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Detection of Hypoglycemic and Antidiabetic Fraction in Ethanol Extract of *Jatropha curcas* Aerial Parts

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Abstract

Diabetes is a global problem and many efforts are being done all over the world searching for new drugs that can manage it more efficiently. The World Health Organization recommended the natural products to be possible source for antidiabetic compounds. *Jatropha curcas* plant was employed in Indian traditional medicine for the treatment of several disorders. So, hexane, chloroform and ethanolic extracts of the aerial part were subjected for evaluation of their hypoglycemic and antidiabetic effects. Oral administration of ethanolic extract at a dose of 400 mg/kg showed hypoglycemic and antidiabetic activities in alloxan induced diabetic mice. Bioassay-guided fractionation used to isolate the active fraction. Moreover, the drug was found to be safe up to dose of 5 g/kg. Further fractionation is recommended to find the active compound(s).

Keywords

Herbal Medicine, *Jatropha curcas*, Hypoglycemic Effect, Antidiabetic Effect, Active Fraction

1. Introduction

Diabetes mellitus is a group of metabolic disorders that results from defects in insulin secretion, insulin action (sensitivity), or both with common clinical manifestation, hyperglycemia [1]. It is estimated that the number of diabetes patients will be 366 million in 2030 [2]. Unfortunately, diabetes is one of the most common diseases in Saudi Arabia [3]. Many efforts are being done all over the world to discover drugs that can manage diabetes

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more efficiently and there is an increase in current interest and demand for herbs. Different parts of the *Jatropha curcas* plant (family Euphorbiaceae) were traditionally used for the treatment of several disorders as anticancer, as an abortifacient, antiseptic, diuretic, purgative and haemostatic [4] [5]. The genus Jatropha contains approximately 170 known species. The genus name Jatropha derived from the Greek jatrós (doctor), trophé (food), which implies medicinal uses [6]. *Jatropha curcas* L. (physic nut or purging nut) is a drought resistant shrub or tree cultivated in Central and South America, South-East Asia, India and Africa [7]. Leaves are alternately arranged, 10 - 15 cm × 7.5 - 12.5 cm; they are broadly ovate, conate, acute usually palmately 3 or 5 lobed, glabrous; flowers in loose panicles of the cymes, yellowish green, fruits are 2.5 cm, long ovoid, black; seeds are ovoid, oblong, dull brownish black. The seeds are collected and exported for the extraction of oil. The seed resembles castor seed in shape but is smaller in size and dark brown in color [4]. Phytochemical analyses have shown that different parts of *Jatropha curcas* plant contain phenolic, flavonoid, saponin and alkaloid compounds [8]. In this study, evaluation of hypoglycemic and antidiabetic activities of *Jatropha curcas* plant will be studied. In addition, bioassay-guided fractionation will be done searching for the active fraction. **Figure 1** shows aerial parts of *Jatropha curcas*.

2. Materials and Methods

2.1. Plant Material

The plant was collected from Ain Al Hara faifa and identified by Dr. Mohammad Yusuf, taxonomist at the College of Pharmacy, King Saud University (KSU). Voucher specimen (voucher no. 15189) was deposited in the Herbarium of College of Pharmacy, KSU.

2.2. Extraction and Isolation

900 g of the aerial parts of the plant were grinded and extracted with hexane (1 L × 3), chloroform (1 L × 3), and finally with 96% ethanol (1 L × 3) at 25˚C for 72 hrs. The extracts obtained were 16.97 g, 14.72 g, 13.76 g respectively. Based on the bioassay result ethanol extract was treated with ethyl acetate giving two portions; ethyl acetate soluble (5.57 g, inactive) and the active ethyl acetate insoluble fractions (7.16 g). The ethyl acetate insoluble fraction was dissolved in methanol and methanol insoluble part was removed. The methanol soluble part was passed over silica gel through Buchner funnel and eluted with chloroform and methanol with increasing

Figure 1. Aerial parts of *Jatropha curcas.*

polarity giving six fractions. The active fraction (no. 4, 5.14 g) was further subjected to diion column giving three fractions; aqueous, methanol-water 1:1 and methanol weighing 3.49 g, 1.07 g and 177 mg respectively.

2.3. Animals

Male Swiss albino mice (20 - 25 g) of approximately the same age were procured from Experimental Animal Care Centre, College of Pharmacy, King Saud University, Riyadh. The conduct of experiments and the procedure of sacrifice (using ether) were approved by the Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.4. Hypoglycemic and Antidiabetic Screening

1) The dose given to each animal was 400 mg/kg weight.
2) 0.5 ml tween 80 was added to each extract or fraction.
3) The experiment was performed according to [9]. For hypoglycemic study, we prepared six groups (each composed of certain number of mice). One group was given normal saline, second was given tween 80 (solvent), the third was given standard hypoglycemic drug; daonil® (glibenclamide) at a dose of 1 mg/kg, the other group was given the examined extract or fraction. The fractions and drugs were given orally to animals after 24 hrs fasting. Blood samples were taken before giving the drug (zero time) and after 2 hrs after giving drugs and glucose blood level was measured using reflotron® instrument.
4) For antidiabetic screening, diabetes was induced by injecting alloxan intra peritoneal (150 mg/kg) in adult mice. After 72 hrs animals became diabetic and the experiment designed as mentioned in hypoglycemic experiment.

2.5. Toxicity Study of *Jatropha curcas*

A pilot study in which high dose of 5 gm/kg was given to six adult male mice orally and their mortality rate was observed for 24 hrs.

2.6. Statistical Analysis

Values are given as arithmetic means ± standard deviation of the mean (S.D.M.). Data was statistically analyzed by using the Student's t-test or ANOVA as appropriate. Significance with respect to control group [10].

3. Results

3.1. Hypoglycemic and Antidiabetic Activities

Blood samples were taken and readings of glucose level by reflotron® instrument for the three extracts of Jatropha curcas and results of the tests are summarized in **Table 1** & **Table 2**.

Based on the above results, the ethanol fraction was treated with ethyl acetate. The soluble and insoluble fractions were tested for the hypoglycemic and antidiabetic activities and the results are given in **Table 3** & **Table 4**.

Table 1. Hypoglycemic effect of plant extracts.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline	1 ml	104.30 ± 6.26	102.83 ± 2.45	
Vehicle (Tween-80)	1 ml	111.53 ± 6.69	110.00 ± 2.51	
Glibenclamide	1 ml	116.00 ± 3.60	4.13 ± 3.58***	53.33
Chloroform extract	400 mg/kg	102.16 ± 5.93	81.63 ± 2.05*	20.09
Ethanol extract	400 mg/kg	112.66 ± 3.48	70.36 ± 6.40***	37.54
Hexane extract	400 mg/kg	105.16 ± 5.16	110.00 ± 1.33	4.59

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 3.

Table 2. Antidiabetic effect of plant extracts.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline + Alloxan	1 ml + 150 mg/kg	301.33 ± 4.70	305.00 ± 7.57	
Vehicle (Tween-80) + Alloxan	1 ml + 150 mg/kg	305.33 ± 13.32	281.33 ± 27.88	
Glibenclamide + Alloxan	1 ml + 150 mg/kg	299.00 ± 9.86	143.66 ± 4.97***	51.95
Chloroform extract + Alloxan	400 mg/kg +150 mg/kg	285.66 ± 13.48	224.33 ± 22.98	21.47
Ethanol extract + Alloxan	400 mg/kg + 150 mg/kg	304.33 ± 11.72	170.66 ± 5.78***	43.92
Hexane extract + Alloxan	400 mg/kg + 150 mg/kg	329.00 ± 8.50	300.66 ± 9.76	8.61

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 3.

Table 3. Hypoglycemic effect of ethyl acetate sol. and insol. fraction.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline	1 ml	108.90 ± 4.47	105.90 ± 3.48	
Vehicle (Tween-80)	1ml	99.92 ± 4.49	101.25 ± 6.56	
Glibenclamide	1 ml	99.92 ± 4.19	49.52 ± 1.75***	50.43
Ethyl acetate Insoluble	400 mg/kg	110.75 ± 3.11	73.57 ± 3.91***	33.56
Ethyl acetate Soluble	400 mg/kg	108.75 ± 4.36	86.32 ± 5.41	20.62

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 4.

Table 4. Antidiabetic effect of ethyl acetate sol. and insol. fraction.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline + Alloxan	1 ml + 150 mg/kg	293.75 ± 11.61	308.25 ± 5.05	
Vehicle (Tween-80) + Alloxan	1 ml + 150 mg/kg	306.25 ± 9.25	291.50 ± 10.75	
Glibenclamide + Alloxan	1 ml + 150 mg/kg	302.00 ± 6.40	156.00 ± 7.86***	48.34
Ethyl acetate Insoluble + Alloxan	400 mg/kg + 150 mg/kg	317.25 ± 10.20	216.50 ± 8.84***	31.75
Ethyl acetate Soluble + Alloxan	400 mg/kg + 150 mg/kg	294.00 ± 17.21	230.75 ± 13.93*	21.51

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 4.

It is found that the ethyl acetate insoluble fraction showed the activities so it was subjected for further fractionation as mentioned and the fraction number four was expected to be the responsible for activity which was confirmed by the biological test results listed in **Table 5** & **Table 6**.

The fraction No. 4 was then subjected to diion column and the two major fractions were tested for the activities. It was found that the aqueous fraction is the responsible fraction for hypoglycemic and antidiabetic activities of the *Jatropha curcas* ethanol extract as reported in **Table 7** & **Table 8**.

3.2. Results of Toxicity Screening

No adverse effects and no death were observed even after 24 hrs of administration of the extract in very high dose 5 g/kg. The extract was found to be considered as safe up to dose of 5 g/kg.

Table 5. Hypoglycemic effect of fraction NO-4.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline	1 ml	112.00 ± 2.33	108.26	
Vehicle (Tween-80)	1 ml	102.46 ± 3.89	117.33 ± 2.90	
Glibenclamide	1 ml	103.35 ± 4.28	58.50 ± 2.57***	43.39
Fraction NO-4	400 mg/kg	114.50 ± 3.48	81.98 ± 4.34***	28.39

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 6.

Table 6. Antidiabetic effect of fraction NO-4.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline + Alloxan	1 ml + 150 mg/kg	309.33 ± 5.09	303.00 ± 12.03	
Vehicle (Tween-80) + Alloxan	1 ml + 150 mg/kg	298.83 ± 6.15	316.50 ± 7.97	
Glibenclamide + Alloxan	1 ml + 150 mg/kg	307.66 ± 7.18	156.66 ± 10.25***	49.40
Fraction NO-4 + Alloxan	400 mg/kg + 150 mg/kg	300.00 ± 8.88	212.00 ± 29.33***	29.33

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 6.

Table 7. Hypoglycemic effect of fractions from diion column.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline	1 ml	108.26 ± 2.54	110.50 ± 2.12	
Vehicle (Tween-80)	1 ml	109.16 ± 3.90	113.83 ± 3.78	
Glibenclamide	1 ml	117.50 ± 3.32	52.48 ± 3.46***	55.33
50% Methanol fraction	400 mg/kg	114.83 ± 9.45	81.55 ± 3.22***	28.72
Aqueous fraction	400 mg/kg	117.33 ± 3.75	67.31 ± 1.61***	42.62

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 6.

Table 8. Antidiabetic effect of fractions from diion column.

Treatment	Dose mg/kg orally	Glucose levels mg/dl		
		0 hour before treatments	2 hours after treatments	% Inhibition
Normal saline + Alloxan	1 ml + 150 mg/kg	292.50 ± 9.59	287.33 ± 10.27	
Vehicle (Tween-80) + Alloxan	1 ml + 150 mg/kg	295.50 ± 9.18	301.66 ± 9.30	
Glibenclamide + Alloxan	1 ml + 150 mg/kg	305.00 ± 8.40	153.00 ± 4.87***	49.83
50% Methanol fraction + Alloxan	400 mg/kg + 150 mg/kg	295.83 ± 7.81	209.16 ± 6.15***	29.29
Aqueous fraction+ Alloxan	400 mg/kg +150 mg/kg	284.33 ± 16.78	167.33 ± 2.59***	41.14

The values marked with asterisks differ significantly from the control group, (*P < 0.05), (***P < 0.001); Number of animals = 6.

4. Discussion

Reviewing the literature revealed that ethanol extract of *Jatropha curcas* aerial parts has hypoglycemic and antidiabetic activities at doses of 250 and 500 mg/kg [11] and nothing was reported concerning species growing in Saudi Arabia. A pilot toxicity study was done to test safety of the plant before doing the screening and it was found safe up to 5 g/kg. Successive extraction was done for aerial parts of the plant using n-hexane, chloroform

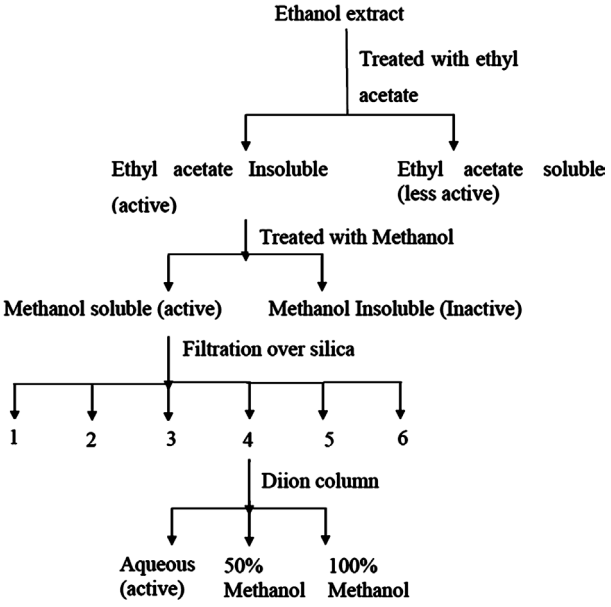

Figure 2. Scheme of bioassay-guided fractionation of the ethanol extract.

and ethanol. It is found that the ethanol extract was also active as that reported for ethanol extract of aerial parts of the plant growing in India and this encouraged us to do bioassay-guided fractionation to detect the fraction responsible for such activities. As mentioned, ethanol extract was portioned using solubility in ethyl acetate and the ethyl acetate insoluble fraction was further filtered over silica gel through Buchner funnel and the fraction number four from this step was passed over diion column and the aqueous fraction was the most active fraction with activity comparable to the standard used. The steps of fractionation was guided by results of the biology tests *i.e*. bioassay-guided fractionation was done. The active fraction was tested using thin layer chromatography and we suppose that it may contain flavonoidal glycosides which may be the responsible compounds for the activity. Hard work is being done in our lab to validate this theory and isolate the active compound(s). **Figure 2** shows scheme of bioassay-guided fractionation of the ethanol extract.

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