

Effects of *Jatropha curcas* root extracts on the metabolic profile of Mature Female rats

Ale Ayotunde Oladunni ^{1*}  , Odesanmi Omolola Selina ² , and Magbagbeola Olubunmi Abiola ² 

¹ Department of Medicine, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria

² Department of Biochemistry, College of Medicine, University of Lagos, Lagos State, Nigeria

* Author to whom correspondence should be addressed

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Abstract: Extracts from *Jatropha curcas*, a tropical shrub, serve as multi-purpose treatments in folk medicine. Previous studies have shown conflicting reports of the metabolic effects of the extract of this plant. This study assessed the effects of *Jatropha curcas* root extracts on metabolic parameters of female Wistar rats, including body weight, glycemic index, and lipid profile. The root extracts of *Jatropha curcas* in water and 80.0% ethanol were prepared using the Soxhlet extraction method. Four groups of mature rats (groups A-D) were administered varying doses of water or ethanol extracts once daily for 15 days. Another group (group E) served as a control and received no extract. After stopping extract administration, the rats were fasted for 18 hours and sacrificed. A venous blood sample was collected from each group for analysis of lipid and glycemic profiles using spectrophotometry. Statistical analysis was performed and the value with $P < 0.05$ was regarded as significant. No significant difference was observed in the overall body weights of the rats of any group before and after the treatment. Furthermore, the rats that were administered with the root extracts (either water or ethanol) exhibited higher levels of fasting blood glucose and serum lipids compared to the rats in the control group. In conclusion, the root extracts of *Jatropha curcas* predispose the rats to hyperlipidemia action and hyperglycemia action.

Introduction

Jatropha curcas (Greek: iatros - doctor, trophe - food, *J. curcas*) also known as Barbados nut, purging nut, physic nut, Ratanjot, Kukui haole, and Purgeer boontjie, is an annual shrub that can grow up to 5 m in highland, has an annual yield of 5-ton seeds per hectare [1]. This plant is hardy and can grow in a wide temperature range. *J. curcas* can remain economical for up to 30 years. It can grow in drought-prone areas and in areas with poor soil conditions. It is highly susceptible to frost [2, 3]. Owing to its tolerance to such a wide range of climatic and soil conditions, this plant is widely distributed within the “*Jatropha* belt”, which stretches from 30° N to 35° S [4]. It is an ornamental, medicinal, and multi-purpose plant that originally belonged to Central and South American countries [5]. From there, it has been documented, that this plant was brought to other tropical and subtropical countries, such as Asia and Africa by the Portuguese seafarers [6]. Taxonomically, *J. curcas* belongs to the kingdom *Plantae*, order *Malpighiales*, family *Euphorbiaceae* [6, 7]. The *Jatropha* genus comprises approximately 175 succulent plants. *J. curcas* generally contains five roots, one central, and four peripherals [8].

Medicinal plants are an important component of traditional and modern medicine [5]. Since ancient times, *J. curcas* has been used as a medicinal plant for the cure of several ailments [9, 10]. All the parts of this plant have been used in this regard [11]. In Nigeria, its fruit has been used to treat diabetes mellitus [12]. The aqueous seed extracts of this plant have been used as abortifacient [13]. The seeds of this plant have been used to make candles, soaps, lubricants, dyes, and detergents. Its seed oil is widely used as biodiesel and bark is used as a fish poison [14]. The leaf extract of this plant has been reported to exhibit beneficial cardiovascular effects [15]. Its latex is used in the management of gonorrhoea, skin ulcer, and ringworm [16]. The aqueous leaf extract of *J. curcas* has been shown to exhibit an anti-helminthic effect against *Pheritimaposthuma* [17]. In developing countries, *J. curcas* root extracts are used for the treatment of different ailments. In females, it is widely used as a contraceptive and abortifacient while studies on oral synthetic contraceptives reported associated adverse effects with its use. Due to safety concerns, this research focused on the different aspects of the oral administration of root extracts which included metabolic effects. Thus, the result showed the hyperglycaemic and hyperlipidaemic properties of this plant. Here, we assessed and compared the effects of aqueous and ethanolic root extracts of *J. curcas* on some metabolic parameters of rats.

Materials and methods

Root extracts of *J. curcas*: Dry roots of *J. curcas* were collected from different traditional Nigerian herbalists and identified at the herbarium of the Department of Botany and Microbiology of the University of Lagos, Lagos, Nigeria. The roots were further dried at 60 °C in a hot air oven for 24 hrs. The dried roots were grounded to a powder using the grinding machine at the Department of Pharmacology, College of Medicine, University of Lagos. The aqueous extract (JCW) and ethanolic extract (JCE) of the dried roots were prepared by dissolving 40 g of the powder in 250 mL of either aqueous or 80.0% ethanol solution in a Soxhlet apparatus for 72 hrs. The extract was then dried using a rotary evaporator at 45 °C and stored in a refrigerator at 4~8 °C until further use.

Treatment of rats: Thirty-five female Wistar rats (about PND 75-90 days) were acquired from the University of Ibadan, Ibadan, Oyo, Nigeria. The rats were kept separately and cleaned regularly under abundant light and ventilation and allowed to acclimatize for 15 days. The animals were fed with rat chow and water *ad libitum*. Rats were then divided into five groups containing seven rats each (groups A-E). All the rats were weighed on the first day of the experiment and then at 3-day intervals until the end of the experiment. The rats in groups A, B, and C received JCW at the concentrations of 5, 10, and 15 mg/200 mg bodyweight of the rats. The rats in group D received JCE at the concentration of 10 mg/200 mg. The rats in group E did not receive either JCW or JCE (control group, n=7). All the extracts were administered orally via stomach tubes. On the 15th day of the experiment, they were fasted for 18 hrs. After that, they were anesthetized using an intraperitoneal injection of 25.0% urethane chloralose. The venous blood samples were collected in fluoride oxalate bottles for fasting glucose analysis and in plain sample bottles for other biochemical analyses. The serum was isolated by centrifuging the samples at 3000 rpm for 20 min. The serum samples for other biochemical analyses were stored at 4°C, while the samples for fasting glucose analysis were examined immediately. This study was approved by the Department of Biochemistry, University of Lagos, Lagos, Nigeria.

Determination of glucose levels and lipid profiling: The serum glucose levels of rats were evaluated using the glucose oxidase method, as described previously [18] and their serum lipid profiling: Total cholesterol (TC) level was assessed using a modified Liebermann-Burchard method [19]. Triglyceride (TG) level was estimated via the enzymatic hydrolysis method [20]. High-density lipoprotein cholesterol (HDL-C) level was estimated using the precipitation method [21], and low-density lipoprotein cholesterol (LDL-C) was measured using Friedwald's equation [22].

Statistical analysis: All the data was presented as mean±SD. One-way ANOVA was used for comparison among more than two groups because the distribution was normal (parametric test). Student *t*-test was used to compare between the two groups. Values with $P < 0.05$ were considered as statistically significant.

Results

Effect of *J. curcas* root extract on the body parameters: As shown in **Tables 1, 2, and 3**, we did not observe any significant change in the overall weight and organ-specific weights of the mature female rats belonging to the five groups (all $P > 0.05$).

Table 1: Effect of different doses of *J. curcas* extracts on the average rat body weight

Group	Dosage/200 g body weight	Day					
		0	3	6	9	12	15
A	5 mg Aqueous extract	130.0±07.0	127.5±06.5	140.0±08.2	146.8±30.0	148.7±10.4	153.0±11.2
B	10 mg Aqueous extract	131.0±16.0	123.8±13.8	132.5±17.6	136.8±18.0	139.5±18.2	143.3±18.3
C	15 mg Aqueous extract	130.0±15.8	131.3±13.1	133.8±14.9	138.0±23.7	147.8±22.6	152.8±21.7
D	10 mg Ethanolic extract	135.0±05.0	145.0±07.6	141.7±07.6	135.7±17.8	157.5±2.91	148.3±20.8
E	0.0	126.0±06.9	141.7±115.0	122.3±07.5	122.0±09.3	131.7±10.4	133.3±12.6
		P=0.86	P=0.34	P=0.34	P=0.44	P=0.62	P=0.54

One-way ANOVA for comparison among the groups, and overall p-value was calculated to find the significance of the difference between the means of the groups

Table 2: Effect of varied doses of *J. curcas* extract on the average weight gain in rats

Group	Dosage/200 g bodyweight of rats	Mean weight gain (g)
A	5 mg (Aqueous extract)	23.00±5.72
B	10 mg (Aqueous extract)	12.00±2.45
C	15 mg (Aqueous extract)	22.75±8.06
D	10 mg (Ethanolic extract)	16.33±15.01
E	0.0	07.33±6.81

One-way ANOVA for comparison among the groups, and overall p-value was calculated to find the significance of the difference between the means of the groups

Table 3: Effect of varied doses of *Jatropha curcas* extract on the organs of mature female rats

Group	Dosage/200 g body weight of rats	Liver weight (g)	Kidney weight (g)	Pancreas weight (g)	Heart weight (g)
A	5 mg Aqueous extract	3.59±0.26	0.68±0.25	0.89±0.27	0.45±0.01
B	10 mg Aqueous extract	3.84±0.89	0.48±0.06	0.92±0.19	0.46±0.01
C	15 mg Aqueous extract	4.41±0.65	0.59±0.08	0.86±0.14	0.48±0.07
D	10 mg Ethanolic extract	3.74±1.02	0.40±0.07	0.76±0.03	0.46±0.07
E	0.0	4.02±0.61	0.52±0.07	0.79±0.06	0.48±0.03
		P=0.44	P=0.05	P=0.69	P=0.57

One-way ANOVA for comparison among the groups, and overall p-value was calculated to find the significance of the difference of the means among the groups

Effect of *J. curcas* root extract on lipid profiles: We examined the HDL-C, LDL-C, TG, and TC levels in all the groups. The results showed that the rats in the control group exhibited significantly lower serum levels of these lipids compared to the rats administered with *J. curcas* extracts (all $P < 0.01$). Among the groups treated with JCW (groups A, B & C), the rats in group C exhibited the highest LDL-C and TC levels ($P < 0.05$). Furthermore, the LDL-C and TC levels in the serum of the rats of groups A and B were comparable ($P = 0.41$ and 0.75 , respectively). In addition, the serum HDL-C levels were comparable between the rats of groups A and B ($P = 1.0$) and significantly higher compared to group C rats ($P < 0.05$ each, **Table 4**). Moreover, among group A-D, the rats in group D exhibited the lowest levels of TG, TC, and HDL-C (all $P < 0.05$) (**Table 5**).

Table 4: Effect of *Jatropha curcas* extracts on the serum lipid profile of mature female rat

Profile	A	B	C	D	E	Overall p value	A vs B	A vs C	B vs C
Total-C (mmol/L)	2.80±0.04	2.82±0.02	3.51±0.01	1.23±0.01	0.95±0.05	0.01	0.41	< 0.0001	< 0.0001
HDL-C (mmol/L)	1.42±0.02	1.42±0.01	1.33±0.02	0.44±0.03	0.31±0.01	0.01	1.00	< 0.001	< 0.001
LDL-C (mmol/L)	0.97±0.12	0.95±0.01	1.80±0.05	1.21±0.02	0.26±0.03	0.01	0.75	< 0.0001	< 0.0001
TG (mmol/L)	2.80±0.06	2.12±1.10	2.05±0.08	1.05±0.13	0.61±0.01	0.01	0.26	< 0.0001	0.90

One-way ANOVA for comparison among the groups. Student's *t*-test for comparison between two groups. Overall p-value was calculated to find the significance of the difference of the means among the groups

Table 5: Effect of *J. curcas* extracts on the serum lipid profile of mature female rat

Profile	A	B	C	D	Overall p value	A vs D	B vs D	C vs D
Total-C (mmol/L)	2.80±0.04	2.82±0.02	3.51±0.01	1.23±0.01	< 0.0001	< 0.0001	< 0.0001	< 0.0001
HDL-C (mmol/L)	1.42±0.02	1.42±0.01	1.33±0.02	0.44±0.03	< 0.0001	< 0.0001	< 0.0001	< 0.0001
LDL-C (mmol/L)	0.97±0.12	0.95±0.01	1.80±0.05	1.21±0.02	< 0.0001	< 0.0001	< 0.0001	< 0.0001
TG (mmol/L)	2.80±0.06	2.12±1.10	2.05±0.08	1.05±0.13	< 0.0001	< 0.0001	< 0.0001	< 0.0001

One-way ANOVA for comparison among three or more groups. Student's *t*-test was used for comparison between two groups. The overall p-value was calculated to find the significance of the difference in the means among the groups

Effect of *J. curcas* root extract on fasting glucose levels: As shown in **Table 6**, the fasting sugar levels were comparable among the rats of the treated groups (groups A-D). However, the fasting glucose levels in rats treated with the root extract (regardless of JCW or JCE) were significantly higher compared to the control group rats (all are $P < 0.05$).

Table 6: Effects of oral administration of varied doses of *J. curcas* extracts on the fasting blood glucose level

Group	Dosage/200 g body weight of rats (mg)	Fasting blood glucose (mmol/l)
A	5 (Aqueous extract)	3.43±0.08
B	10 (Aqueous extract)	3.78±0.06
C	15 (Aqueous extract)	3.78±0.02
D	10 (Ethanolic extract)	3.83±0.47
E	0.0	1.37±0.29
		P = 0.0004

One-way ANOVA for comparison among three or more groups and overall p-value was calculated to find the significance of the difference in means among the groups

Discussion

Although all the parts of *J. curcas* are used, in this study, we focused on the root extracts of this plant. Previous studies have reported that *J. curcas* can be used to treat several kinds of ailments, including jaundice, cough, malaria, paralysis, neuralgia, scabies, dermatitis, stomachache, rheumatism, snake bites, toothache, sores, and ringworms [23-25]. Different parts of this plant have been shown to exhibit anti-oxidant, anti-inflammatory, anti-cancer, coagulative, anti-diarrheal, anti-microbial, and anti-leukemic effects [9, 23, 26, 27]. It has been explored as an abortifacient, laxative, anodyne, depurative, vulnerary and styptic agent [28, 29]. Apart from medicinal values, this plant is also an excellent source of biodiesel fuel [30]. The seed oil serves as a fungicide, insecticide, and pesticide [9, 31]. Its bark and sap are used for manufacturing fish poisons and histological stains [32, 33]. Overall, the findings indicated that administration of the root extracts did not change the mean overall weights of the rats significantly. However, we did observe a mild increase in the overall weights of the rats that were treated with the extracts compared to the rats of the control group. Many researchers have reported dose-dependent histological changes in the lungs, heart, spleen, liver, etc., following the administration of *J. curcas* extracts [34-37]. We assessed the changes in the weights of the liver, heart, kidney, and pancreas after the administration of the root extracts in the rats. However, we did not observe any significant differences between the weights of these organs compared to the control group.

Administration of the root extract of *J. curcas* led to significantly elevated TC, TG, HDL-C and LDL-C levels. These findings were similar to those of Johnson *et al.* [38] who reported an increase in the levels of TC. It has previously been reported that *J. curcas* extracts play a significant role in insulin inhibition. Such insulin inhibition might lead to stimulation of lipolytic hormones on fat depots, which, in turn, might lead to elevation in TC [39-40]. It has also been reported that an increase in the TC levels is primarily responsible for death in the birds treated with *J. curcas* extract [38]. Various studies have shown that *J. curcas* extracts significantly increased HDL-C levels [41-43]. However, in contrast, some studies have demonstrated the hypolipidemic effects of *J. curcas* extracts. Due to its hypolipidemic effect, *J. curcas* was proposed to be effective in diabetes mellitus management [5]. We also observed that the fasting glucose levels of the rats that were administered with the root extracts were significantly higher than in the control group. Whether the extract was aqueous or ethanolic, it did not affect the increase in the glucose levels, since the rats in group A-D exhibited comparable levels of glucose. Although we observed such hyperglycemic action of *J. curcas*, the previous study demonstrated the hypoglycemic effects of this plant [15]. However, these agents are administered orally, they often encounter several physiological processes that often reduce the amount of the agent that reaches the circulatory system of the host; a lower amount leads to a lower level of activity [44]. Compared to other studies, the dosage used was low. With such doses, we observed a mild increase in the weight of the rats treated with the root extracts. Larger doses might have led to significant changes in the overall weight. *J. curcas* may serve society as a medicinal plant, food source, and protein supplement for animals. However, its extracts have previously been reported to be toxic after consumption and not fit for human use.

Conclusion: Root extracts of *Jatropha curcas* induce hyperlipidemic and hyperglycemic effects in rats.

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