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ANTIHYPERGLYCEMIC EFFECT OF ETHANOLIC EXTRACT AND FRACTIONS OF ANACARDIUM OCCIDENTALE L. STEM BARK IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT: The numbers of adults with diabetes in the world is estimated to rise 300 millions in the year 2025 and this leads to increasing search for better anti-diabetic drug. The effects of the ethanol extract (1.25 g/kg) and fractions (Ethyl acetate, dichloromethane and butanol in the dose of 1 g/kg) of *Anacardium occidentale* stem bark on the blood glucose levels in streptozotocin-induced types 1 and 2 diabetic rats at different prandial states were studied. The ethanol extract of *A. occidentale* had no hypoglycemic effect in type 1 diabetic rats in fasting and postprandial glucose load conditions and, in type 2 diabetic rats in fasting condition. However, the extract, significantly lowered blood glucose levels in type 2 diabetic rats when fed simultaneously with glucose. The ethyl acetate fraction showed a significant opposing effect in serum glucose rise after administration of glucose. Additionally, its dichloromethane extract also exhibited a significant reduction in serum glucose level compared to control after glucose administration while its butanol fraction was devoid of this activity. These findings conclude that the active principles responsible for the antihyperglycaemic effect might be concentrated in the ethyl acetate and dichloromethane fractions of the extract.

KEYWORD:

Anacardium occidentale
Non insulin dependent diabetes,
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INTRODUCTION

The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years [1]. Few of plant treatments used in traditional medicine for diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrant attention [2]. It has reported that aqueous extracts of *Anacardium occidentale* has antihyperglycemic effects [3]. Therefore the present work was undertaken to study the glucose-lowering effects of the ethanol extract and fractions of *A. occidentale* stem bark in streptozotocin-induced types 1 and 2 diabetic rats at different prandial states.

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MATERIALS AND METHODS

Animals

Swiss Albino (Wistar) rats of weight, 200–230 g, bred at animal house Sri Sai Institute of Pharmaceutical Education & Research (SSIPER), Badhani, Pathankot were used for this study. The animals were maintained on 12 hours light-dark cycle at room temperature, fed on a standard laboratory pellet diet and with water supplied *ad libitum*.

Plant Material

A. occidentale stem bark was collected from Badhani, Pathankot and identified by Dr. N.N. Sharma at (SSIPER), Badhani, Pathankot. Herbarium voucher specimen number SAI-76 has been deposited for reference in future. The bark was dried under sunlight and ground into powder.

Extraction procedure and Fractionation

A 2.5 kg powdered *A. occidentale* bark was extracted (4 times, 24 hours each time) with 80% ethanol at room temperature, filtered and concentrated. It is further freeze-dried to obtain a 200 g extract (8% w/w). The dried powder was suspended in water (500 ml) and then partitioned successively with CH₂Cl₂, EtOAc and 1-BuOH to give 12.92 g, 20.60 g and 30.82 g of extracts, respectively. The parent extract (80% EtOH) and its dichloromethane (DCM), ethyl acetate (EA), 1-butanol (BU) soluble parts were tested on IDDM and NIDDM model rats at different prandial states.

Effects on the fasting blood glucose levels

Type 1 (IDDM) diabetes was induced by intraperitoneal injection of streptozotocin (stz, 65 mg/kg body weight) dissolved in citrate buffer (pH 4.5) immediately before use to 3 month old adult rats which were remain fasted for 18 hours. Type 2 diabetes (NIDDM) was induced by intraperitoneal injection of streptozotocin (90 mg/kg body weight) to 48-hour old pups rats as previously described by Bonner-Weir et al [4]. Groups of 6 to 8 rats each were used for the experiments. The ethanol extract of *A. occidentale* (1.25 g/kg body weight) were fed orally by gastric intubation to overnight fasting (12 hours) rats at 0 min and blood samples were drawn at 0, 60 and 120 min. Negative control group received only

water (10 ml/kg body weight) and another group of rats received glibenclamide (5 mg/kg body weight) as a standard drug (Positive control group). The animals were kept unfed throughout the period.

Effects on blood glucose levels at concomitant administration of the fractions and glucose

The *A. occidentale* (1.25 g/kg body weight) extract and its fractions (1 g/kg body weight) were fed simultaneously with glucose (2.5 g/kg body weight) by gastric intubation to overnight fasting (12 hours) rats at 0 min and blood samples were drawn at 0, 30 and 60 min. Negative control group received only glucose (2.5 g/kg body weight) and the positive control group of rats received glibenclamide (5 mg/kg body weight) along with glucose (2.5 g/kg body weight). The animals were kept unfed throughout the period.

Blood collection and biochemical analysis

Blood samples were collected from the tail tip under mild ether anesthesia. The serum was separated by centrifugation and the glucose level was measured immediately by digital glucometer (CT-X10, Elegance, Germany) with glucostix (Elegance, Germany).

Statistical analysis

Results were expressed as mean blood glucose levels ± S.E.M. Data were analyzed using One-way ANOVA followed by Dunnett's test. The level of significance was set at 0.05.

RESULTS

The ethanol extract of *A. occidentale* had no hypoglycemic effect in IDDM and NIDDM rats on fasting condition (Table 1). However, glibenclamide showed a significant (P<0.05) decrease of blood glucose levels in NIDDM rats on fasting state (36% and 38% reduction respectively 60 and 120 min after administration).

Figure 1 shows the effect of *A. occidentale* 80% ethanol extract on postprandial blood glucose levels of IDDM rats when fed simultaneously with glucose (2.5 g/kg body weight). The extract showed a tendency to oppose the blood glucose rise but this effect was not significant compared to control (4% reduction 30 min after glucose load). Glibenclamide treated rats showed the same tendency.

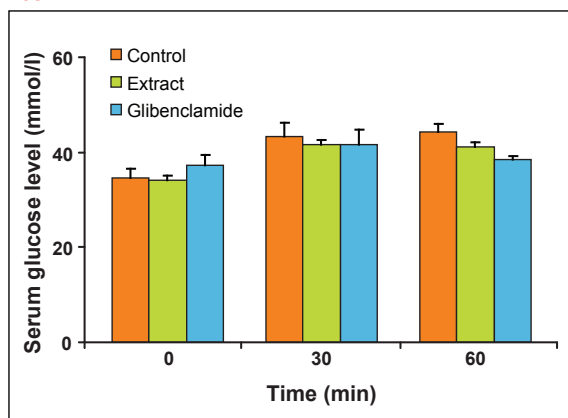
However, in NIDDM diabetic model rats (Figure 2), the extract had a strong and significant (p<0.001)

TABLE 1: EFFECT OF ANACARDIUM OCCIDENTALE ETHANOLIC EXTRACT ON FASTING SERUM GLUCOSE LEVELS OF IDDM AND NIDDM DIABETIC MODEL RATS

Group	Blood glucose levels, mmol/l (percent of 0 min value)		
	0 min	60 min	120 min
A. IDDM model rats (n=6)			
Control	35.35 ± 1.30 (100)	31.97 ± 0.40 (91.12 ± 3.81)	30.20 ± 1.37 (86.42 ± 6.13)
Extract (1.25g/kg)	34.46 ± 1.66 (100)	29.29 ± 1.77 (85.30 ± 4.20)	29.22 ± 1.95 (85.20 ± 5.24)
Glibenclamide (5 mg/kg)	35.46 ± 1.21 (100)	35.46 ± 1.21 (100)	28.73 ± 1.41 (82.15 ± 6.88)
B. NIDDM model rats (n=8)			
Control	9.45 ± 0.40 (100)	8.90 ± 0.36 (95.24 ± 5.10)	9.97 ± 0.43 (107.38 ± 7.98)
Extract (1.25g/kg)	10.03 ± 0.41 (100)	8.88 ± 0.45 (89.96 ± 6.44)	9.26 ± 0.33 (93.97 ± 6.59)
Glibenclamide (5 mg/kg)	9.75 ± 0.42 (100)	6.24 ± 0.47*** (64.34 ± 4.62)**	6.02 ± 0.44*** (61.70 ± 3.24)***

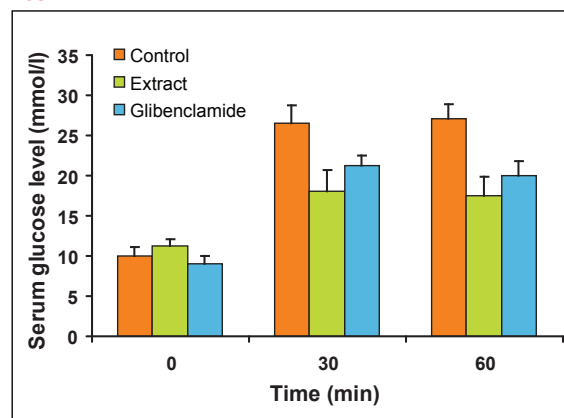
Values are mean blood glucose levels ± S.E.M. (n=number of rats per group). Significantly different from control at different periods: **p<0.01 and ***p<0.001

FIGURE 1:



Effect of *Anacardium occidentale* ethanolic extract on serum glucose levels of IDDM diabetic model rats when fed simultaneously with glucose load. Values are mean blood glucose levels \pm S.E.M. (n = 6, number of rats per group)

FIGURE 2:



Effect of *Anacardium occidentale* ethanolic extract on serum glucose levels of NIDDM diabetic model rats when fed simultaneously with glucose load. Values are mean blood glucose levels \pm S.E.M. (n=8, number of rats per group). Significantly different from control at identical periods: *p<0.05 and ***p<0.001.

opposing effect on the rise of serum glucose level when fed simultaneously with glucose (32% and 35% inhibition of glucose rise respectively 30 and 60 min after glucose load). Glibenclamide also opposed the rise (20% inhibition, $p < 0.05$) of serum glucose 30 min after simultaneous administration with glucose.

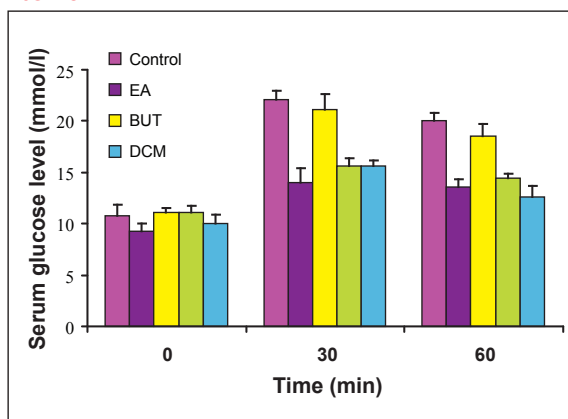
As shown in Figure 3, the ethyl acetate fraction showed a significant ($P < 0.001$) opposing effect in serum glucose rise 30 min after administration (36% inhibition of glucose rise compared to control rats, the ethyl acetate fraction showed a significant reduction (32%, $P < 0.01$) of serum glucose level 75 min after simultaneous administration with glucose. The dichloromethane extract also showed a significant ($P < 0.05$) reduction in serum glucose level compared to control (29% and 28% respectively 30 and 60 min

after administration). In contrast, when compared to the control group, the butanol fraction was, however, devoid of this activity.

DISCUSSION

In adult animals, streptozotocin selectively destroys the pancreatic insulin-secreting β -cells, leaving less active cells and resulting in type 1 diabetic state [5-6]. Our results show that neither the 80% ethanol extract of *A. occidentale* nor glibenclamide demonstrated hypoglycemic or antihyperglycemic effects in IDDM rats in both fasting and glucose load states respectively. The main mechanism of action of glibenclamide is by the stimulation of insulin release. It has been described that glibenclamide is effective in moderate diabetic state, and ineffective in severe diabetic animals where pancreatic β -cells are almost totally destroyed [7-8]. The similar inactivity of the extract and glibenclamide may indicate that the extract also act by stimulation of the Islet cells and thus requires functional pancreatic β -cells for its action. This was confirmed by the inactivity of the extracts on fasted IDDM rats (Table 1). In the NIDDM diabetic model rats, the ethanol extract of *A. occidentale* showed an antihyperglycemic effect comparable to that of glibenclamide when fed simultaneously with glucose. Thus, the extract may act on β -cells like sulfonylurea drugs to stimulate insulin secretion. As the ethanol extract of *A. occidentale* did not show any hypoglycemic effect in NIDDM rats on fasting condition, it can be assumed that this extract like tetraethyl ammonium (TEA), may stimulate insulin secretion in a glucose-dependent manner

FIGURE 3:



Effect of fractions obtained from the ethanol extract of *Anacardium occidentale* (1 g/kg) on serum glucose levels in NIDDM rats when fed simultaneously with glucose. Values are mean blood glucose levels \pm S.E.M. (n=6). EA: Ethyl Acetate, DCM: Dichloromethane, BUT: Butanol extract. Significantly different from control at identical periods: *p<0.05; **p<0.01 and ***p<0.001

[9]. On the other hand, the hypoglycemic effect of the extract in the glucose-fed rats may be accounted in part, by an inhibition of intestinal glucose absorption and the stimulation of the glucagon-like peptide (GLP-1) which is also a glucose-dependent insulin secretagogue [10].

In this study, the ethyl acetate and dichloromethane fractions obtained from the ethanol

extract, produced important hypoglycemic effects in NIDDM rats when fed simultaneously with glucose, indicating that the hypoglycemic components of the plant are concentrated in these two fractions. Further chemical and pharmacological investigations are in progress to elucidate in detail the active principles and the real mechanism of action of this plant extract.

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