Comparative Study of the Effects of Methanolic Extracts of *Spondias mombin* Leaves and *Curcuma longa* Rhizomes on Serum Lipid Profile and Electrolytes in Alloxan Induced Diabetes in Male Wistar Rats

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Introduction:** Diabetes mellitus is an important risk factor for cardiovascular diseases; the possible uses of *Spondias mombin* and *Curcuma longa* rhizomes for the treatment of diabetes and cardiovascular disorders have become prevalent in our environment.

**Aim:** The present study attempts a Comparative assessment of the effects of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes on serum lipid profile and electrolytes in alloxan induced diabetes in male wistar rats.
Methodology: 90 male wistar rats were randomly divided into 9 groups of 10 rats each. Diabetes was induced intraperitoneally using alloxan at 200 mg/kg-bw. The different rat Groups were treated with extracts and glibenclamide orally for 42 days as follows: Group 1: untreated non diabetic; Group 2: untreated diabetic; Group 3: diabetic + low dose Spondias mombin; Group 4: diabetic + high dose Spondias mombin; Group 5: diabetic + low dose Curcuma longa; Group 6: diabetic + high dose Curcuma longa; Group 7: diabetic + low dose combined Spondias mombin and Curcuma longa; Group 8: diabetic + high dose combined Spondias mombin and Curcuma longa; and Group 9: diabetic + glibenclamide. Blood was collected on day 43 by cardiac puncture for determination of serum lipid profile and electrolytes. 

Results: Compared to Group 2, total serum cholesterol, triglyceride, low density lipoprotein and electrolytes were significantly reduced while high density lipoprotein was significantly increased in all treated Groups (p<0.05). Compared to Groups 3 to 6, Groups 7 and 8 rats showed a significant reduction in total cholesterol, triglyceride and low density lipoprotein as well as electrolytes (p<0.05); however, high density lipoprotein was significantly increased (p<0.05).

Conclusion: Spondias mombin showed better hypolipidemic effects compared to Curcuma longa. However, results show that combined treatment with both extracts had better hypolipidemic effects than administration of individual extracts. Further research is recommended to evaluate the possible mechanism of action of these extracts.

Keywords: Spondias mombin; Curcuma longa; Alloxan; diabetes mellitus; glibenclamide.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder constituting a major public health concern the world over: with an increasing worldwide prevalence over the past few decades [1,2,3]. Epidemiological studies have shown that diabetes mellitus is one of the most important risk factors for cardiovascular diseases [4,5]; currently, more than 150 million people worldwide are afflicted with diabetes and it is expected that the number will increase to 366 million by 2030 [5]. Diabetes posses an unprecedented economic burden on third world countries presently estimated at approximately USD 372 billion [6]. Associated abnormalities in serum lipid and electrolytes profile is a common complication of diabetes [6]. Although insulin is the major medication used to remedy diabetes, there is an urgent need to find new agents with minimal side effects [7,8,9,10]. In traditional medicine, herbs, due to their ease of access and fewer side effects have been the main stay for several diseases including diabetes [7]. In recorded history, medicinal plants have been in use for the treatment of man and animal diseases [9,10]. A plant becomes a medicinal herb due to its antioxidant and anti-inflammatory properties and has received interest from both the medical/scientific world and from culinary enthusiasts, as it is the source of the polyphenol curcumin [16,17]. Curcuma longa (turmeric) on the other hand, is a rhizomatous herbaceous perennial plant of the ginger family; It has been traditionally used in Asian countries as a medical herb due to its antioxidant and anti-inflammatory potentials [18]. It aids in the management of metabolic syndrome, arthritis, anxiety, and hyperlipidemia [19,20,21]. It may also help in the
management of exercise-induced inflammation and muscle soreness, thus enhancing recovery and performance in active people [22]. Reports on the use of these plants either individually or in combination for the possible treatment of diabetes have become common in our environment. These reports have been fairly validated by several authors [23,24,25,26] for Spondias mombin, Curcuma longa and Capparis ovate desf. Var. palaestina zoh respectively. The aim of the present study is therefore, to compare the anti-diabetic potential of methanolic extracts of Spondias mombin leaves and Curcuma longa rhizomes on some lipid parameters and serum electrolytes in alloxan induced diabetes using male wistar rats as models.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extract Preparation

Fresh leaves of Spondias mombin were obtained from the University of Port Harcourt botanical garden; while C. longa rhizomes were obtained from a local market in Rivers State, Nigeria. Both specimens were identified and authenticated by Dr. C. Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria. Voucher specimens of both plants were deposited in the herbarium. The research protocol for the study was approved by the Ethics Committee of our institution vides a communication referred: UPH/R&D/REC/04 and dated 3rd July, 2018. The study was conducted in accordance with the guidelines for the care and use of Laboratory animals issued by the United States Institute for laboratory and Animal Research [27].

The leaves of Spondias mombin were dried at room temperature for a minimum of 14 days and extracted using the percolation method as described by Saronee et al. [1]. Briefly, the Spondias mombin leaves were ground into powder; 8.8 kg was properly macerated with 98% methanol for three days and the percentage yield was 93%. It was then filtered and concentrated using rotary evaporator at 40°C. The obtained extract was kept in air tight containers and stored at room temperature before use. Rhizomes of C. longa were oven dried at 40°C for 72 hours to a constant weight. The dried rhizomes were then pulverized using a household blender. 7.5 g of the powder obtained was dissolved in 98% methanol for about two days and the percentage yield was 96%. The extraction procedure employed was percolation as described by Saronee et al. [1].

2.2 Experimental Animals and Drugs

90 male wistar rats weighing between 120–250 g were used for this study. The animals were kept at the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The rats were fed with normal rat pellet and tap water ad libitum. The experimental animals were acclimatized for a period of two weeks after which they were properly grouped. Alloxan monohydrate was obtained from Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, USA. While glibenclamide was obtained from Swiss Pharm Nigeria Ltd, 5, Dopemu Road, Agege, Lagos, Nigeria.

2.3 Acute Toxicity Study (LD_{50})

The LD_{50} value of the leaf extract of Spondias mombin was assumed to be greater than 1000 mg/kg-bw as reported by Olatunde et al. [24]. Similarly, the LD_{50} value of the methanol extract of Curcuma longa rhizomes was regarded as the value obtained by Yuandani and Edy (2016), which was regarded to be greater than 5000 mg/kg-bw.

2.4 Experimental Design

The rats were subsequently randomly divided into 9 groups of 10 each. Diabetes was induced in all rat groups except Group 1 using alloxan at a dose of 200 mg/kg-bw administered intraperitonially. Diabetes was confirmed after 72 hours of alloxan administration if the blood glucose is ≥ 11.1 mmol/L (200 mg/dl) [27,1]. Each rat group was subsequently treated as follows:

**Group 1:** Untreated non diabetic; rats in this group had free access to only normal rat chow and tap water ad libitum.

**Group 2:** Untreated diabetic; rats in this group received no further treatment after induction of diabetes.

**Group 3:** Diabetic + low dose Spondias mombin; rats in this group were treated with 200 mg/kg-bw of Spodias mombin extract daily after the induction of diabetes.

**Group 4:** Diabetic + high dose Spondias mombin; rats in this group were treated with 400 mg/kg-bw of
Spodias mombin extract daily after the induction of diabetes.

**Group 5:** Diabetic + low dose Curcuma longa; rats in this group were treated with 500 mg/kg-bw Curcuma longa extract daily after the induction of diabetes.

**Group 6:** Diabetic + high dose Curcuma longa; rats in this group were treated with 1000 mg/kg-bw Curcuma longa extract daily after the induction of diabetes.

**Group 7:** Diabetic + combined low doses of Spodias mombin and Curcuma longa; rats in this group were treated with both 200 mg/kg-bw of Spodias mombin and 500 mg/kg-bw of Curcuma longa extracts daily after the induction of diabetes.

**Group 8:** Diabetic + combined high doses of Spodias mombin and Curcuma longa; rats in this group were treated with both 400 mg/kg-bw of Spodias mombin and 1000 mg/kg-bw of Curcuma longa extracts daily after the induction of diabetes.

**Group 9:** Diabetic + glibenclamide; rats in this group were treated with 0.6 mg/kg-bw of glibenclamide daily after the induction of diabetes.

The glibenclamide and the extracts of Spodias mombin and Curcuma longa were administered daily using an oral cannula for 42 days. On day 43, blood was collected by direct cardiac puncture for determination of lipid profile and serum electrolyte concentrations.

### 2.5 Determination of Some Serum Lipid Parameters and Electrolytes

Serum lipid and electrolytes were determined using Randox kits of Randox Biosciences, Randox Korea Ltd. 415, Heungan-daero, Dongan-gu, Anyang-si, Gyeonggi-do, Republic of Korea. Total cholesterol was estimated according to the method described by Stein [28], serum triglycerides were determined using the method described by Chawla [29]. The low and high density lipoprotein (LDL/HDL) was evaluated according to the method described by Mire and Snow (1986). Serum electrolytes were determined using the method described by Shahid et al. [30].

### 2.6 Statistical Analysis

Results are presented in Tables 1 and 2 and are expressed as mean ± standard error of mean. Significance differences were determined using one way ANOVA. A p value of less than 0.05 was considered statistically significant.

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**Table 1. Effect of methanolic extract of Spodias mombin leaves and Curcuma longa rhizomes on serum lipid parameters of male wistar rats**

| Groups                              | Total cholesterol (Mmol/l) | Triglyceride (Mmol/l) | High density lipoprotein (Mmol/l) | Low density lipoprotein (Mmol/l) |
|-------------------------------------|-----------------------------|-----------------------|-----------------------------------|----------------------------------|
| Group 1: Untreated non diabetic     | 2.68±0.01                   | 0.62±0.007            | 0.76±0.07                         | 1.63±0.007                      |
| Group 2: Untreated diabetic         | 3.33±0.007b                 | 0.98±0.007b           | 0.41±0.07b                        | 2.47±0.07b                      |
| Group 3: Diabetes + low dose Spondias mombin | 3.28±0.007ab               | 0.91±0.007ab          | 0.45±0.005ab                      | 2.42±0.007ab                     |
| Group 4: Diabetes + high dose Spondias mombin | 3.12±0.007a                | 0.80±0.007a           | 0.52±0.007a                       | 2.24±0.007a                      |
| Group 5: Diabetes + low dose Curcuma longa | 3.30±0.007b                | 0.93±0.007ab          | 0.47±0.007a                       | 2.41±0.007ab                     |
| Group 6: Diabetes + high dose Curcuma longa | 3.16±0.007ab               | 0.83±0.007a           | 0.54±0.007ab                      | 2.24±0.007a                      |
| Group 7: Diabetes + Combined low doses of Spodias mombin and Curcuma longa | 2.89±0.007ab               | 0.69±0.005ab          | 0.63±0.007ab                      | 1.95±0.007ab                     |
| Group 8: Diabetes + Combined high doses of Spodias mombin and Curcuma longa | 2.70±0.007ab               | 0.52±0.007ab          | 0.72±0.007ab                      | 1.74±0.007ab                     |
| Group 9: Diabetes + Glibenclamide   | 3.10±0.007a                 | 0.81±0.007a           | 0.50±0.007a                       | 2.23±0.007a                      |

a significant difference compared to Group 2 (untreated diabetic) (p<0.05)

b significant difference compared to Group 9 (Diabetes + glibenclamide) (p<0.05)
3. RESULTS

3.1 Effects of Methanolic Extract of *Spondias mombin* Leaves and *Curcuma longa* Rhizomes on Some Serum Lipid Parameters

Table 1 shows the effect of methanolic extract of *Spondias mombin* leaves and *Curcuma longa* rhizomes on some serum lipid parameters of male wistar rats. Compared to Group 2 (untreated diabetic) rats, administration of extracts of *Spondias mombin* to Groups 3 and 4 rats significantly reduced total cholesterol, triglyceride and low density lipoprotein concentrations, but increased high density lipoprotein concentrations (p< 0.05); suggesting a possible hypolipidemic potential. This hypolipidemic effect of *Spondias mombin* is comparable to that of glibenclamide observed amongst Group 9 rats. Furthermore, a comparison of the reduction of some serum lipid parameters particularly; total cholesterol, triglyceride and low density lipoprotein but increased high density lipoprotein at both low and high doses suggests that *Spondias mombin* is perhaps of higher potency compared to *Curcuma longa*; however, difference was not significant. Comparatively, *Curcuma longa* extract at both low and high doses also caused a significant and dose dependent reduction in these serum lipid parameters amongst Groups 5 and 6 rats respectively compared to Group 2 (untreated diabetic) rats (p< 0.05). This effect of *Curcuma longa* is similarly comparable to the effect of glibenclamide administration amongst Group 9 rats. The combined administration of both extracts amongst Groups 7 and 8 rats, also caused a significant and dose dependent reduction in total cholesterol, triglyceride, low density lipoprotein but increased high density lipoprotein compared to Group 2 (untreated diabetic) and Group 9 (glibenclamide treated) rats (p< 0.05). The reduction following combined administration of both extracts is greater than the reduction observed following the single administration of either extract: suggesting a possible synergistic effect of both extracts on serum lipid parameters.

Table 2. Effect of methanolic extract of *Spondias mombin* leaves and *Curcuma longa* rhizomes on electrolytes of male wistar rats

| Groups                                      | Sodium (Mmol/l) | Potassium (Mmol/l) | Magnesium (Mmol/l) | Phosphate (Mmol/l) | Chloride (Mmol/l) | Bicarbonate (Mmol/l) |
|--------------------------------------------|-----------------|--------------------|--------------------|-------------------|-------------------|---------------------|
| Group 1: Untreated non diabetic            | 143.16±3.59     | 12.06±0.27         | 2.07±0.01          | 5.24±0.17         | 34.15±0.18        | 24.20±0.19          |
| Group 2: Untreated Diabetic                | 154.88±1.73     | 14.33±0.15         | 2.40±0.20          | 10.76±0.17        | 62.33±0.15        | 29.08±0.02          |
| Group 3: Diabetes + low dose *Spondias mombin* | 151.54±0.17    | 12.52±0.14         | 2.32±0.15          | 10.86±0.03        | 58.43±0.16        | 27.29±0.17          |
| Group 4: Diabetes + high dose *Spondias mombin* | 147.46±0.21     | 11.46±0.14         | 2.24±0.15          | 8.45±0.14         | 49.28±0.24        | 26.17±0.35          |
| Group 5: Diabetes + low dose *Curcuma longa* | 152.42±0.30     | 12.87±0.03         | 2.30±0.09          | 11.05±0.01        | 58.82±0.03        | 27.71±0.05          |
| Group 6: Diabetes + high dose *Curcuma longa* | 149.22±0.13     | 11.70±0.11         | 2.21±0.04          | 6.96±0.01         | 50.37±0.02        | 26.09±0.005         |
| Group 7: Diabetes + Combined low doses of *Spondias mombin* and *Curcuma longa* | 140.15±0.01 | 11.01±0.02         | 2.13±0.03          | 6.05±0.01         | 40.15±0.04        | 25.05±0.02          |
| Group 8: Diabetes + Combined high doses of *Spondias mombin* and *Curcuma longa* | 130.27±0.04 | 10.05±0.01         | 2.02±0.008         | 5.47±0.06         | 33.74±0.14        | 21.06±0.01          |
| Group 9: Diabetes + Glibenclamide          | 145.07±0.02     | 11.34±0.03         | 2.26±0.09          | 7.96±0.02         | 48.84±0.01        | 25.46±0.01          |

*a* significant difference compared to Group 2 (untreated diabetic) (p<0.05); *b* significant difference compared to Group 9 (Diabetes + glibenclamide) (p<0.05)
3.2 Effects of Methanolic Extract of *Spondias mombin* Leaves and *Curcuma longa* Rhizomes on Serum Electrolytes

Table 2 shows the effect of methanolic extract of *Spondias mombin* leaves and *Curcuma longa* rhizomes on serum electrolytes in male wistar rats. Compared to Group 2 (untreated diabetic) rats, administration of *Spondias mombin* extract at low and high doses significantly reduced serum electrolytes in a dose dependent manner amongst Groups 3 and 4 rats (p< 0.05). This is similar to the effect of administration of glibenclamide at 0.6 mg/kg-bw seen in Group 9 rats (p< 0.05). Similarly, administration of *Curcuma longa* extract caused a significant and dose dependent reduction in serum electrolytes amongst Groups 5 and 6 rats compared to Group 2 (untreated diabetic) and Group 9 (glibenclamide treated) rats (p< 0.05). This effect of *Curcuma longa* is comparable to that of glibenclamide observed in Group 9 rats. The combined administration of both extracts also caused a significant reduction in serum electrolytes amongst Groups 7 and 8 rats compared to all other rat Groups (p< 0.05).

4. DISCUSSION

The present study is a comparative evaluation of the effects of methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes on some serum lipid parameters and electrolytes in alloxan induced diabetic male wistar rats. Elevated serum insulin increases hormone sensitive lipase and lipoprotein lipase activity in adipose tissue thereby promoting fuel storage in the form of triglycerides in normal metabolism [23,1]. The deficiency of insulin increases blood glucose concentration. The increased glucose concentration causes an osmotic diuresis of electrolytes depleting the activity of lipoprotein lipase and hormone sensitive lipase, leading to a deranged lipoprotein metabolism characteristic of diabetes [24,1]. As compared to Group 1 (untreated non-diabetic) rats, untreated diabetic rats showed significant increased values of all serum lipid indices assessed except high density lipoprotein which was reduced. The significant reduction in total cholesterol, triglyceride and low density lipoprotein with increased high density lipoprotein concentrations seen as a result of the administration of both extracts, suggests that these extracts possess a possible hypolipidemic potential: indicating a potential beneficial effect in alloxan induced diabetes. Furthermore, the effects of both extracts on serum lipid and electrolytes in alloxan induced diabetic wistar rats are comparable to the effect of glibenclamide a known anti-diabetic agent. Noteworthy, the hypolipidemic effects of the combined extracts is greater than the effect of single administration of either extract: suggesting a likely synergism of effect. By comparison, the total cholesterol, triglyceride and low density lipoprotein of rats administered *Spondias mombin* leaves was marginally lower than those administered *Curcuma longa* rhizomes at both doses. This perhaps suggests a better hypolipidemic effect of *Spondias mombin* compared to *Curcuma longa*.

Phytochemical examination of *Spondias mombin* and *Curcuma longa* has revealed an array of compounds comprising: flavonoids, tannins, curcumin, triterpenoid, glycosides and sterols compounds [31,32,33,34,35]. These compounds have demonstrated therapeutic potential in ameliorating the possible complications associated with diabetes [36]. These compounds are believed to act through: stimulation of insulin from remnant beta cells, [36,37] enhancement of glucose transport to marginal tissues, [37,38] and inhibition of gastrointestinal absorption of glucose [39,40,41].

As compared to Group 2 (untreated diabetic) rats, all treated groups showed significant reduction in serum concentration of sodium, potassium, phosphate, chloride and bicarbonate except magnesium; suggesting that *Spondias mombin* leaves and *Curcuma longa* rhizomes extracts could individually ameliorate complications associated with diabetes. Rats administered *Spondias mombin* leaves showed lower serum electrolytes than those administered *Curcuma longa* rhizomes suggesting a greater effect of *Spondias mombin* compared to *Curcuma longa*. Importantly, the effect of the combined administration of both extracts on serum electrolytes is greater than the effect of single administration of either extract further suggesting a synergism of effect.

5. CONCLUSION

In conclusion, the present study reports that methanolic extracts of *Spondias mombin* leaves and *Curcuma longa* rhizomes administered either singly and in combination elicits a concomitant dose dependent reduction in electrolytes, total cholesterol, triglyceride, low density lipoprotein but increased high density lipoprotein in alloxan
induced diabetes in male wistar rats. *Spondias mombin* showed better hypolipidemic effects compared to *Curcuma longa*. However, results show that combined treatment with both extracts had better hypolipidemic effects than administration of individual extracts. The obtained results suggest that extracts of *Spondias mombin* leaves possess greater hypolipidemic potentials compared to *Curcuma longa* rhizomes and the effects of combined administration is greater than the individual administration of either extract. These effects are comparable to that of glibenclamide a popular anti-diabetic agent. Our results conform to recent and current status of oral medications and future perspectives in diabetes mellitus management [42]. It also validates the use of these plants extracts in traditional folklore medicine in the treatment of diabetes and previous reports of the anti-diabetic potentials of *Spondias mombin* leaves and *Curcuma longa* rhizomes in animal models [23,24] We recommend further studies on the possible mechanism of action of these extracts.

**ETHICAL APPROVAL**

The research protocol for the study was approved by the Ethics Committee of our institution vide a communication referred: UPH/R&D/REC/04 and dated 3rd July, 2018. The study was conducted in accordance with the guidelines for the care and use of Laboratory animals issued by the United States Institute for laboratory and Animal Research [27].

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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