/L, respectively, as well as the other groups. The enzymatic activity in G2 and G3 considerably reduced (P < 0.05) compared to G1.

3.1 Impact of Tamarind Fruit Extracts on Renal Function in the Serum of Rats

Table 2 displays the results of several kidney parameters, including blood creatinine and urea levels, from rats administered tamarind fruit extracts in comparison to diabetic controls. The mean blood creatinine and serum urea concentrations in the control group (G0) were 0.43 ± 0.03 & 25.0 ± 2.25 mg/dL, respectively, whereas in the diabetic group (G1), its values were 0.85 ± 0.01 & 63.0 ± 1.35 mg/dL, respectively. The treated rats with tamarind fruit extracts exhibited improved results compared to the opposite diabetic group.

3.3 Influence of Tamarind Fruit Extracts on Anti-Oxidant Parameters

Reducing oxidative stress and improving organismal health are two of the primary functions of antioxidants including SOD, CAT, and GPx When comparing the diabetic group (G1) to the nondiabetic (G0), the levels of (SOD, CAT, and GPx) in the former were essentially lower (11.2 \pm 1.41, 12.0 ± 1.31 , & 17.7 ± 1.23 IU/L, respectively) (p < 0.05). The efficacy of the treatment group (G5) in terms of plasma SOD, CAT, and GPx was substantially different (p < 0.05). With comparison to G0 and other groups, they are relatively high at $(55.8 \pm 2.53, 65.5 \pm 3.39, \& 61.7 \pm 3.06)$ IU/L. The diabetic rats in G1 had a considerably higher plasma MDA level (87.5 \pm 2.19 mmol/L) than the nondiabetic rats in G0 (32.0 ± 2.98 mmol/L), according to the results in Table 3 and Figure 1.

Table 1: Liver enzymes activity in the serum of rats (n=7), following 30 days treatment with tamarind fruit extracts.

Groups	Liver enzymes IU/L				
	AST	ALT	ALP		
	Mean ± S. E				
G0	40.4 ± 3.49 c	37.7 ± 2.79 c	$108.8 \pm 2.79 \text{ c}$		
G1	157.4 ± 2.45 a	172.4 ± 1.77 a	$453.5 \pm 2.97 \text{ a}$		
G2	45.7 ± 4.17 c	37.8 ± 3.38 c	$116.4 \pm 3.70 \mathrm{c}$		
G3	41.1 ± 2.56 c	$36.4 \pm 2.77c$	$109.5 \pm 4.80 c$		
G4	$39.5 \pm 0.71c$	36.0 ± 3.52 c	$109.0 \pm 4.11c$		
G5	$39.0 \pm 1.74 \text{ c}$	33.8 ± 0.89 c	$108.0 \pm 3.28 c$		
G6	66.7 ± 3.44 b	54.8 ± 4.10 b	153.0 ± 1.96 b		
LSD	8.31	8.49	10.1		

The whole of the data is shown as mean \pm standard error. Means designated by various superscript letters are remarkably different (p < 0.05).

Table 2: Kidney function in the serum of rats (n=7), following 30 days of treatment with tamarind fruit extracts.

Groups	Creatinine mg/dL	Urea mg/dl	
	Mean ± S.E		
G0	$0.43 \pm 0.03 \text{ c}$	$25.0 \pm 2.25 \text{ cd}$	
G1	0.85 ± 0.01 a	$63.0 \pm 1.35 \text{ a}$	
G2	$0.47 \pm 0.02 \text{ c}$	27.7 ± 1.03 c	
G3	$0.45 \pm 0.04 \text{ c}$	$25.6 \pm 2.12 \text{ cd}$	
G4	$0.42 \pm 0.02 \text{ c}$	$23.5 \pm 2.18 \text{ cd}$	
G5	$0.40 \pm 0.01 \text{ c}$	$33.3 \pm 0.63 d$	
G6	$0.58 \pm 0.04 \text{ b}$	41.3 ± 1.74 b	
LSD	0.091	4.98	

The whole of the data shown as mean \pm standard error. Means designated by various superscript letters are remarkably different (p < 0.05).

Table 3: Antioxidant parameters and malondialdehyde levels in the serum of rats (n=7), following 30 days treatment with tamarind fruit extracts.

	1	1		
Groups of study	SOD (IU/L)	CAT (IU/L)	GPX (IU/L)	MDA (mmol/L)
	$Mean \pm S.E$			
G0	42.5 ± 4.44 b	55.2 ± 2.87 b	44.8 ± 2.62 bc	$32.0 \pm 2.98 \text{ c}$
G1	11.2 ± 1.41 d	12.0 ± 1.31d	17.7 ± 1.23 e	87.5 ± 2.19 a
G2	41.0 ± 2.31 b	50.7 ± 4.34 b	40.6 ± 1.55 c	$39.7 \pm 3.08 c$
G3	41.8 ± 2.75 b	52.2 ± 4.00 b	$46.2 \pm 3.50 \text{ bc}$	$37.3 \pm 2.20 \text{ c}$
G4	44.6 ± 1.48 b	57.9 ± 1.55 ab	48.9 ± 2.17 b	$32.9 \pm 2.28 \text{ c}$
G5	55.8 ± 2.53 a	65.5 ± 3.39 a	61.7 ± 3.06 a	21.4 ± 6.16 d
G6	$23.9 \pm 2.74 \text{ c}$	$40.5 \pm 3.52 \text{ c}$	31.8 ± 2.99 d	$52.0 \pm 4.06 \text{ b}$
LSD	7.80	9.24	7.44	10.2

The whole of the data shown as mean \pm standard error. Means designated by various superscript letters are remarkably different (p < 0.05).

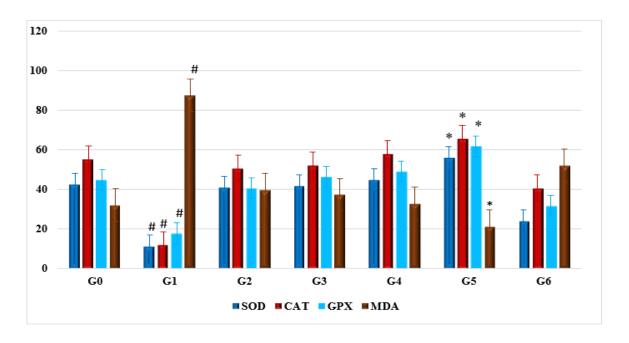


Figure 1: Antioxidant parameters (SOD, CAT & GPX) (IU/L) and MDA levels in the serum of rats (n=7), administered tamarind fruit extracts for a duration of 30 days, sig = #,* at (p < 0.05).

4 DISCUSSION

The findings show that consumption of tamarind fruit affected liver enzymes, renal function, antioxidants, and the oxidative stress marker MDA. Compared with the control group and other groups, the diabetic group showed a significant increase in the activity of liver enzymes (AST, ALT, and ALP). Consistent with previous research, the current study found that injecting male rats with 150 mg/kg of alloxan induced diabetes [10]. The elevated levels of the oxidative stress factor (MDA) in this group indicate that increased free radical production resulting from drug toxicity likely leads to increased lipid peroxidation. When the selective permeability of liver cell membranes is impaired due to oxidative stress, a large amount of liver enzymes is released into the blood [11].

Diabetic groups treated with either an aqueous or ethanolic extract of the fruit showed significant improvements in liver enzyme activity compared to the diabetic group. In a previous study, rats were given a toxic dose of paracetamol (1 g/kg orally) for 7 days and were treated with aqueous extracts of various parts of the tamarind plant for 9 days, including 350 mg/kg orally for the fruit and leaves and 700 mg/kg orally for the seeds. This resulted in the plant's hepatoprotective effects; there was a significant decrease in liver weight and necrosis, and

the results showed that serum bilirubin, ALP, and AST levels were significantly reduced [12]. The liver benefits observed in this study are believed to be due to the antioxidant polyphenolic chemicals present in the fruit. These may protect liver cells from necrotic damage caused by free radicals resulting from drug toxicity [13].

Furthermore, compared to the diabetic group, the insulin-treated diabetic group showed decreased liver enzyme activity, which is consistent with some studies [14] that found similar results in diabetic rats. This improvement, which explains the observed decrease in enzyme activity, is believed to be due to insulin's effects on liver glucose levels and lipid metabolism.

The findings showed that, compared to the healthy group (G0), creatinine and urea levels in diabetic male rats (G1) were significantly higher. Multiple studies [10, 15, 16] have found similar increases in these markers in the alloxan-injected group compared to the control group, and our results are consistent with those findings. This is likely due to vascular damage to the kidneys and nephrons caused by elevated blood glucose levels associated with diabetes [17]. Renal damage may occur as a result of non-enzymatic protein accumulation and excessive free radical formation resulting from high glucose levels [18]. The glomerular filtration process is impaired when the functional units are damaged,

reducing their ability to function. Kidney function is responsible for excreting creatinine (a product of muscle metabolism) and urea (a product of protein metabolism), with the kidneys excreting more than 90% of both. Renal damage reduces their excretion of creatinine and urea, leading to their accumulation in the blood and elevated serum levels [17].

In addition, the diabetic groups receiving tamarind fruit extracts, either aqueous or ethanolic (G2 & G3), showed significantly lower creatinine and urea levels compared to the diabetic group (G1). The kidney- and liver-protective effects of tamarind (T. indica) extract and its antioxidant properties are said to be due to its high concentration of polyphenols, which effectively neutralize intracellular free radicals [18]. Insulin may eliminate free radicals, thus reducing damage to the renal cortex, which showed lower kidney function indicators (creatinine and urea) (G6) compared to the control diabetic group (G1).

According to the results of [19], diabetic male rats (G1) showed decreased activity of SOD, CAT, and GPx, while malondialdehyde levels were increased, in contrast to the control group. The main reason for decreased antioxidant enzymes and increased malondialdehyde levels excessively high blood glucose levels, which produce large amounts of free radicals, leading to decreased antioxidant efficacy and, consequently, oxidative stress [20]. However, the results were opposite for diabetic rats treated with the aqueous extract (G2) and diabetic rats treated with the ethanolic extract (G3) of tamarind fruit at a dose of 500 mg/kg, as the antioxidant enzymes SOD, CAT, and GPx showed increased activity, while MDA levels decreased compared to the diabetic group. The antioxidant activity is likely due to the synergistic interaction of the many phytochemicals that the extract may contain. This interaction prevents free radicals, such as hydrogen peroxide, from initiating or stopping their chain reactions and may indirectly act as an antioxidant by increasing the activity of free radical-scavenging enzymes or enhancing their expression [21].

In comparison to the control and other groups, the rats that received ethanolic tamarind extract (G5) showed significantly higher levels of antioxidant enzymes and significantly lower levels of malondialdehyde (MDA). This effect is attributed to the presence of two phenolic compounds in the extract: orcinol (1-(2-furanyl)-1-propanone) and furyl hydroxymethyl ketone. Flavonoids in the extract, such as succinic acid, 3-methylbut-2-yl 3-heptyl ester, tartaric acid, 1,2-benzenedicarboxylic

acid, and butyl acid, also have antioxidant properties [22]-[24]. The structural properties and hydroxyl groups of these compounds are likely responsible for their ability to neutralize free radicals by donating hydrogen atoms.

These findings highlight the therapeutic potential of tamarind fruit in improving liver and kidney function and reducing oxidative stress levels in diabetic patients. However, further clinical research is needed to confirm these effects and determine safe and effective doses.

5 CONCLUSIONS

The findings of this study demonstrate that T. indica extracts, especially the ethanolic extract at a dose of 500 mg/kg body weight over 30 days, significantly improved liver and kidney functions in alloxaninduced diabetic rats. These improvements were evidenced by the reduction of elevated liver enzymes (AST, ALT, and ALP) and normalization of kidney biomarkers (creatinine and urea levels), indicating recovery of organ function. The ethanolic extract showed superior efficacy compared to the aqueous extract, likely due to its higher retention of bioactive antioxidants such as polyphenols and flavonoids. These compounds appear to mitigate oxidative stress (MDA) and enhance the activity of endogenous antioxidant enzymes (SOD, CAT, and GPx). The protective effects observed suggest that the ethanolic extract of T. indica may counteract the hepatotoxic and nephrotoxic effects associated with diabetes or its pharmacological treatments. Therefore, this extract shows promise as a complementary therapeutic agent in preventing or reducing diabetes-induced organ damage. These results provide scientific justification for the traditional use of T. indica in managing chronic conditions and support further investigations into its potential clinical applications.

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