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ANTIDIABETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *ANANAS COMOSUS* L. LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study was to investigate the presence of Antidiabetic activity on the hydro-alcoholic extract of *Ananas comosus* L. Leaves. The extracts were obtained using soxhelation method and the Antidiabetic activity tested using streptozotocin induced diabetic rats. After the oral administration of hydro-alcoholic extract at doses of 200 mg/kg, 400mg/kg and 600 mg/kg body weight blood glucose levels and body weights were monitored at specific intervals. In chronic model of diabetic, hydro-alcoholic extract of *Ananas comosus* L.leaves (HEAC) at a dose of 200 mg/kg, 400 mg/kg, 600 mg/kg and glibenclamide (5 mg/kg) were administered for 21 days. In our study, both glibenclamide and HEAC significantly decreases fasting blood glucose and increases the body weight in streptozotocin induced diabetic rats as compared to the animals in the diabetic control group. The antidiabetic activity of HEAC was comparable to that of standard drug glibenclamide at a dose of 5 mg/kg. The present investigation reveals that hydroalcoholic leaves extract of dose 600mg/kg showing good antidiabetic property in streptozotocin induced diabetic rats. This investigation concludes *Ananas comosus* L. Leaves hydroalcholic extract possess Antidiabetic activity against streptozotocin induced diabetic rats.

Keywords: *Ananas comosus,* Antidiabetic activity, Streptozotocin and Hydro-alcoholic extract.

INTRODUCTION

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Defective insulin secretion is the major cause for chronic hyperglycemia resulting in impaired function or serious damage to many of the body's systems, like eyes, kidneys, nerves, heart and blood vessels[1,2].

The common signs and symptoms are excessive thirst and urination, weight loss or gain, fatigue, and influenza–like symptoms. Early diabetes symptoms can be very mild and often even unnoticeable. Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world[3, 4].

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases. Most patients can be classified clinically as having either Type 1 diabetes mellitus (IDDM).

It is an auto immune type I the main cause of this beta cell loss is a T-cell mediated autoimmune attack. It is also characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to a deficiency of insulin[5]. Type 2 diabetes mellitus (NIDDM) is characterized differently due to insulin resistance or reduced insulin sensitivity, combined with reduced insulin secretion. Variants in 11 genes significantly associated with the

risk of Type 2 diabetes of these 8 genes are responsible for impaired beta-cell function[6].

The World Health Organisation has estimated that perhaps 80% of earths 6 billion inhabitance rely upon traditional medicine for their primary health care needs, and a major part of this therapy involves the use of plant extracts or their active principles. Nearly all 'wonder drugs' in use today are derived from natural products of about 120 plant derived drugs commonly in use in one or more countries, 74% were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine[7]. Well known examples include the cardiac glycosides from Digitalis purpurea, the antihypertensive agent and tranquillizer reserpine from Rauwolfia serpentine, the antimalarial agent quinine from Cinchona spp and the analgesics, codeine and morphine from Papaver somniferum. Secondary metabolites isolated from medicinal plants have also served as precursors or models for the preparation of effective agents through semisynthesis or lead-based total synthesis. Examples include in anticancer agents, etoposide a semisynthetic derivative of epipodophyllotoxin isolated from Podophyllum spp[8,9] and anticholinergic drugs modeled on the Belladonna alkaloids isolated from Atropa belladonna.

Ananas comosus (L.) MERRILL. (Family: Bromeliaceae), also named pineapple, has long been one of the most popular of tropical and subtropical fruits. It is grown extensively in Hawaii, Philippines, Caribbean area, Malaysia, Taiwan, Thailand, Australia, Mexico, Kenya, South Africa, India, Hainan province of China and so on. Besides agricultural utilities such as being a fruit with nutritional value, some folk medicinal uses have been found. In Thailand, *A. comosus* was used as an indigenous medicinal plant[10,11] for the treatment of dysuria. In China, *A. comosus* cortexes served as alexipharmic, antitussive, and antidiarrheal agents and *A. comosus* leaves were usually used as an antidyspepsia or antidiarrheal agent in Chinese Traditional Medicine[12].

In Australia, some molecules from *A. comosus* leaves are claimed to have an antitumor effect. Some studies claim that, the extract of *A. comosus* leaves (AC) enriched with phenols has anti-hyperlipidemic, antidiabetic[13], and anti-oxidative effects. However, its antidiabetic mechanism remains undetermined in streptozotocin induced diabetic rats. Considering the complexity of both antidiabetic mechanisms and components of AC, we will investigate the activities of AC in multi-models of rats to elucidate its antidiabetic mechanisms.

MATERIALS AND METHODS

Plant Material: Leaves of *A. comosus* were collected from the pineapple fields Ernakulam kerala in the month of June 2011 and the identity was confirmed by Mrs. Vasundara Professor Horticulture department GKVK Bangalore. The leaves were shade dried at room temperature.

***Preparation of Extract*[14]:** The leaves after drying were coarsely powdered, passed through sieve and extracted with hydroalcoholic solution (70% ethanol) using soxhelt apparatus.

Animals:Albino rats weighing 150-180 g were procured from Biogen Bangalore. The animals were kept in polypropylene cages 6 in each cage under standard laboratory conditions (12:12 hour light / dark cycle) and had a free access to commercial pellet diet and water *ad libitum.* The animal house was maintained at 27^{o} $\pm 2^{o}$ C temperature and humidity was maintained at 50% ±2. The study was conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Gautham College of Pharmacy, Bangalore, Karnataka, India. Registration No. 491/01/c/CPCSEA.

Acute Toxicity Studies[15]: Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose level of 100 mg/kg body weight by intragastric tube and observed for 14 days. Mortality was not observed, and the procedure was repeated for further higher dose such as 200, 500 and 2000 mg/kg body weight. Then intermittently and at the end, number of deaths was noted to calculate LD_{50}.

Induction of Diabetes in Rats[16]: After one week of acclimatization, the rats were subjected to overnight fasting. Diabetes was induced with a single intraperitoneal injection of STZ, freshly dissolved in citrate buffer 0.01M, pH 4.5 at a dose of 50 mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. After 3 days, blood glucose levels were measured and the animals with a glucose

concentration of more than 230 mg/dL were classified as diabetic[17] and taken for the experiment. Administration of the single and composite plant extracts was started on 4th day after STZ injection and this was considered the 0^{th} day of treatment, which was continued for 21 days.

Experimental Design[18]: Group-I: Distilled water was supplied and served as normal control (for this group Streptozotocin was not administered).

Group-II: Saline was supplied and served as diabetic control.
Group-III: Standard drug Glibenclamide[19] was supplied at a dose of 5 mg/kg orally.
Group-IV: A dose of HEAC 200mg/kg body weight was supplied orally in distilled water.
Group-V: A dose of HEAC 400mg/kg body weight was supplied orally in distilled water.
Group-VI: A dose of HEAC 600mg/kg body weight was supplied orally in distilled water.

The body weight and blood glucose level were measured at about every 7 days interval. Blood samples were obtained by tail vein puncture of both the normal and STZ induced diabetic rats. Blood glucose levels were measured by using the glucose oxidase-peroxidase reactive strips and a glucometer (Sugarchek, Wockhardt, India).

Statistical Analysis: The mean ± S.E.M values were calculated for each group. The data was analyzed using one way ANOVA followed by Dunnett's multiple comparison tests. $P< 0.05$ was considered to be statistically significant.

RESULTS

In acute toxicity study the hydroalcoholic extract of *Ananas comosus (L.)*. leaves did not produce lethality up to the dose level of 2000mg/kg. In the antidiabetic activity, the effects of hydroalcoholic extract of *Ananas comosus (L.)*. leaves on body weight is measured on 0^{th}, 7^{th}, 14^{th} and 21^{st} day of post induction and first diabetic control groups were compared with normal control and then treated groups were compared with diabetic control groups. The values are shown in Table No-1. Streptozotocin induced diabetic rats showed a significant decrease ($P<0.05$) in body weight compared to normal rats. Oral administration of HEAC at the dose of 400 mg/kg showed extremely significant increase ($P<0.001$) in body weight on 21^{st} day and oral administration of HEAC at the dose of 600 mg/kg showed a significant increase ($P<0.01$) in body weight on 14^{th} day and extremely significant increase ($P<0.001$) in body weight on 21^{st} day of post induction when compared to untreated diabetic rats.

The effects of hydroalcoholic extract of *Ananas comosus* L. leaves on fasting blood glucose level is measured on 0^{th}, 7^{th}, 14^{th} and 21^{st} day of post induction and first diabetic control groups were compared with normal control and then treated groups were compared with diabetic control groups. The values are shown in Table No-2. Streptozotocin induced rats showed extreme significant increase ($P<0.001$) in fasting blood glucose level compared to normal rats. Oral administration of HEAC at the dose of 400 mg/kg body weight showed a very significant decrease ($P<0.01$) in blood glucose level on 14^{th} day of treatment and extremely significant decrease ($P<0.001$) in blood glucose level on 21^{st} day of treatment. The fasting blood glucose level on 14^{th} and 21^{st} days of post induction were 244.0 ±2.869 and 235.5 ±1.323 respectively and oral administration of HEAC at the dose of 600 mg/kg body weight showed a extremely significant decrease ($P<0.001$) in blood glucose level on 14^{th} and 21^{st} day of treatment. The fasting blood glucose level on 14^{th} and 21^{st} days of post induction were 238.0 ±0.707 and 153.3 ±1.109 compared to fasting blood glucose of diabetic control animals 258.5 ±1.90 and 257.3 ±1.250 respectively. The group treated with Glibenclamide 5 mg/kg showed fasting blood glucose level of 104.0 ±1.225 mg/dl on 21^{st} day of post induction.

DISCUSSION

The hypoglycemic effect of plants has been paid more attention because of increasing incidence of diabetes and predominance of traditional plants in the therapy. The effective components of herbs that have antidiabetic property include alkaloids, oligosaccharides, polysaccharides, organic acids and flavonoids etc[20]. Streptozotocin induced diabetes has been described as a useful experimental model to study the activity of antidiabetic agents[21]. Streptozotocin induced diabetes is characterized by severe weight loss [22]. Hence, the weight gain after administration of the extract in severely diabetic rats is simply due to the ability of the extract to reduce hyperglycemia. In the present study the hypoglycemic activity of hydroalcoholic extract of *Ananas comosus* L. leaves was evaluated in Streptozotocin induced diabetic rats. The control of blood glucose level in diabetic rats was produced by continuous treatment of leaf extract for a period of 21 days which is comparable to that of standard drug Glibenclamide which is used in treatment of type-II diabetes mellitus.

Glibenclamide stimulates insulin secretion from beta cells of islets of langerhans. From the study, it is suggested that the possible mechanism by which the plant extract decreases the blood glucose level may be by potentiation of insulin effect either by increase in peripheral glucose uptake or by increase in pancreatic secretion of insulin from beta cells of islets of langerhans The above experimental model is sensitive and relatively specific to all major classes of oral hypoglycemic drugs.

CONCLUSION

Thus, it may be concluded that *Ananas comosus* L. leaves produced significant antidiabetic activity in streptozotocin induced diabetic rats. The efficacy of the *Ananas comosus* L. leaves was comparable to that of Glibenclamide. Further work is necessary to elucidate the mechanism of action involved in the antidiabetic activity of *Ananas comosus* L. Leaves with special references to phytochemicals.

Table 1: Effect of *Ananas comosus (L.)* leaves extract on body weight in STZ induced diabetic rats.

Groups	Treatment	Body weight (gms)			
		0^{th} Day	7^{th} Day	14^{th} Day	21^{st} Day
Group-I	Saline	183.5 ±3.379	185 ± 3.136	184.5 ± 3.17	185. 5 ±3.27
Group-II	Saline + STZ (50mg/kg)	175.8 ±5.543	165.3 ± 4.956**	157.8 ± 4.15***	148.5 ± 3.27***
Group-III	Glibenclamide (5mg/kg) + STZ (50 mg/kg)	187 ± 2.582	181.3 ± 2.323*	185.3 ± 2.17***	189 ± 2.483***
Group-IV	HAEC (200 mg/kg) + STZ (50 mg/kg)	167.3 ±4.230	158.5 ± 3.304	156.5 ± 3.304	158.0 ± 3.367
Group-V	HAEC (400 mg/kg) + STZ (50 mg/kg)	174.5 ±2.754	166.8 ± 3.092	168 ± 2.041	169.3 ±2.394***
Group-VI	HAEC (600 mg/kg) + STZ (50 mg/kg)	173.3±2.016	172±2.198	170.3±1.702*	174.0±1.958***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** represent extremely significant P<0.001, **represent very significant at P<0.01, * represent significant at P<0.05. All the values are compared with the diabetic control group.

Table 2: Effect of *Ananas comosus (L.)* leaves extract on blood glucose levels in STZ induced diabetic rats.

Groups	Treatment	Blood glucose levels (mg/dl)			
		0^{th} Day	7^{th} Day	14^{th} Day	21^{st} Day
Group-I	Saline	97.75± 3.568	98.50± 3.403	97.75±3.092	98.75± 3.902
Group-II	Saline + STZ (50 mg/kg)	256.3± 1.10***	257.5± 1.555***	258.5± 1.190***	257.3± 1.250***
Group-III	Glibenclamide (5mg/kg) + STZ (50 mg/kg)	256.5± 2.102	248.0± 1.080**	134.8±3.637**	104.0± 1.225***
Group-IV	HEAC (200 mg/kg) + STZ (50 mg/kg)	257.0± 1.080	255.0± 0.5774	254.3±2.869	248.0± 1.291*
Group-V	HEAC (400 mg/kg) + STZ (50 mg/kg)	254.8± 2.955	252.0± 0.7071	244.0± .8165**	235.5± 1.323***
Group-VI	HEAC (600 mg/kg) + STZ (50 mg/kg	256.3± 1.250	250.0± 0.7071*	238.0± 0.707***	153.3± 1.109***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** represent extremely significant P<0.001, **represent very significant at P<0.01, * represent significant at P<0.05. All the values are compared with the diabetic control group.

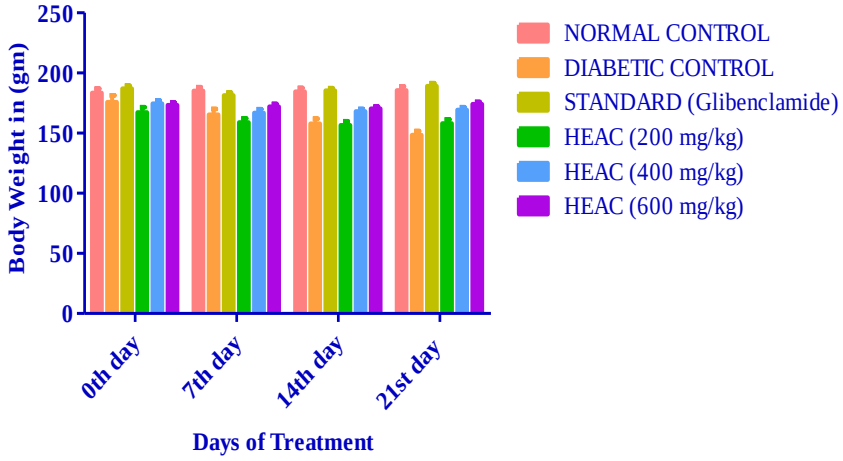

Figure 1: Effect of *Ananas comosus* L. leaves extract on body weight of STZ induced diabetic rats.

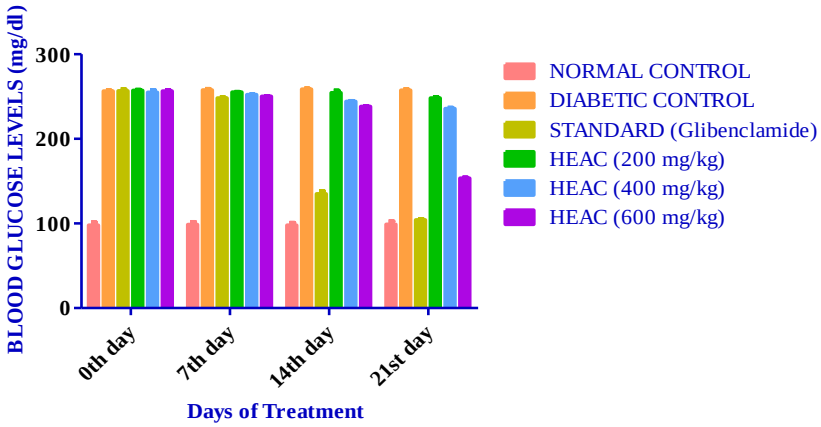

Figure 2: Effect of *Ananas comosus (L.)* leaves extract on blood glucose levels in STZ induced diabetic rats

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