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Toxicological profiles of methanol stem bark extract of *Tamarindus indica* on experimental hyperglycemic wistar rats

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Abstract

Introduction: Drinking behavior after water deprivation is one of the standard tests used to study thirst in humans and animals and in addition, diurnal cycle, food availability are known to influence water intake.
Methodology: A diurnal cycle of 24 h water deprivation with free access to food from 13.00 h one day to 13.00 h the next day, followed by free access to both food and water was used. To determine the level of haematological parameters alloxan was used to induce hyperglycaemia and graded doses of the extract were administered orally for 28 days.
Results: The three doses of the extract used significantly ($p < 0.05$) improved the feeding behaviour of the hyperglycaemic animals after seven days. Water consumption was significantly reduced by all the doses of the extract used in the hyperglycaemic treated animals. The WBC, neutrophils, monocytes, lymphocytes, and eosinophils levels were also analyzed. Administration of graded doses of the extract caused a significant improvement in the level of RBC and its related indices, the 1000 mg/kg dose being the most effective. The 250 mg/kg dose and metformin caused a significant ($p < 0.05$) improvement in the white blood counts and the level of neutrophils, monocyte and lymphocyte were also significantly ($p < 0.05$) restored to near normal values.
Conclusion: The doses of the extract used and metformin also significantly ($p < 0.05$) reduced the water consumption of the animals. Decreases in haematological parameters were restored to near normal levels in the groups treated with the extract.
Keywords: *T. indica*, hyperglycaemia food and water intake, hematology

1. Introduction

Drinking behavior after water deprivation is one of the standard tests used to study thirst in humans and animals and in addition, diurnal cycle, food availability are known to influence water intake [1]. The lateral nuclei of the hypothalamus serve as the feeding centre, and stimulation of this area causes animals to eat voraciously (hyperphagia). Conversely, destruction of the lateral hypothalamus causes lack of desire for food and progressive inanition, a condition characterized by weight loss, muscle weakness and decreased metabolism.

Medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited. They may have recognizable therapeutic effects [2]; and they may also have toxic side-effect [3].

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. 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.

2. Materials and Methods

2.1. Materials

The materials used for the study include the following:

- Stem-bark of *Tamarindus indica* Linn
- Male and female Wistar albino rats (weight 150-200 g)

2.1.1. Solvents

- Methanol 90% BDH Chemicals Ltd England

2.1.2. Drugs

- Alloxan Sigma-Aldrich Germany
- Metformin Sigma-Aldrich Germany

2.2. Methods

2.2.1. Induction of experimental hyperglycemia

Hyperglycaemia was induced by a single intraperitoneal injection of 150 mg/kg body weight of alloxan to 12 hours fasted rats [4, 5]. 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 [6]. 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 [7].

2.2.2. Determination of Food and Water Intake

For this experiment, the method of Kooi *et al*., 2001 as described by Alewu and Anuka 2009 was used. The animals were divided into six groups of five (5) rats each. The first group served as control group (normal animals). The second group served as hyperglycaemic control and animals were given normal saline. Groups III, IV and V received 250, 500, and1000 mg/kg body weight of *T. indica* respectively and the group VI animals were administered metformin 250 mg/kg. The animals were given *T. indica* daily in addition to food and water. A diurnal cycle of 24 h water deprivation with free access to food from 1300 h one day to 1300 h the next day, followed by free access to both food and water (Night-with-Food) was used. This was followed by feeding with water for 6 hours i.e. from 1300 h to 1900 h the same day. Approximately one hundred milliliters (100ml) of water in the rats' drinker was offered to the animals in each group. After 6 hours, the final level of water was recorded. This was repeated for seven (7) times and the average taken. The animals had free access to a weighed amount of molded feed for thirteen (13) hours i.e. from 19.00 h to 8.00 h the following day. The cages were cleared of sawdust and food leftover by the 13th hour and the animals were deprived of food for 10hours. The animals were fed again for 13 hours then the amount of food consumed was measured by subtracting the food leftover from the amount given. The experiment was repeated for seven (7) times.

2.2.3. Determination of Haematological Parameters

In this experiment the following groupings as shown was used

Group I : Received normal saline orally
Group II : Received 250 mg/kg body weight of methanol stem bark extract of *T. indica* orally
Group III : Received 500 mg/kg body weight of methanol stem bark extract of *T. indica* orally
Group IV : Received 1000 mg/kg body weight of methanol stem bark extract of *T. indica* orally
Group V : Received metformin 250mg/kg body weight orally [8, 9].
Group VI : Normoglycemic

Alloxan was used to induce hyperglycaemia as mentioned above (except group VI), the animals were administered the extract and the standard agent orally every morning 30 min before feeding for 28 days. On the 28th day the animals were sacrificed under slight chloroform aneasthesia. Blood was collected from the jugular vein into EDTA sample bottles for the haematological analysis which include hemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC). White blood cell (WBC) count, neutrophils, monocytes, lymphocytes, eosinophils investigated.

2.3. Data Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA). Student's t-test at 95% level of significance was used to assess significant difference between the control and treated group. The results are presented in tables and charts.

3.0. Results

3.1. Feed and Water Intake

All the doses of the extract used and metformin improved the feeding behaviour of the animals, but the extract produced a significant difference in the feeding. The doses of the extract used and metformin also significantly ($p < 0.05$) reduced the water consumption of the animals (Table 1).

Table 1: The effect of methanol stem-bark extract of *T. indica* on feed intake and water consumption after 7 days of treatment

Treatment	Feed intake (g)		Water consumption (ml)	
	Day 1	Day 7	Day 1	Day7
Normal control	15.8±0.7	19.5±0.4	23.3±0.2	27.4±0.3
Hyperglycaemic + N/ saline	11.3±0.2	14.0±0.9	22.6±0.5	30.2±0.1
Hyperglycaemic + extract 250mg/kg	14.0±0.9	18.4±0.3*	22.0±0.9	26.3±0.2*
Hyperglycaemic + extract 500mg/kg	14.6±0.5	19.3±0.2*	23.4±0.3	21.5±0.4*
Hyperglycaemic + extract 1000mg/kg	13.3±0.2	17.5±0.4*	24.1±0.0	22.6±0.5*
Hyperglycaemic + MFN 250mg/kg	12.6±0.5	16.3±0.2	22.9±0.8	23.2±0.1*

n = 5, MFN = Metformin, * = sig. at $p < 0.05$ Vs hyperglycaemic + normal saline group, *Student's t-test*

3.2. Haematological Indices

Table 2 below shows decreases in the levels of RBC, PCV, Hb, and MCHC in the hyperglycaemic untreated group when compared to normal control. The decreases in these parameters were restored to near normal levels in the groups treated with the extract. The WBC and the monocyte levels were significantly lowered as compared to the normal animals, but 250 mg/kg of the extract (WBC) and all the doses of the extract used and the standard agent significantly ($p < 0.05$) improved the level of the monocyte.

Table 2: The effect of methanol stem-bark extract of *T. indica* on haematological parameters in alloxan-induced hyperglycaemic Wistar rats

Parameter	Normal control	Hyperglycaemic Control	*T. indica* (250 mg/kg)	*T. indica* (500 mg/kg)	*T. indica* (1000 mg/kg)	Metformin (250 mg/kg)
RBC $^{X\ 1012/L}$	10.8±0.4	9.4±0.6	10.12±0.3	10.7±0.3	11.8±0.4*	10.14±0.6
PCV (%)	45.3±4.4	40.7±3.2	48.1±1.7	51.1±2.6*	50.9±2.1*	43.9±4.8
Hb (g/dL)	13.5±0.5	12.3±0.7	14.3±0.4	15.0±0.4*	15.0±0.5*	13.5±0.7
MCHC (g/dL)	30.4±0.6	25.5±0.5	30.6±0.4	29.7±0.6	33.8±0.5	30.9±2.0
WBC x 10^9	12.13±2.0	5.4±1.0	7.2±2.0*	5.5±0.5	6.2±2.0	7.4±3.0*
Neutrophil (%)	35.5±1.0	25.7±2.5	32.6±2.0*	43.7±1.0*	35.6±1.5*	35.3±3.0*
Lymphocyte (%)	60.3±3.5	30.2±2.0	56.6±3.0*	52.2±2.5*	54.4±3.0*	55.6±4.0*
Monocyte (%)	9.11±3.0	2.6±0.5	7.5±0.0*	5.5±0.0*	6.3±0.0*	10.2±0.0*
Eosinophil (%)	3.3±0.5	1.0±0.0	2.5±0.0*	1.5±0	2.0±0.0	1.1±0.0

n = 5, * = significant at $p < 0.05$ Vs hyperglycaemic untreated group *Student's t-test*

RBC = Red blood cell, PCV = Packed cell volume, Hb = Haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cell

4. Discussion

Feeding behaviour is the final result of intricate relationships between central nervous system and peripheral tissues. Some aspects of feeding, such as meal size or satiety, seem to be mainly dependent on the interplay between general oropharyngeal sensations and the action of nutrients and peptidic and nervous signals from the gastrointestinal tract [10]. The three doses of the extract used significantly ($p < 0.05$) improved the feeding behaviour of the hyperglycaemic animals after seven days. Water consumption was significantly reduced by all the doses of the extract used and metformin in the hyperglycaemic treated animals.

It has been shown that experimental diabetes induced by streptozotocin causes hypophagia for a few days immediately after the treatment, followed by a continued hyperphagia and remarkable changes in feeding behaviour [11-13]. Since its known that streptozotocin and alloxan have similar mechanism of action, the reduction in feeding behaviour observed in the untreated animals could be due to partial destruction of the β-cells responsible for insulin production. Under basal conditions, insulin and leptin are thought to inhibit hypothalamic arcuate nucleus (ARC) neurons that coexpress neuropeptide Y (NPY) and agouti generelated protein (AgRP), peptides that potentially stimulate food intake. Conversely, basal insulin and leptin levels also activate an adjacent ARC neuronal population that expresses preproopiomelanocortin (POMC), which reduces food intake and increasing energy expenditure [14]. The water intake of hyperglycaemic untreated animals was significantly higher than the treated rats. This result is similar to the work of Kim *et al* 2006 who demonstrated the effect of *Morus alba* in controlling the desire for food and water intake under diabetic condition.

It is important to assess the haematological parameters of animals, this is to help in determining any deleterious effect of foreign compounds (alloxan) including plant extracts on the blood constituents of the animals.

Anaemia has been reported in diabetes which is said to be due to the increased non-enzymatic glycosylation of RBC membrane proteins [15]. Oxidation of membrane proteins in RBC and the hyperglycaemia in diabetes mellitus results in an increase in the production of lipid peroxides that lead to haemolysis of RBC [16].

In this study, the red blood cells parameters such as Hb, MCHC, and PCV were studied to investigate the beneficial effect of *T. indica* extract on the anaemia caused by untreated hyperglycaemic states in rats. The levels of RBC, PCV, Hb, and MCHC in the hyperglycaemic untreated animals were reduced which may result from damages caused by alloxan on the normal body systems. This observation is similar to the work of Baskar in 2006 [17] who reported antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin-induced diabetic rats. An alteration of RBC parameters is known to cause anaemic condition in man [18]. Administration of graded doses of the extract caused a significant improvement in the level of RBC and its related indices, the 1000 mg/kg dose being the most effective. The improvement in the level of RBC and its parameters indicates that the extract of *T. indica* may possess some phytochemicals that can stimulate the formation or secretion of erythropoietin. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [19]. Improvement in the level of RBC is supported by the improved level of MCHC [20].

The extract's ability to improve RBC level may also be attributed to its ability to lower lipid peroxidation level which causes haemolysis of erythrocytes [21]. Phytochemical screening of this plant (*T. indica*) revealed the presence of flavonoids, and tannins, these compounds have been reported to possess strong antioxidant property [22]. This antioxidant property of the plant could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in hyperglycaemic animals reported earlier [23, 24].

There was a significant reduction in the WBC count and the level of its differentials such as monocytes, eosinophils, lymphocytes and neutrophils were also reduced in the hyperglycaemic untreated group. Following administration of the extract, the white blood counts was improved significantly ($p < 0.05$) at 250 mg/kg dose and metformin and the level of neutrophils, monocyte and lymphocyte were significantly ($p < 0.05$) restored to near normal values. However, 500 and 1000 mg/kg doses of the extract did not have any significant effect on the WBC count and eosinophil level in this study.

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Competing of interest: None declared

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