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THE EFFECT OF DRYING CONDITIONS ON THE ANTIDIABETIC ACTIVITY OF THE ETHANOLIC LEAF EXTRACT OF *VERNONIA AMYGDALINA*

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Abstract
Ethanolic extracts of the leaves of *Vernonia amygdalina* were obtained by maceration from the freshly -harvested leaves, leaves dried in the shade, and leaves dried at 40 ^{O}C in the hot air oven. Solvent removal was achieved with the aid of a rotary evaporator. White albino rats of both sexes weighing 90-145 g were used. Diabetes was induced with alloxan given *i.p.* at a dose of 150 mg/kg body weight. The animals were randomized into 8 groups of 5 animals per group. After a 12 hr overnight fast, each group treated as follows: group 1 received 50 mg/kg of fresh leaves extract; group 2 received 50 mg/kg of shade-dried leaves extract; group 3 received 50 mg/kg of oven-dried leaves extract; group 4 received 100 mg/kg of fresh leaves extract; group 5 received 100 mg/kg of shade-dried leaves extract; group 6 received 100 mg/kg of oven-dried leaves extract; group received Glibenclamide 10 mg/kg while group 8 received 2 ml/kg distilled water. Fresh blood obtained from the rat tail vein was analyzed for blood glucose level at 0, 1,2,4,6,8,12 and 24 hr from the time of administration with a glucometer. Free access to both food and water was allowed immediately after drug administration. Analysis of variance of the results was carried out with Microsoft SPSS® software at a 5 % level of significance. The results show that at 50 mg/kg dose of extract, the hypoglycemic activities were in the order: shade-dried leaves extract > fresh leaves extract> glibenclamide (10 mg/kg)> oven-dried leaves extract > water. The hypoglycemic effects of the leaves extracts were significant up till 6 hr; thereafter the blood glucose started rising again. Hypoglycemic activity significantly ($p<0.05$) increased with increase in extract dose. Thus, the shade-dried leaves extract was more effective weight for weight than either the oven-dried or fresh leaves extracts as an antidiabetic agent when compared weight per weight.

Keywords: Diabetes mellitus, *Vernonia amygdalina*, drying conditions, blood glucose-lowering,

INTRODUCTION

Diabetes mellitus is a major endocrine disorder affecting nearly 10 % of the population all over the world (Burk *et al.*, 2004). In spite of the introduction of oral hypoglycemic agents, diabetes and its related complications continue to be a major medical problem. Patients with non-insulin dependent diabetes mellitus have been treated orally with a variety of plant extracts (Rahman and Zaman, 1989). *Vernonia amygdalina* (bitter leaf) is a shrub belonging to the family Compositae and grows predominantly in a range of ecological zones in tropical Africa (Farombi, 2003). It has

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received tremendous attention from researchers (Aka and Okafor, 1992; Gyang *et al.*, 2004*;* Okolie *et al.*, 2008). Among most plants with antidiabetic activity, *V. amygdalina* is second only to *Cassia alata* as the most frequently used (Abo *et al.*, 2000). From many research works carried out on the plant, its antidiabetic activity has been established (Aka and Okafor, 1992; Gyang *et al.*, 2004*;* Okolie *et al.*, 2008). The median lethal dose (LD_{50}) according to Ojiako and Nwanjo (2006) was found to be 500 mg/kg.

The present work is designed to ascertain the effects of different drying conditions of *Vernonia amygdalina* leaves on the antidiabetic activity of the ethanolic extract. This could serve as a good guide in the formulation of the extract into dosage forms. No such work could be found in the literature.

MATERIALS AND METHODS

Plant materials and preparation of extracts

Vernonia amygdalina leaves were collected from the medicinal plant farm of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. The leaves were identified by Dr Mrs Margaret Bassey of the University of Uyo. The Department keeps voucher specimens of the plant in its herbarium. The extraction was done in the Laboratory of the Department of Pharmacognosy and Natural Medicine as follows: the freshly collected leaves (6 kg) were divided into three parts of 2 kg each. One part was left fresh, the second part was shade-dried and the third portion was oven dried at a temperature of 40^{o}C. Extraction was done by maceration with 70 % ethanol for 72 hr. Solvent removal was achieved using a rotary evaporator. The resultant dry extracts were stored at 4 ^{o}C in a refrigerator until use.

Animals

Approval for the use of the animals in the study was obtained from the Animal Ethical Committee, Faculty of Pharmacy, University of Uyo. Albino rats weighing between 90-145 g were used. They were housed in standardized environmental conditions of 29 ± 2.5 ^{o}C, at a relative humidity of 80-85 %, and allowed to acclimatize for two weeks. During that period, they were fed with standard rodent diet (Unfailing Veterinary Services, Uyo, Nigeria), and water ad *libitum*.

Methodology

Alloxan purchased from SIGMA Company through one of its agents was used to induce diabetes in the animals. The diabetogenic agent was given to each of the rats at a dose level of 150 mg/kg body weight by intraperitoneal administration. The rats were treated with alloxan after an overnight fast (they had access to only water) of 12 hr to make them more susceptible to developing diabetes (following the method of Szkudelski, 2001). The alloxan –treated rats were left undisturbed for 48 hr during which time diabetes developed and reached a steady state in the animals. Alloxan-treated rats with blood glucose levels persistently above 200 mg/dL were considered diabetic and were used for the study. All the rats used in this study were fasted for a period of 12 hr (but still allowed free access to drinking water) before they were treated with distilled water, the plant extract or glibenclamide (in solution) warmed to 37 ± 1^{o}C. The 40 diabetic rats were divided into 8 groups (1-8) of five rats each. Fresh blood obtained from the rat tail vein was analyzed for blood glucose level at 0 hr with a glucometer (One Touch®, Johnson and Johnson).They were then treated as follows: group 1 received fresh leaves extract at 50 mg/kg body weight dose

level; group 2 received shade-dried leaves extract, 50 mg /kg ; group 3 received oven-dried leaves extract, 50 mg/kg; group 4 received fresh leaves extract, 100 mg/kg; group 5 received shade-dried leaves extract, 100 mg/kg; group 6 received oven-dried leaves extract, 100 mg/kg; group 7 received glibenclamide, 10 mg/kg; while group 8 received distilled water at the level of 2 ml/kg. The animals were now allowed free access to food and water. The blood glucose was sampled and analyzed as above at 1, 2, 4, 6, 8, 12 and 24 hr from the time of drug administration in each case.

Data analyses

Blood glucose levels were expressed as % of baseline blood glucose calculated with Microsoft EXCEL® and plotted as graphs. The group means were calculated and compared by ANOVA or T-test using the Microsoft SPSS® software.

RESULTS AND DISCUSSION

The results of the effect the leaves extracts given at 50 mg/kg body weight (Figure 1, Table 1) show that between 0 and 4 hr, the hypoglycemic activities were in the order- shade-dried leaves extract > fresh leaves extract> glibenclamide (10 mg/kg)> oven-dried leaves extract > distilled water. Thus, there was more potent hypoglycemic effect weight for weight with the shade-dried leaves extract than with either the fresh leaves or the oven-dried leaves. The implication of this is that one would need less quantity of the shade –dried leaves extract than the other extracts to produce equivalent hypoglycemic effect in formulations. This suggests that it is preferable to shade-dry the leaves before extraction than to extract in the fresh or after oven-drying. The blood glucose levels of the rats treated with each extract significantly reduced up to 6 hr before starting to rise again. This shows that the hypoglycemic activity of each extract lasted up to 6 hr. However, blood glucose levels of the rats treated with glibenclamide continued to drop throughout the period. Thus, there might be need for a more frequent dosing with the extracts than with glibenclamide, or the addition of a longer-acting hypoglycemic agent to the extract for a more sustained effect in the body.

At 50 mg/kg body weight of extract the results (Figure 2, Table 2) show that between 0 and 3 hr, the hypoglycemic activities were in the order- shade-dried leaves extract > fresh leaves extract> oven-dried leaves extract ≈ glibenclamide (10 mg/kg) > distilled water. After 4 hr, the % baseline blood glucose levels with the shade-dried and fresh leaves extracts were not significantly distinguishable in value from each other. The blood glucose levels of the rats treated with each extract significantly reduced up to 6 hr before starting to rise again. This shows that the hypoglycemic activity of each extract lasted for 6 hr. However, the hypoglycemic activity of the oven-dried extract lasted till the end of the experiment at both dose levels. It has been reported that some plants have both hyper- and hypoglycemic agents (Iwu, 1980). It might be that the hyperglycemic principles have been destroyed by heating leaving only the hypoglycemic agents to act.

At both 50 mg/kg and 100 mg/kg of the shade-dried leaves extract the blood glucose reduction lasted for up to 6 hr with that of the higher dose being progressively more (Figure 3, Table 3). At 8 hr, blood glucose levels with the 50 mg/kg dose had significantly increased almost equal to the same level as the baseline values, while blood glucose levels with the 100 mg/kg dose remained fairly constant and less than the baseline values till the end of the experiment. The results indicate that the hypoglycemic activities of the extracts

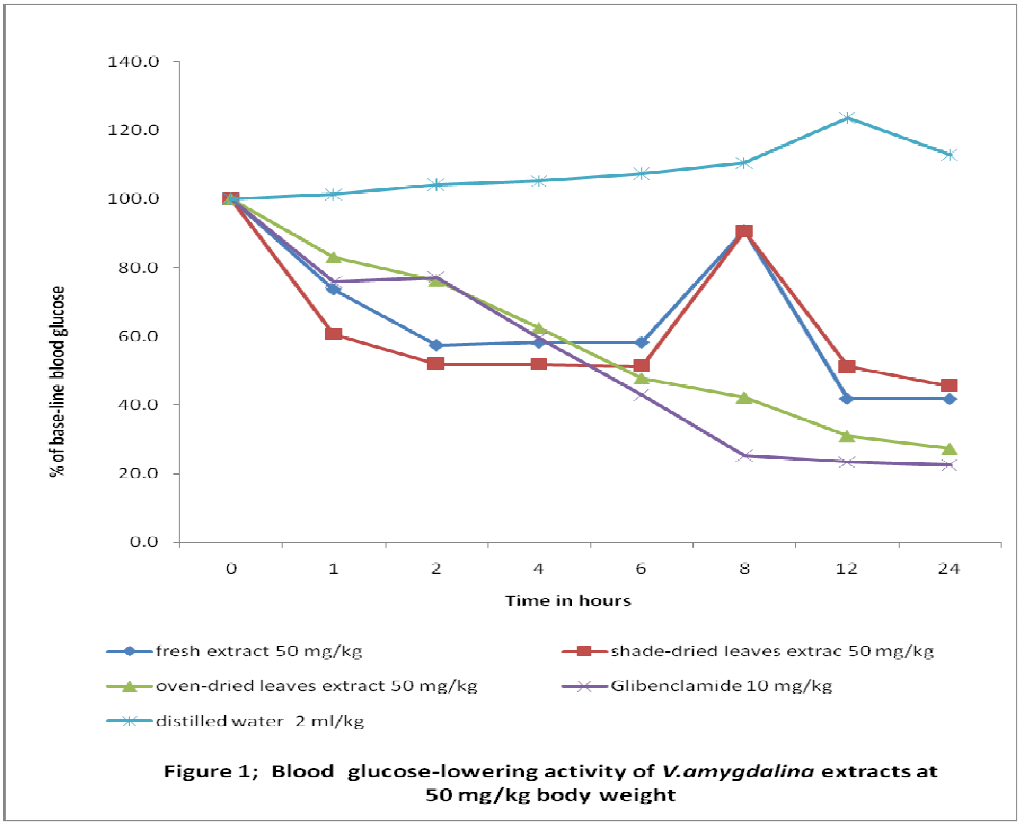

Figure 1; Blood glucose-lowering activity of *V.amygdalina* extracts at 50 mg/kg body weight

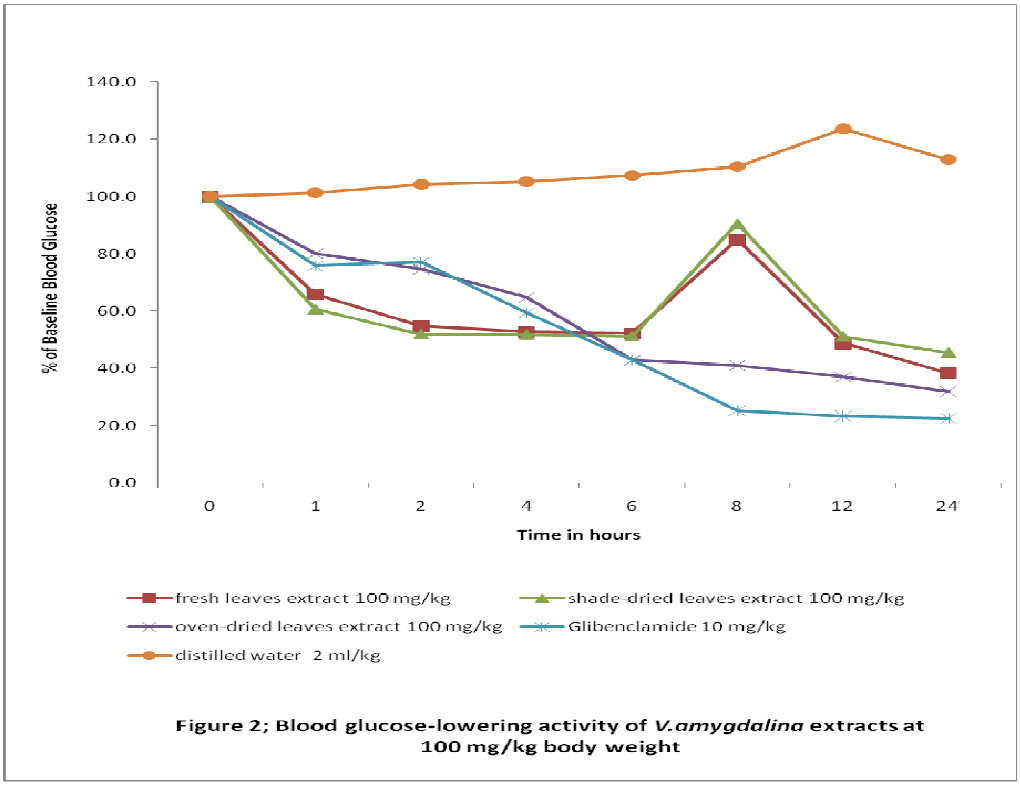

Figure 2; Blood glucose-lowering activity of *V.amygdalina* extracts at 100 mg/kg body weight

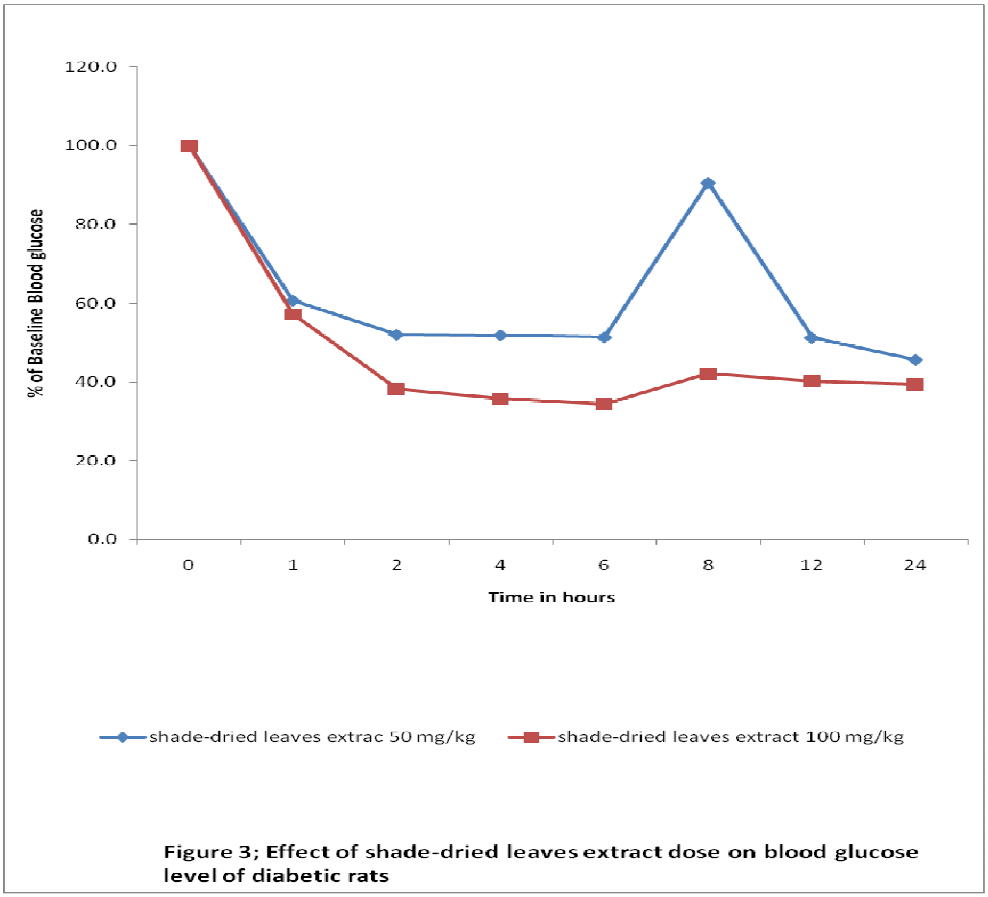

Figure 3; Effect of shade-dried leaves extract dose on blood glucose level of diabetic rats

Table 1: ANOVA for hypoglycemic activity of extracts at 50 mg/kg

Time (hour)	F	*p*	Remarks
One	3.689	.021	*
two	8.672	.000	*
four	12.437	.000	*
six	14.302	.000	*
Eight	15.992	.000	*
Twelve	25.377	.000	*
Twenty-four	21.984	.000	*

*Significant at p< 0.05

Table 2: ANOVA for hypoglycemic activity of extracts at 100 mg/kg

Time (hour)	F	*p*	Remarks
One	4.325	.011	*
Two	14.204	.000	*
Four	36.362	.000	*
Six	29.294	.000	*
Eight	19.949	.000	*
Twelve	26.320	.000	*
Twenty-four	26.340	.000	*

*Significant at p< 0.05

		Levene's Test for Equality of Variances		t-test for Equality of Mear	
		F	Sig.	t	Sig. (2-ta
1 h	Equal variances assumed	.003	.959	.613	.557
	Equal variances not assumed			.613	.557
2 h	Equal variances assumed	1.924	.203	3.949	.004
	Equal variances not assumed			3.949	.007
4 h	Equal variances assumed	1.393	.272	4.586	.002
	Equal variances not assumed			4.586	.003
6 h	Equal variances assumed	3.462	.100	5.245	.001
	Equal variances not assumed			5.245	.003
8 h	Equal variances assumed	22.960	.001	3.486	.008
	Equal variances not assumed			3.486	.021
12 h	Equal variances assumed	3.172	.113	1.313	.226
	Equal variances not assumed			1.313	.243
24 h	Equal variances assumed	4.719	.062	.608	.560
	Equal variances not assumed			.608	.568

are dose-dependent thus confirming the finding of Gupta *et al*., (2005). The elevation in blood glucose after 8hr and its subsequent fall might be as a result of change in physical activities of the animals as that falls within the dark period thereby reducing the physical activities of the animals which would lead to less energy being expended, and hence causing a rise in blood glucose.

CONCLUSION

The study reveals that at 50

mg/kg and 100 mg/kg dose of the ethanolic extracts of the leaves of *Vernonia amygdalina* and in terms of speed of response, the hypoglycemic potencies are in the order: shade-dried leaves extract > fresh leaves extract >> oven-dried leaves extract and there are significant differences ($p<0.05$) between the treatment group results.

The shade-dried leaves extract showed more activity than either the oven-dried or fresh leaves extracts as an antidiabetic agent when compared weight per weight. Thus, it might be preferable to dry the leaves in the shade before extraction than to extract from the fresh or oven-dried leaves. The hypoglycemic effects of the leaves extracts were significant up to the 4^{th} hour; thereafter the blood glucose started rising again. The hypoglycemic effect of the oven-dried leaves extract was sustained throughout the experiment, though. Hypoglycemic activity significantly ($p<0.05$) increased with increase in extract dose.

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