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ANTIDIABETIC ACTIVITY OF AQUEOUS EXTRACT FROM *VIGNA RADIATA* IN STREPTOZOTOCIN INDUCED DIABETIC MICE.

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ABSTRACT

Vigna radiata is an important medicinal plant that belongs to family Fabaceae, which is widely used in the traditional medicine all over the world. Behind its nutritional acceptance, the grain of this plant is cooked traditionally and consumed for the purpose of lowering the blood glucose level in diabetic patients especially in rural areas. This study was designed to scientifically validate its antidiabetic activity in diabetic mice. Mice obtained from Ethiopia public health institute were allowed to adapt the experimental room for 3 days before the actual experiment. They consumed the standard food (pellet) throughout the experiment. After dissolving streptozotocin with 0.9% normal saline, the fresh solution was injected to all mice through intraperitoneal route at a dose of 35 mg/kg. The diabetic mice were divided into four groups. The first group was treated with glibenclamide (5mg/kg), the second group was treated with normal saline (10 ml/kg) and the last two groups were treated with aqueous extract of *v.radiata* at 200 and 300 mg/kg. Finally, blood glucose levels were measured 1hr, 2hr, 3hr and 4hr after administration of each treatment. The aqueous crude extract at 200 and 300 mg/kg decreased the blood glucose level as compared to the control group ($p < 0.05$). The antidiabetic activity of aqueous extract of *v.radiata* at 200mg/kg was lower than that of the aqueous extract at 300mg/kg. In conclusion, the aqueous crude extract of *v.radiata* possesses a significant antidiabetic activity.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased blood glucose level (hyperglycemia) resulting from the defects in insulin secretion, insulin action, or both. It is one of the alarming worldwide health problems at present leading to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications [1]. The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus and unexpected weight loss [2]. DM is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. The two broad categories of DM are designated type 1 and type 2 [3].

Diabetes currently is a major health problem for the people of the world and exerts a significant burden in Sub-Saharan region, and this is expected to increase. Many diabetic patients face significant challenges accessing diagnosis and treatment, which contributes to the high mortality and prevalence of complications observed. In Ethiopia Prevalence of diabetes is increasing and estimated to be 2% nationally related to lifestyle changes and the resulting surge in obesity. More than one-third, but only less than half, of diabetic patients in Ethiopia receives standard diabetes care [4].

Insulin is the mainstay for treatment of virtually all type 1 DM and many type 2 DM patients. When necessary, insulin may be administered intravenously or intramuscularly; however, long-term treatment relies predominantly on subcutaneous injection of the hormone. Preparations of insulin can be classified according to their duration of action into short, intermediate, and long acting and by their species of origin human or porcine. A serious complication of intensive therapy was an increased incidence of severe hypoglycemia. Type 2 DM can be managed apart from life style modification by oral hypoglycemic agent with insulin or alone. There is considerable proportion of patients, experiencing episodes of hypoglycemia during anti-diabetic treatment on mono- or dual oral combination therap. The risk of hypoglycemia associated with insulin and oral hypoglycemic agents might be substantially reduced by properly selecting anti-diabetic pharmacotherapy [4].

Proper control of blood sugar in type 2 diabetes mellitus (T2DM) is not adequate and challenging to the medical system till now in spite of use of well-planned dosage regimens containing oral hypoglycemic agents/insulin or both. Due to improper control, side effects and availability of ant diabetic drugs, people in some parts of the world are found to use herbal treatments either alone or in combination with other forms of treatments. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects and relatively low cost [5]. Since, Ethiopia is rich in fauna and flora, investigation on such agents from traditional medicinal plants has become more important.

Phytomedicines obtained from herbal sources are in great demand as they are able to cure many diseases. *V. radiata* is one of the most widely used medicinal plants in the world and is cultivated in Africa including Ethiopia [6]. As traditional medicine, it is used to treat cancer, diabetes, hypertension, hepatitis, gastritis and heat rash [7]. Even though many positive results were reported from previous studies, the antidiabetic activity is not well investigated and to the best of my knowledge, there is no any published report on antidiabetic activity of *v. radiata* in Ethiopia. Therefore, this study was intended to evaluate the effect of aqueous extract of flours from *V. radiata* on blood glucose level in mice model.

MATERIALS AND METHODS

Chemicals

During the whole course of this study, the following chemicals and drugs were utilized. Glibenclamide (5 mg/kg), streptozotocin (35 mg/kg), 0.9% normal saline and distilled water.

Plant material collection and preparation of the extract

Around 400 gram of dried grain of *v. radiata* was bought from mung-bean market. The grain was powdered coarsely. The powdered herbal material was macerated with distilled water with occasional shaking for about 7 days. The aqueous extract was filtered with Whatman filter paper and then after placed in deep freeze to solidify. The solidified aqueous extract was placed in lyophilizer machine and finally a powder was obtained for administration into mice.

Experimental animals

Healthy mice of either sex weighing between 25 and 30 g were obtained from Ethiopia public health institute, Ethiopia. After approval by animal ethics committee of college of medicine, Debre berhan university, Ethiopia, animals were acclimatized for 3 days; and had access to food (pellet) and water ad libitum under 12-h light/12-h dark cycle [9].

Induction of diabetes

Mice fasted overnight were injected with streptozotocin solution intraperitoneally at a dose of 35 mg/kg and then observed for 3-days. After three days observation, fasting blood glucose levels were measured. Mice that showed blood glucose levels of 11 mmol/l and above were included in this study [8].

Antidiabetic test in streptozotocin induced diabetic mice

Diabetic mice were equally divided in to four groups (n=6). The first group was administered with glibenclamide (5mg/kg). The second group was treated with 0.9 % normal saline (10ml/kg). The last two groups were treated with 200 and 300 mg/kg of aqueous extract of *v. radiata*. All administrations were through oral route. Finally blood glucose levels were measured 1hr, 2hr, 3hr and 4 hr after administration of each treatment [10].

Statistical Analysis

Each data was expressed as mean \pm standard error of the mean .one way analysis of variance (ANOVA) was used to indicate mean differences between groups. The location of mean difference between groups was analyzed by post hoc Dunnett's test. P value < 0.05 was considered as significant.

RESULTS

The result from the present study indicates that the aqueous extract from *v.radiata* decreased the blood glucose level in diabetic mice when it is compared with the negative control. The antidiabetic activity of the aqueous extract was found to be dose dependent. As it can be seen from the table, the antidiabetic activity at 300mg/kg showed significant decrement in blood glucose level in diabetic mice when compared with 200 mg/kg. The antidiabetic activity of the aqueous extract at 200 mg/kg exhibited time dependent activity. To the end of the experiment, the antidiabetic activity for 200 mg/kg was not significant in comparison with negative control ($P>0.05$). Comparing the antidiabetic activity of glibenclamide at 5mg/kg and aqueous extract of *v.radiata*, the aqueous extract at 300 mg/kg showed similar antidiabetic activity throughout the experiment period but the antidiabetic activity of aqueous extract at 200 mg/ kg was lower than that of glibenclamide (Table 1).

Table 1: Effect of aqueous extract of *v.radiata* in diabetic mice.

Treatment groups	Dose (mg/kg)	Blood glucose level(mmol/l)			
		1hr after	2 hr after	3 hr after	4 hr after
Glibenclamide	5	23.15 \pm 2.19 ($p<0.01$)	22.76 \pm 3.12 ($p<0.01$)	22.87 \pm 2.75 ($p<0.01$)	23.89 \pm 1.51 ($p<0.02$)
Normal saline	10ml	29.86 \pm 1.33	29.79 \pm 1.00	29.88 \pm 1.77	28.89 \pm 2.33
AEVR	200	25.43 \pm 2.19 ($p<0.03$)	25.00 \pm 3.76 ($p<0.04$)	26.14 \pm 2.65 ($p>0.05$)	27.75 \pm 1.17 ($p>0.05$)
AEVR	300	24.74 \pm 2.98 ($p<0.023$)	23.07 \pm 4.33 ($p<0.01$)	24.78 \pm 3.11 ($p<0.02$)	24.70 \pm 1.12 ($p<0.01$)

AEVR: aqueous extract of *v.radiata*, $P<0.01$: extremely significant, $P>0.05$: not significant
 $P<0.02$: moderately significant, $P<0.04$: significant,hr:hour.

DISCUSSION

The present study was conducted in diabetic mice to investigate antidiabetic activity of aqueous extract of bean flours from *v.radiata*. Glibenclamide was used as positive control for the current study. It acts by increasing insulin secretion from the pancreatic beta cells by closing the adenosine triphosphate sensitive potassium channels [9]. This indicates that the probable mechanism for the aqueous extract of *v.radiata* might be similar with glibenclamide. Previous phytochemical screening done in Chinese agricultural food sciences, China showed that extracts from *v.radiata* contain free phenolic acids, bound phenolic acids and anthocyanin and this constituents were reported to responsible for antidiabetic activity of this plant[7].

From the current study , the p value for antidiabetic activity of aqueous extract of *v.radiata* at 200 mg/kg was < 0.03 and < 0.04 administration 1hr and 2hr after respectively. But the value of p for the aqueous extract at 300 mg /kg was <0.02 and <0.01 administration 1hr and 4 hr after respectively. A research done by college of pharmacy in India on antidiabetic activity of *v.radiata* in alloxan induced diabetic model in wistar rats indicated that the value of p for lowering blood glucose level was less than 0.001[10].This indicates that the active ingredients that are present in *v.radiata* found in India have greater antidiabetic activity than *v.radiata* in Ethiopia.

CONCLUSION

In conclusion, the aqueous extract of *v.radiata* possesses significant antidiabetic activity. The antidiabetic activity of this plant is dose dependent i.e. as dose increases; its antidiabetic activity also increases. This positive result invites further to investigate active constituents responsible for antidiabetic activity.

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