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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (5): 828-830, 2007
ISSN 1028-8880

Screening of the Seed of *Picralima nitida* for Hypoglycaemic Activity

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Abstract: The seed extract of *picralima nitida* was screened for hypoglycaemic activity. The hypoglycaemic activity was evaluated in both normoglycemic and alloxan-induced diabetic rats, using glibenclamide as a reference drug. In both normoglycaemic and alloxan diabetic groups, 250 mg kg^{-1} of the extract, 5 mg kg^{-1} glibenclamide and a mixture of the extract (125 mg kg^{-1}) and glibenclamide (2.5 mg kg^{-1}) were administered orally, respectively. Blood glucose was measured with a glucometer at 0, 1, 2 and 4 h. In the sub acute group, the same doses as above were administered to diabetic rats once daily for 14 days and blood glucose was determined on days 1, 5, 10 and 15. The seed extract did not show any hypoglycaemic effect in both normoglycaemic and alloxan diabetic rats. Rather, the extract increased blood glucose concentration in the sub acute group on days 5, 10 and 15. These results indicate that, unlike the fruit pulp, the seed extract of *Picralima nitida* did not possess hypoglycaemic activity.

Key words: *Picralima nitida*, glibenclamide, hypoglycaemic, alloxan

INTRODUCTION

Picralima nitida (staphf) is a member of Apocynaceae family. The plant is popularly used in West Africa as an antipyretic and antimalarial (Ansa *et al*., 1990; Magilu *et al*., 1996). Pronounced inhibitory activities against asexual erythrocytic forms of *plasmodium falciparum* was highest in the root, stems bark and fruit rind-extracts. Leaf and seed extracts yielded much lower activity or were completely in active (Francois *et al*., 1996; Iwu and Klayman, 1992). The antimalarial activity of the plant has been attributed to its alkaloidal components (Iwu *et al*., 1992; Kspsdia *et al*., 1993; Moeller *et al*., 1972). The trypanocidal (Wosu and Ibe, 1989) and antileishmanial activities of the plant have been reported (Iwu *et al*., 1992b). It is used medicinally as an arrow and a fish poison (Omino, 1996). The plant possesses opium analgesic (Menzies *et al*., 1998; Arens *et al*., 1982) and anticholinesterasic (Levy and Collin, 1978) properties. The hypoglycaemic effect of the pulp has been established (Inya-Agha, 1999). The present study was conducted to determine wether the seed, like the pulp, possesses hypoglycaemic effect.

MATERIALS AND METHODS

Plant material: Pods of *P. nitida* were obtained from Ubulu in Imo State, Nigeria. They were identified by Dr. Eshiet of Botany Department and voucher specimens were deposited at Faculty of Pharmacy, University of Uyo.

Extraction: The pod of *P. nitida* were sliced and the seeds removed. The seeds were crushed in a mortar. The crushed seed material was extract with 96% ethanol. The extracts was concentrated in vacuo and dried in a dessicator.

Preparation of diabetic rats: Albino rats of both sexes, obtained from the University of Uyo animal house weighing 105.66±11.54 g were made diabetic by intraperitoneal injection of alloxan monohydrate (150 mg kg^{-1}). These were allowed to rest for 7 days to allow glucose levels to stabilise. The rats had free access to standard laboratory rat chow and water and were kept under standard laboratory conditions.

Administration of extract:
Normoglycemic rats: Twenty normoglycemic rats fasted overnight, were divided into four groups of 5 rats each and treated as follows: Groups I (control) received 1 ml of saline water orally only. Groups II received orally 1ml of seed extract of *P. nitida* (250 mg kg^{-1}). Group III received orally 1 mL of glibenclamide (2.5 mg kg^{-1}).

Group IV received orally a mixture of 0.5 mL seed extract of *P. nitida* (125 mg kg^{-1}) and 0.5 mL glibenclamide (2.5 mg kg^{-1}).

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Diabetic rats: Twenty overnight-fasted alloxan- induced diabetic rats were divided into four groups (5 animals per group). Each group was treated as in (a) above.

Subacute group: Twenty diabetic rats were divided into four groups of 5 rats each and treated as in (a) above. The various doses of extract and drugs were administered once daily for 15 days.

Estimation of blood glucose: Blood collected from the tail vein of the rats was analysed for glucose using the One Touch^{R} glucose analyser. In both the normal and diabetic rats (i.e., a and b above), blood glucose was determined at time 0, 2 and 4 h. While in the subacute group (i.e., c above) blood glucose determination was carried out on the 1st, 5th, 10th and 15th day.

Statistical analysis: Data were expressed as mean±SEM, n = 5. Student's t-test was used to check the level of significance. $p<0.05$ indicates significant difference from control group.

RESULTS AND DISCUSSION

The percent change in glucose level is given in parenthesis and was calculated as follows:

Percent change = G_T/G_O x100%
Where G_T = blood glucose concentration at time t
G_O = blood glucose concentration at time zero.

Hypoglycemic agents reduce blood glucose level in normoglycemic rats by stimulating the beta cell of the pancreas to produce and release insulin (Table 1). The extract of seed of *P. nitida* did not show hypoglycaemic effect in the normoglycaemic and diabetic rats when administered alone at 250 mg kg^{-1}. This shows that the extract did not stimulate insulin secretion by the pancreatic B cell. Glibenclamide (reference antidiabetic drug) reduced glucose level when administered alone. But co-administration of the extract with glibenclamide did not reduce glucose level, showing that the extract did not enhance or complement the hypoglycaemic effect of glibenclamide. The extract when administered alone elevated blood glucose level ($p<0.05$) during sub-acute administration to the alloxan-induced diabetic rats (Table 2). In the sub-acute co-administration of the extract and glibenclamide, the hyperglycemic effect of the extract was mitigated or countered by the hypoglycaemic activity of glibenclamide. The reasons for this blood glucose elevation of *P. nitida* seed is not known to us yet and should be a matter for further research. However, the plant is known to contain alkaloids such as picratidine, akuammine, akuammicine, akuammidine, akuammigine and pseudoakuammigine. Any of these alkaloids or a yet to be identified component may be responsible for the hyperglycemic activity of the seed by increasing the production and release of glucose from liver (i.e., gluconeogenesis) in the diabetic rats or through some other mechanism.

The fruit pulp of this plant has been shown to exhibit hypoglycaemic activity, but this present study shows that the seed of *P. nitida* does not possess hypoglycaemic activity in both normoglycemic and alloxan diabetic rats either when administered alone or in combination with glibenclamide. Contrary to expectation, the plant exhibited hyperglycemic activity when administered alone sub-acutely in the diabetic rats.

This study shows that the seed of *P. nitida* may not be useful in reducing blood glucose level in the diabetic patient and may be unsafe for a diabetic patient to consume as doing so may raise the blood glucose concentration to a fatal level.

Table 1: Effect of seed of *P. nitida* on the blood glucose levels in normoglycaemic and alloxan-diabetic rats

	0 h		2 h		4 h	
	Normo-glycaemic	Diabetic	Normo-glycaemic	Diabetic	Normo-glycaemic	Diabetic
Control	43.4±8.28 (100)	173±17.8 (100)	45.2±4.49 (105)	143.6±20.3 (83)	43.6±11.52 (101)	112.5±16.7 (63)
Glibenclamide	35±4.85 (100)	143±56.4 (100)	31.2±8.11 (89)*	94±22.5 (57)*	30.4±6.65 (87)*	54.4±16.3 (33)*
P. nitida	50.16±6.84 (100)	140.25±81.3 (100)	54.73±6.34 (109.2)	131.84±31.7 (94)	58.08±10.99 (115.8)	116.41±34.6 (83)
P. nitida + Glbenclamide	51.26±8.27 (100)	251.9±8.27 (100)	52.14±10.26 (100)	137.04±41.0 (54.4)	51.7±8.63 (100.9)	121.67±29.9 (48.3)

* $p<0.05$ Mean±SEM; Percent change in glucose level is given in parenthesis

Table 2: Effect of seed of *P. nitida* on the blood glucose level of diabetic rats (mg/dl) in subacute administration

	Day 1	Day 5	Day 10	Day 15
Control	173±17.8 (100)	147.1±30.1 (85)	196±46.2 (113)	160.9±33.6 (93)
Glibenclamide	164±14.1 (100)	81.5±12.1 (50)*	121.4±14.4 (74)*	114±16.7 (70)*
P. nitida	240.5±81.3 (100)	334.28±61.7 (139)*	360.75±81 (150)*	339.11±68.8 (141)*
P. nitida + Glibenclamide	298±34.7 (100)	358.49±41.2 (120)	424.22±61 (142)	313.68±37.6 (105)

* $p<0.05$ Mean±SEM; Percent change in glucose level is given in parenthesis

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