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RESEARCH ARTICLE

STUDY OF ANTIDIABETIC ACTIVITY OF ALLI CHOORANAM (NYMPHAEA NOUCHALI)BURM.F)

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Abstract

Present study was undertaken to evaluate the antidiabetic activity of hydroalcoholic extract of powder of rhizome & flower of the plant Alli (Nymphaea nouchali burm.f.) in rats. Diabetes was induced by Single intraperitoneal injection of Streptozotocin monohydrate (STZ)(150mg/kg) and randomly divided in to five groups. Animals were treated with low (200mg\kg) & high dose (400mg\kg) of hydroalcoholic extract of powder of rhizome & flower of the plant Alli (Nymphea nouchali burm.f.)up to 28 days. Body weight & Blood glucose level (BGL) were measured in weekly basis. After 28 days of treatment animal were sacrificed by mild ether anesthesia blood & vital organ were collected to estimate biochemical parameters & to study histopathological changes. A single-dose administration of Streptozotocin induced hyperglycemia in all the groups. A regular rise in BGL was observed in toxic control groups when compared with the normal control. Daily oral administration of rats with extracts of Alli chooranam (Nymphaea nouchali burm.f.)and standard drug (Glibenclamide, 10 mg/kg), lowered elevated BGL significantly ($\mathbf{p} < 0.001$), and body weight was retrieved in diabetic rats.. The study revealed that Alli chooranam (Nymphea nouchali burm.f.)possesses .promising antidiabetic activity.

Introduction:-

Diabetes mellitus (DM), a disease affecting the metabolism of carbohydrates, fats, and proteins occurs when the pancreatic β-cell does not produce a sufficient amount of insulin that the body needs for proper dispersal of blood glucose in different tissue cells. Polydipsia, polyuria, polyphagia, and weight loss are some of the common symptoms of DM. It has been reported in various studies that diabetes are more vulnerable to papillary necrosis and glomerular lesions, urinary tract infection, and renal atherosclerosis [MohdNazam et al 2021].The universal prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. Moreover, the prevalence of diabetes has also been found to steadily increase for the past 3 decades and has risen faster in low- and middle-income countries compared to high-income countries. The increase in the prevalence of diabetes is parallel with an increase in associated risk factors such as being overweight or obese. [Bahare et al,2019].. Invitro, in vivo and clinical studies are focused on uncovering the mechanisms by which altered glucose converge to affect peripheral nerve function and health to support the development of potential mechanism-based therapies. It is proved that N.nouchali hydroalcoholic seed extract has DDPH scavenging activity, nitric oxide scavenging activity & lipid peroxidation inhibitory activity.[Mable et al2014]

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The Preparation and standardization of medicinal herbs are urgently need for future studies and therapies. The Alli (Nymphaea nouchali Bum,f) large aquatic herb of the family Nymphaeaceae, commonly known as Water lily (Alli in Tamil).Aquatic perennial herb lactiferous rooted. Flowers bisexual floating &solitary. It is native to tropical Asia, tropical Africa & Australia. Siddha medicine recommended flower & rhizome of this plant has astringent & emollient action can be used in the treatment of Diabetes mellitus,urinary diseases,eye diseases & for healing ulcers. Although antidiabetic activity of Alli (Nymphaea nouchali Bum,f) have been reported, lack of sufficient literature on flower & rhizome. This study was focused on evaluating antidiabetic activity of hydroethanolic extract (HEE) of powder of rhizome & flower of the plant

Materials And Methods:-

Collection and Authentication of Plant

The flower & rhizome of Alli (Nymphaea nouchali Burm.f)freshly collected from various places of Kerala. Identified and authenticated by the Medicinal Botanist at Government Siddha Medical College and Hospital, Palayamkottai. These herbal formulations purified according to the suitable procedure methods described in Siddha classical literature. The drug is dried and subjected to size reduction to get uniform coarse powder. The powdered material then subjected to excessive extraction using water & ethanol solvents in a Soxhlet extractor.

Selection and acclimatization of animals

Wistar strains of albino rats weighing between 180-200g are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water ad libitum. The animals were well acclimatized to the standard environmental condition of temperature (22 ± 5^0c) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

Evaluation of Antidiabetic activity

Assessment of antidiabetic activity in Streptozotocin monohydrate (STZ) rats

Diabetes mellitus was induced in Wister rats by single intraperitoneal injection of freshly prepared solution of STZ monohydrate (150mg/kg Body Weight) in physiological saline after overnight fasting for 12hr.STZ is commonly used to produce Diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as H_2O_2,O_2. The development of hyperglycemia in rats is confirmed by plasma glucose estimation 72 hr post STZ injection. The rats with fasting plasma glucose level of 200-260mg/dl was used for this experiment.

The rats were divided into 5 groups after the induction of STZ diabetes.
Group-I: Normal control treated with normal saline (10ml/Kg,PO)
Group-II: Diabetic control STZ monohydrate (150mg/kg) & normal saline (10ml/Kg,PO)
Group-III: Diabetic rat treated withGlibenclamide (10mg/Kg,PO)
Group-IV: Diabetic rat treated with low dose of Alli (Nymphaea nouchali Burm.f)(200mg/Kg, PO)
Group-V: Diabetic rat treated with high dose of Alli (Nymphaea nouchali Burm.f) (400mg/Kg, PO)

Treatment once started continued up to 28 days. Both, vehicle and the drug solution were administered daily through gastric intubation at a specified time. The BGL was measured on 0, 7, 14, and 28 days to evaluate the effect of extract and standard drug. At the end of the study, maximum blood was collected from the retro-orbital plexus, and animals were sacrificed by cervical dislocation under light ether anesthesia. The abdominal cavity was opened and liver, pancreas, and kidney tissues were extracted to examine the effect of hyperglycemia at the cellular level by histopathology. Various biochemical parameters such as blood glucose, Lipid profile Hepatic glucokinase and hexokinase activity, Glucose-6-phosphatase activity, were evaluated to assess the normal functioning of the vital organs such as liver and kidneys. Histopathological examination of pancreas also carried out.

Statistical Analysis

All the values were expressed as mean ± standard error of mean. The data were statistically analyzed by one-way ANOVA followed by Dennett's t-test, and value $P < 0.05$ was considered to be significant. Statistical analysis was performed using INSTAT- V3 Software programme.

Results:-

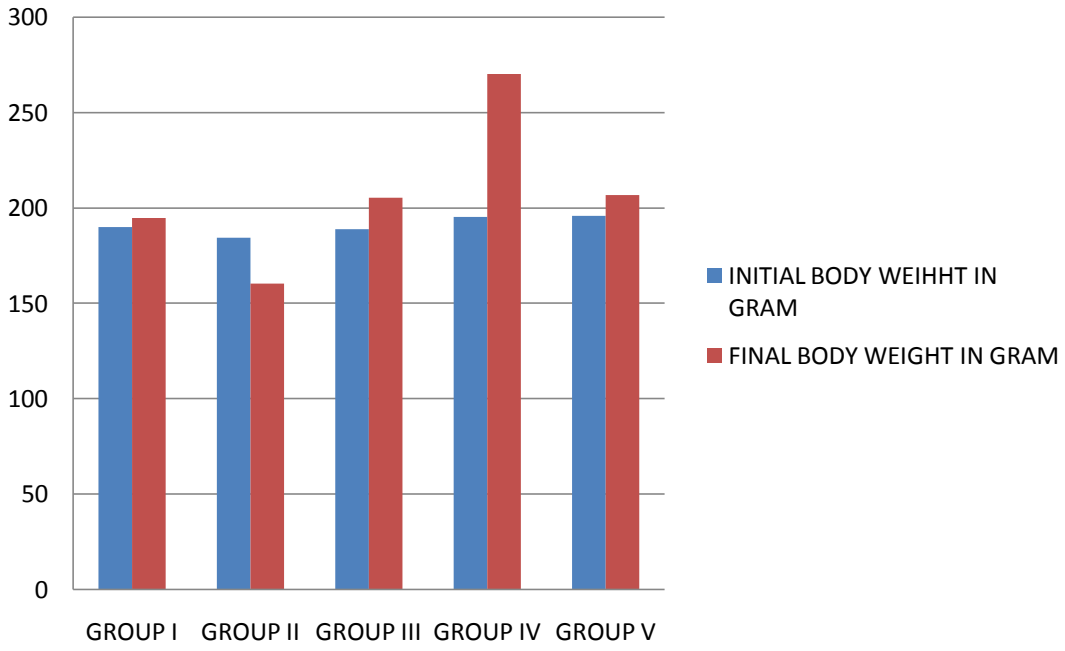
Evaluation of Antidiabetic activity

Effect of HEE on body weight of normal and experimental animals in each group

Groups	Initial Body Weight (g)	Final Body Weight (g)
Group I	190.06 ± 0.24	194.90 ± 0.85
Group II	184.55 ± 0.84	160.42 ± 0.80
Group III	188.98 ± 0.90	205.50 ± 0.45
Group IV	195.40 ± 0.85	207.40 ± 0.42
Group V	195.92 ± 0.16	206.67 ± 0.60

All values expressed as mean± SEM. * = p < 0.001, when compared to control. (G-I).

Figure 3.1.a:- Effect of HEE on body weight of normal and experimental animals in each group



The body weight of rats was reduced after STZ administration significantly (P<0.001) than normal control rats. A significant (P<0.001) increased bodyweight was noticed in type 2 diabetic rats after the oral administration of HEE on 0^{th}&28^{th} day, compared to the control group. (Table 3.1.a).

Table 3.1.b:- Effect of treatment with various doses of HEE on glucose levels (mg %) in STZ diabetic rats.

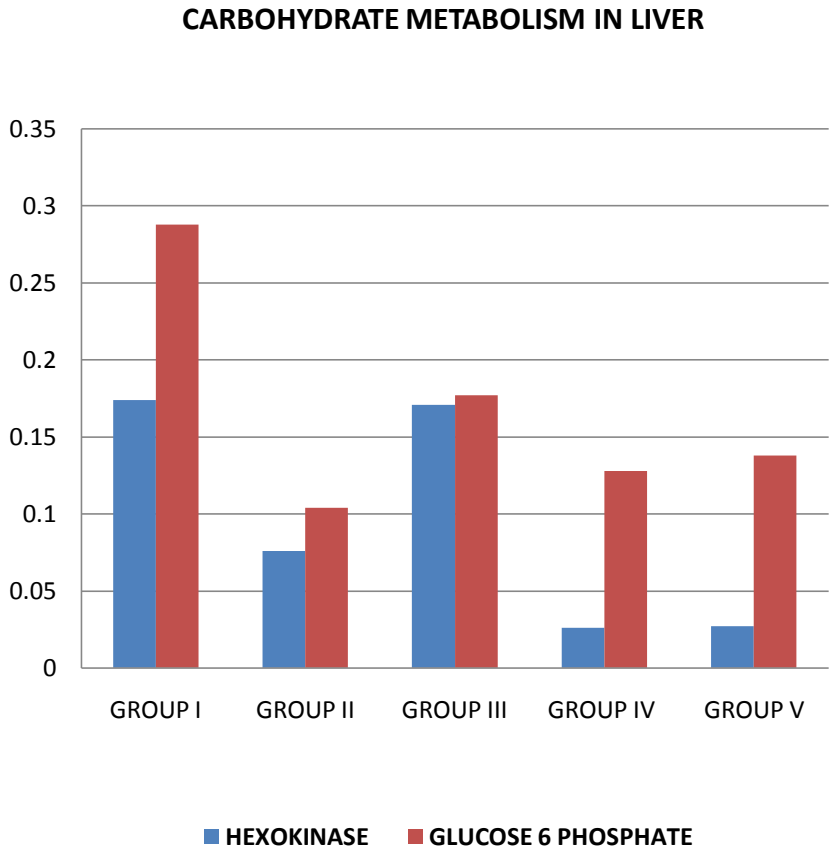
Groups	0Day		14^{th} Day		28^{th} Day	
	Before Meals	After Meals	Before Meals	After meals	Before Meals	After Meals
G I	72.1±0.72	77.6 ±0.72	67.75±0.25	72.80 ± 0.65	67.07±0.23	73.12 ± 0.23
G II	144.55±0.70	149.60 ± .70	156.0±0.20	170.05 ± 0.20	105.1±0.55	210.6 ± 0.45
G III	77.44±0.20	82.92 ± 0.60	74.30±0.18	79.35 ± 0.28	64.17±0.40	69.42 ± 0.40
G IV	115.53±0.15	120.28 ±0.05	105.30±0.92	110.35 ± 0.52	91.40±0.40	96.55 ± 0.40
G V	113.55±0.18	118.60 ± .28	78.41±0.46	83.56 ± 0.46	76.45±0.80	81.50 ± 1.10

Administration of STZ significantly (P<0.001) increased the blood glucose level compared to normal control rats. A significant reduction (P<0.001) of blood glucose level was noticed in type 2 diabetic rats after the oral administration of HEE on 0^{th},14^{th}& 28^{th} day, compared to the control group. (Table 3.1.b)

Table 3.1.c:- Effect of HEE on enzymes involved in carbohydrate metabolism in rats.

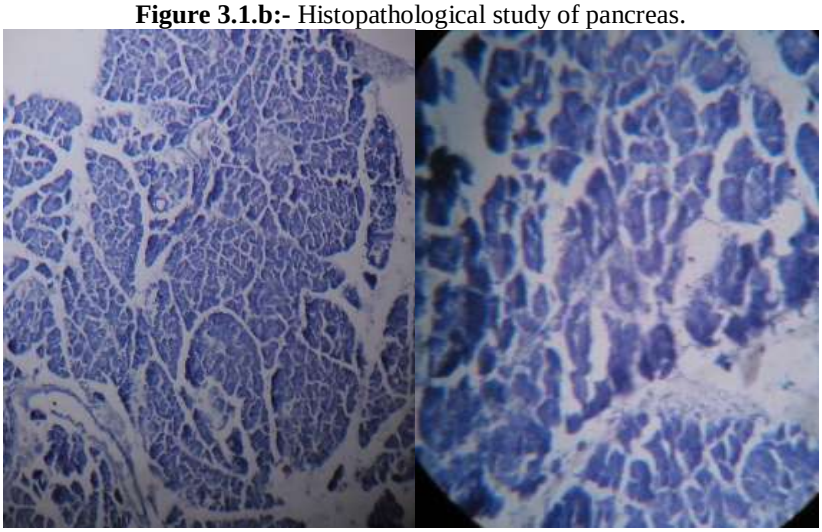
Groups	Hexokinase (µg/mg)	Glucose-6-Phosphate (µg/mg)	Glucokinase (µg/mg)
Group I	**0.174 ± 0.27**	**0.288 ± 0.36**	**18.90 ± 0.24**
Group II	**0.076 ± 0.03**	**0.104 ± 0.23**	**15.79 ± 0.24**
Group III	**0.171 ± 0.05**	**0.177 ± 0.06**	**11.20 ± 0.88**
Group IV	**0.026 ± 0.04**	**0.128± 0.03**	**8.15 ± 0.46**
Group V	**0.027 ± 1.06**	**0.138 ± 0.04**	**7.16 ± 0.86**

Figure 3.1.a:- Effect of HEE on enzymes involved in carbohydrate metabolism in rats.

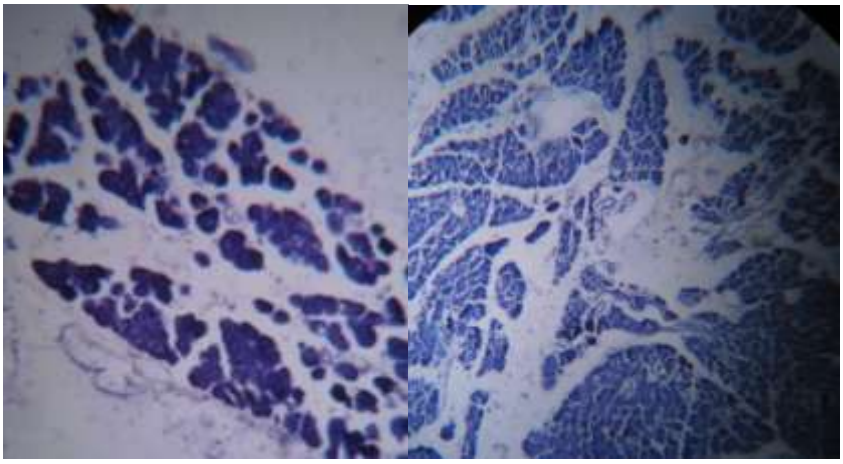


Enzyme Levels of the diabetic control group (G-II) has shown a significant decrease on the 28th day, once compared to the normal group (G-I). The enzyme has shown a significant rise in the standard group (G-III) which received Glibenclamide, compared to the diabetic control group (G-II). With the management of HEE in three different doses (G-IV and GV,) on the 28th day, a significant increase in enzyme levels also noted, compared to the diabetic control group (G-II) (Table 3.1.c**)**.

Figure 3.1.b:- Histopathological study of pancreas.

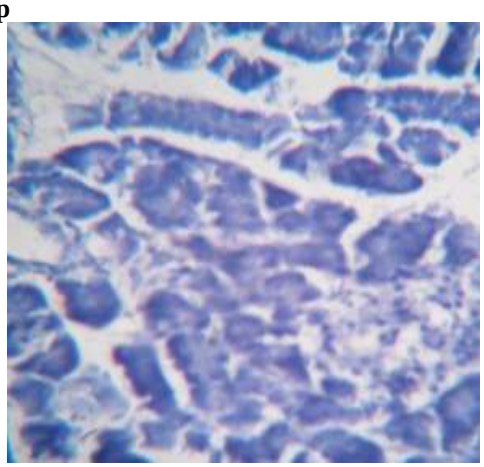


G I: Normal Control **G II: Toxic Contol**



G III: Positive Control **G IV: Treatment group**

G V: Treatment group



Histopathological studies revealed that HEE and Glibenclamide significantly improved the histological architecture of the islets of langerhans. The groups treated with HEE (200mg/kg and 400mg/kg) and Glibenclamide (10mg/kg) showed greater persistence of islets of langerhans and lesser degree of necrotic changes as compared to the untreated STZ-induced diabetic rats.

Discussion:-

Diabetes mellitus is a collection of disorders, which results from either lack of insulin or factors which interfere with the action of this hormone.The disease is progressive and is associated with high risk of atherosclerosis, kidney and nerve damage as well as blindness.[Rajagopal et al2008]).

HEE at high dose (400mg/kg) and low dose (200mg/kg) exhibited significant anti-hyperglycemic activity in normal and STZ-diabetic rats. This powder showed improvement in the parameters like body weight and carbohydrate metabolizing enzymes as well as regeneration of β-cells of pancreas.In mild diabetes, the maximum percent reduction in glucose level was seen in groups receiving per day. This could be due to potentiation of insulin effect of plasma by increasing their pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of extract in STZ-induced diabetic rats is also due to enhanced glucose utilization by peripheral tissues

The body weights were decreased in STZ-induced diabetic rats**.** The administration of two doses shows increased body weight in STZ-induced diabetic rats. The ability of to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.Decreased enzymatic activity of Hexokinase, Glucokinase and substrate glucose-6-phosphate has been reported in diabetic animals resulting in depletion of liver and muscle glycogen**.** ([Hikino et al] 1989)The present study also had similar results. Treatment with HEE significantly increased the hexokinase, Glucokinase activity and glucose-6-phosphate level in the liver, indicating an overall increase in glucose influx to have an overall effect of increase in glucose utilization.

Histopathological studies revealed that HEE and Glibenclamide significantly improved the histological architecture of the islets of langerhans. The groups treated with HEE(200mg/kg and 400mg/kg) and Glibenclamide (10mg/kg) showed greater persistence of islets of langerhans and lesser degree of necrotic changes as compared to the untreated STZ-induced diabetic rats.

Conclusion:-

The results of the study demonstrated study hydroethanolic extract of powder of rhizome & flower of the plantAlli (Nymphaea nouchali Burm.f) possess antidiabetic effect.These effects were statistically analyzed by ANNOVA & found to be significant. ($p<0.05$)

Further studies are required to identify, isolate & characterize active principle responsible for the antidiabetic activity of the plant.

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Conflict of interest:

The author declares no conflict of interest in the present work.

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