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EXTRACTION AND EVALUATION OF ANTIDIABETIC EFFECT IN FOLK MEDICINAL PLANT AND DIABETIC INDUCED RAT BY EXTERNAL CHEMICAL SUBSTANCE

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ABSTRACT

Diabetes *mellitus*, it's not a disease and its group of metabolic disorders characterized by an imbalanced blood sugar level or hyperglycemia over a prolonged period. For in this study diabetes was induced by injection of a single intra-peritoneal dose of Alloxan monohydrate (freshly prepared in 0.1% normal saline). Overnight fasted rats were injected with Alloxan (alloxan; 120mg/kg body wt., *i.p*) to induce diabetes. Diabetic was confirmed by glucose estimation. Animal with plasma glucose level > 200mg/dl were selected for the study. Diabetic induced Animals were grouped for further study. After 3 days of alloxan induction, treatment was started. Weak preliminary evidence hints that Tylophora might have anti-inflammatory, reduces asthma symptoms, antiallergic, and antispasmodic actions and regulate blood glucose level. Preliminary phytochemical studies revealed the presence of alkaloids, carbohydrates, steroids, saponins, and triterpenes.

KEYWORDS

Diabetes, Alloxan, Alkaloids, Carbohydrates, Steroids, Saponins and Triterpenes.

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INTRODUCTON

The term diabetes mellitus portrays a metabolic issue of numerous etiology described by constant hyperglycemia with aggravations of sugar, fat and protein digestion coming about because of imperfections in insulin emission, insulin activity, or both. The long-term effects of diabetes mellitus include organ failure, dysfunction, and damage. Typical signs of diabetes mellitus can include thirst, polyuria, blurred vision, and weight loss. Ketoacidosis or a non-ketotic hyperosmolar state can develop to the point of stupor, coma and death

if no effective treatment is provided. As a result, hyperglycemia sufficient to cause pathological and functional changes may be present for a considerable amount of time before the diagnosis is made because symptoms may be mild or absent[1-4]. The progressive development of the specific complications of retinopathy, which may result in blindness, nephropathy, which may result in renal failure, and/or neuropathy, which may result in foot ulcers, amputation, Charcot joints, and autonomic dysfunction, including sexual dysfunction, are some of the long-term effects of diabetes mellitus. Cardiovascular, peripheral vascular, and cerebrovascular disease are more common in diabetics[5-7].

Diabetes is the result of a number of pathogenetic processes. These include processes that cause the pancreas' beta cells to be destroyed, resulting in insulin deficiency and resistance to insulin action. Deficient insulin action on target tissues as a result of insulin insensitivity or deficiency is the root cause of the abnormalities in protein, carbohydrate, and fat metabolism[8-10].

TYPES OF DIABETES

Type 1

Results from the body's failure to produce insulin. It is estimated that 5-10% of Americans who are diagnosed with diabetes have type 1 diabetes. Presently most persons with type 1 diabetes take insulin injections.

Type 2

Results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with relative insulin deficiency. Most Americans who are diagnosed with diabetes have type 2 diabetes. Many people destined to develop type 2 diabetes spend many years in a state of Pre-diabetes: Termed "America's largest healthcare epidemic, "pre-diabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. As of 2009 there are 57 million Americans who have pre-diabetes[10,11].

Gestational diabetes

It is known as gestational diabetes when pregnant women who have never had diabetes before have high blood sugar glucose levels during pregnancy. About 4% of pregnant women suffer from gestational diabetes. It may occur before type 2 or rarely type 1 develops. Apart from these, there are many other types of diabetes mellitus. Several types of monogenic diabetes, steroid diabetes caused by high doses of glucocorticoids, and congenital diabetes caused by genetic insulin secretion defects are examples[12].

MATERIAL AND METHODS

Material

Tylophora indica powder, Methol, Ethanol, Ether, Wistar Albino Rats.

Extraction

Ethonolic Maceration Extract of Tylophora-indica has prepared.

Animals

The Animal House provided 150-200g Wistar Albino Rats; Rats were fed a standard pellet diet and ad libitum tap water. They were acclimatized to their surroundings for two weeks prior to the experimental use, and they were kept in clean cages with a 12 hour light/dark cycle and a room temperature of 22-24^{o}C. The guidelines that were approved by the Institutional Animal Ethics Committee were followed during the course of this study.

INDUCTION OF DIABETES

Alloxan monohydrate (freshly prepared in 0.1% normal saline solution) was injected into the peritoneum to cause diabetes. Alloxan was injected into rats that had fasted overnight (alloxan; 120mg/kg of body weight, to bring about diabetes. Glucose estimation confirmed diabetes. The animals selected for the study had plasma glucose levels greater than 200mg/dl. Animals with diabetes were grouped for further research. The induction with alloxan lasted three days before treatment began.

Experimental design

The rats were divided in to 5 groups having 5 animals.

I-Group: Control
II-Group: Alloxan (120mg/kg)
III-Group: Glibenclamide (150mg/kg)
VI –Group: Plant extract (300mg/kg)
V-Group: Total alkaloid of Plant (3gm/kg).

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The ethonolic extract of tylophora-indica was examined preliminary phytochemical screening of all the test conducted and the result was showed in Table No.1.

Determination of total ASH

In a tarred platinum or silica dish, approximately 2grams of air-dried crude drug were accurately weighed before being incinerated at a temperature not exceeding 4500 degrees Celsius until carbon was removed, after which it was cooled and weighed. The drug was air-dried when the percentage of ash was calculated.

Determination of water soluble ASH

The total ash was boiled for 5.0 min, with 25.0ml of water. The insoluble matter was collected in a gooch crucible or an ash less filter paper. It was washed with hot water and ignited for 15min, at a temperature not exceeding 4500^{o}C. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in the weight of the ash, represent the water soluble ash. The percentage of water soluble ash calculated with reference to the air dried drug.

Determination of acid in solulbe ASH

The was boiled with 25ml of 2M HCL for 15min. the insoluble matter was collected in a Gooch crucible or an ash less filter paper. It was washed with hot water and ignited; it was then cooled in a desiccators and weighed. The percentage of water soluble ash calculated with reference to the air dried drug.

Alcohol soluble extractive

Took 5 grams of the powder were macerated for 24 hours in a closed flask with 100 milliliters of the specified strength of alcohol, shaking frequently for 6 hours, and left to stand for 18 hours. It was quickly filtered to prevent alcohol loss, and 25 milliliters of the filtrate were weighed after being evaporated to dryness at 105 degrees Celsius. With regard to the drug that had been air dried, the percentage of water-soluble extractive was gathered.

Water soluble extractive

In a closed flask, 5g of the powder was macerated with 100ml of water for 24 hours, shaking frequently for 6 hours, and allowed to stand for 18 hours. It was quickly filtered to prevent alcohol loss, and 25 milliliters of the filtrate were weighed after being evaporated to dryness at 105 degrees Celsius. With regard to the drug that had been air dried, the percentage of water-soluble extractive was gathered.

Loss on drying

The percentage of weight lost after drying is referred to as the loss on drying procedure. To be used in the determination, a glass weighing bottle with a stopper that had been dried for 30 minutes in the same conditions was weighed. The bottle and its contents were precisely weighed after the sample was placed inside and covered. The sample was evenly distributed up to a maximum depth of 10mm. The stopper was removed before the loaded bottle was placed in the oven's drying chamber. At a temperature of 110 degrees Celsius in hot air, the sample was dried to a constant weight.

Fluorescence analysis

Fluorescence characteristics of the powdered drug with different chemicals were observed in day light and ultraviolet light. Various solvent extracts were also subjected to day light and ultraviolet light for its fluorescence characteristics. The powder was treated with neutral solvents like methanol, water and acid like 1N HCL, 50%HCL, 50%H_2SO_4, 50%HNO_3, alkaline solution like 1N NaOH an alcoholic IN NaOH.

Quantitative determination of glucose in serum

The overnight fasted normal, diabetic control (DC) and drug-treated diabetic animals' serum glucose levels were estimated to be 200mg/kg and 400mg/kg, respectively. To separate serum, vials of blood were taken from the tail vein and allowed to clot. To obtain clear serum, it is then centrifuged for ten minutes at 2500rpm. With 1ml of kit reagent and 10 minutes at 37°C, 10 l of serum was added. Mix and determine the absorbance at 505nm. Table No.2 depicts the effect of Tylophora Indica on the serum glucose levels of diabetic alloxane-induced rats.

Oral glucose tolerance test

Ordinary and alloxan diabetic rodent, abstained for the time being nevertheless given water not obligatory, were managed the test tests orally one hand and a half hour preceding the oral glucose heap of 2mg/kg b.w. The glucose concentration was

measured before and after the glucose load, at 30, 60, 90 and 120 minutes. Only the glucose load was given to the control group. Effect of Tylophora Indica on Oral glucose tolerance test in alloxane induced diabetic rats showed in Table No.3.

ESTIMATION OF CREATININE IN BLOOD

Pipetted out 100mL of the sample, 1ml of the working reagent was added.The same treatment was given to Standard. The absorbance taken at 510nm for both the Standard (ST) and the Test (TS) at 0 seconds, 20 seconds (ST1, TS1) and 80 seconds (ST2, TS2) against distilled water. The serum creatinine concentration was expressed in mg/dl.

Discussion

Alloxan is frequently utilized in studies of experimental diabetes to measure healthy individuals' fasting blood glucose levels because it kills pancreatic islet cells with high specificity. Alloxan's own free radicals start a chain reaction that eventually leads to cell death and hypoinsulinemia, which is the most common explanation for diabetes. It has been discovered that glucose kinase, in contrast to the higher affinity hexo-kinase types I-III, is a low affinity glucose phosphorylating enzyme that is extremely sensitive to sulphydryl group reactive reagents like alloxan and highly susceptible to sulphydryl group oxidation. Alloxan additionally annihilated cell capability by decreasing the movement of the glucokinase catalyst by oxidizing two thiol bunches situated in the compound's glucose restricting site.

The typical blood glucose levels are between 70-100mg/dl. In diabetics, this level may rise to 500mg/dl or higher. This expansion in glucose levels is alluded as hyperglycemia. This happens principally because of lack of insulin. Hyperactivity of the pituitary, thyroid, and adrenal glands also causes slight increases. Hormonal disorders such as hypothyroidism can occasionally result in hypoglycemia. On diabetic rats, the continuous 28-day treatment with the ethanolic extract of Tylophora indica, but not tylophorine, resulted in a significant decrease in blood glucose levels, whereas on normal treated rats, no such effect was observed. This is a fascinating observation because neither hypoglycemic shock nor an accidental overdose of the extract will occur. The lower dose of the extract itself exhibits its activity, and the effect was observed to be dose dependent. It was discovered that this drug significantly decreased the level of glucose (p 0.001).

Table No.1: Preliminary phytochemical screening (Preliminary-Chemical Test)

S.No	Test	Result
1	Test for carbohydraes Test for starch	(+) (+)
2	Tests for proteins and aminoacids	(-)
3	Test for alkaloids	(-)
4	Test for flavonoids	(+)
5	Test for tannins	(+)
6	Test for phytosterols	(+)
7	Test for saponons	(-)
8	Test for glycosides	(-)
9	Test for quinones	Positive
10	Test for anthocyanins	Positive

Table No.2: Effect Tylophora Indica on serum glucose levels in alloxane induced diabetic rats

S.No	Treatment (mg/kg)	0 DAY	7th DAY	14th DAY	21st DAY	28th DAY
1	Control	112.66±0.7	96.16±1.19	95.66±1.20	101.33±1.9	96.83±0.79
2	D.control	307±3.93	319±4.50	324.33±5.4	311.81±5.1	302.83±6.1
3	Standard	315.66±6.5	119.83±2.1	96±1.18	95.83±0.88	92.50±0.99
4	TY600mg/kg	301.88±169	115.83±1.2	105.16±0.7	103.33±0.4	95.66±0.66
5	TY-500mg/kg	304.16±1.6	137.83±1.4	126.16±0.6	120.66±0.7	110.66±2.3
6	TY-400mg/kg	294.16±0.9	166.50±2.2	145.50±1.11	132.66±0.80	118.50±1.33

Results are expressed as mean±SEM, n=6, $p<0.001$ vs alloxane induced group using Two way ANOVA followed by Bonferroni-post test.

Table No.3: Effect of Tylophora Indica on oral glucose tolerance test in alloxane induced diabetic rats

S.No	Treatment (mg/kg	-30(min)	0	30	60	90	120
1	Control	108.5±37.8	124.3±2.24	257±6.03	295.8±5.6	243.8±6.84	209.3±4.4
2	D.control	278±8.35	298.5±4.23	326±2.2	362.6±4.64	32.3±2.51	294.1±3.82
3	Standard	93.66±28.9	151.3±3.07	172.6±1.70	108.8±1.35	137±1.63	89.16±2.94
4	TY-400mg/kg	89.83±1.07	86.5±1.23	159±1.78	228.5±12.72	115.3±1.49	87±1.13
5	TY-500mg/kg	98.83±1.57	87.6±1.56	158.1±1.22	227.5±1.91	128.8±1.66	93.5±1.47
6	TY-600mg/kg	93.83±1.24	29.6±1.33	160.1±0.79	220.8±1.51	147.1±1.35	104±1.86

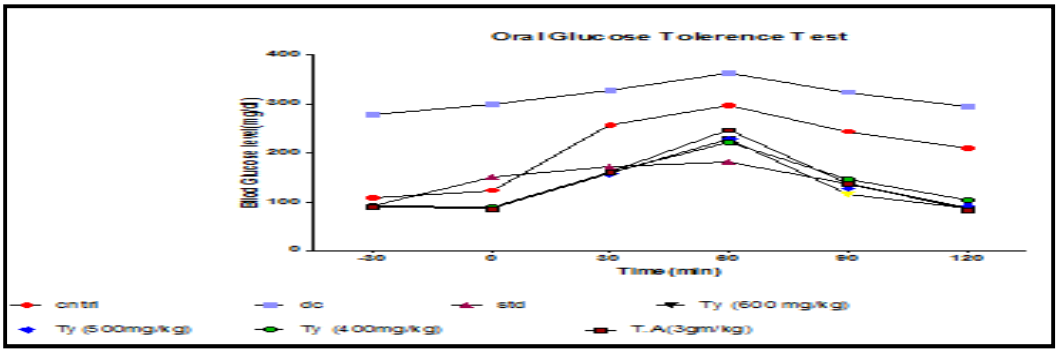

Figure No.1: Effect of Tylophora indica on oral glucose tolerance test in alloxane induced diabetic rats

CONCLUSION

The current study demonstrated that rats treated with alloxan had a higher serum TC concentration than normal rats, while oral administration of methanolic extract total alkaloid content and of Tylophora Indica (600mg/kg) significantly decreased tylophorine's TC level (500 and 400mg/kg) was not. According to the findings of this study, Tylophora Indica extracts protect against macrovascular complications.

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CONFLICT OF INTEREST

There is no conflict of interest in our review article.

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