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Estimation of glycogen content in liver, skeletal muscle and cardiac muscle of niddwin, a polyherbal formulation in alloxan induced diabetic rats

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The objective of present study was focused to estimate the glycogen content in liver, skeletal muscle and cardiac muscle of NIDDWIN in diabetic rats. Alloxan induced diabetic rats were divided into four groups of five each. Group-I was given 2% gum acacia, Group-II and III was given NIDDWIN 50mg/kg and 100mg/kg, Group-IV was given Glibenclamide 10mg/kg were given orally to each group, 2hours after administration of drugs into different groups they were scarified liver, skeletal muscle, cardiac muscle were isolated and taken into test tube containing 5% trichloroaceticacid with 5ml ethanol and allowed to stand for overnight at room temperature and the precipitate obtained is estimated for glycogen content. NIDDWIN 100mg/kg showed significant to Glibenclamide 10mg/kg in increasing the glycogen content in muscles. NIDDWIN concluded that it possess reduction in blood glucose levels by increasing glycogen synthesis as that of Glibenclamide.

Keyword: NIDDWIN, Glibenclamide, Trichloroaceticacid, Anthrogen Reagent, Ethanol, Calorimeter.

1. Introduction

Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism which causes hyperglycemia resulting from insufficient insulin secretion, insulin action or both [1, 2]. It is one of the refractory diseases identified by Indian council of medical research for which an alternative medicine is a need for the treatment. Diabetes mellitus has become a growing problem in the contemporary world [3]. Today India has become

the diabetic capital of the world with over 20million diabetic patients and this number is likely to increase to 57 million by 2025 [4]. This astronomic increase in the prevalence of diabetes has made diabetes a major public health challenge for India and is become important human ailment afflicting many from various walks of life in different countries and once again the whole world being looked upon ayurvedic the oldest healing system of medicine for the treatment of diabetes [2]. Although there are many

synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered that totally from diabetes [5]. The modern oral hypoglycemic agents showed undesirable side effects thus in the recent years considerable attention has been directed towards the antidiabetic potential of medicinal plants and their herbal formulation in the management of disease.

The concept of polyherbalism is peculiar to ayurveda although it is difficult to explain in term of modern parameters. It is evident that there are many herbal formulations of varying potency since these preparation act by different mechanism, it is theoretically possible that different combination of these extract will do better job in reducing blood glucose. In the traditional system of plant medicine it is usual to use plant formulation and combined extract of plant are used as a drug of choice rather than individual ones [6] to get the benefit of synergism. NIDDWIN a polyherbal formulation which include 11 antidiabetic herbs and 1 mineral the 12 constituents of NIDDWIN were individually proved to have antidiabetic activity but the combination of these 12 constituents called NIDDWIN for its antidiabetic activity was not yet reported. Each 500mg of NIDDWIN consists of following formulation: *Tinospora cordifolia* – 50mg, *Gymnema sylvestre* – 50mg, *Terminalia tomentosa* – 50mg, *Asphaltum* – 50mg, *Tribulus terrestris* – 50mg, *Emblica officinalis* – 58mg, *Mucuna pruriens* – 50mg, *Sida cordifolia* – 50mg, *Withania somnifera* – 25mg, *Terminalia belerica* – 8mg, *Terminalia chebula* – 8mg, *Momordica charantia* – 10mg.

Therefore, the present study was focused to estimation of glycogen content in liver, skeletal muscle and cardiac muscle of NIDDWIN, a polyherbal formulation in Alloxan induced diabetic rats.

2. Materials and Methods

2.1 Plant Material

NIDDWIN a polyherbal formulation containing 11antidiabetic herbs and 1minerial was manufactured by IMIS pharmaceuticals Pvt ltd.,

Vijayawada is evaluated for hypoglycemic activity.

2.2 Animals

Male albino wistar rats weighing 180-200gms were obtained from authorized animal house (Albino research center, Hyderabad). Animals were housed at room temperature 25 °c with a 12hrs light and 12hrs dark cycle. The animals had free access to standard rat pellet diet and tap water. After one week of acclimatization, the animals were considered for suitable study and the experiments were conducted according to CPCSEA guidelines no GNIP (TKR)/CPCSEA/IAEC/2013/11.

2.3 Acute toxicity study

The animals were divided into four groups each containing 5animals NIDDWIN a polyherbal formulation was given orally in logarithmic doses 30, 100, 300 and 1000mg/kg. The rats were observed continuously for 2hrs for behavioural, neurological and autonomic profiles and after 24hours and 72hours for any lethality [7, 8, 9].

2.4 Experimental design:

2.4.1 Experimental induction of diabetes mellitus:

The rats were injected Alloxan monohydrate dissolved in sterile normal saline at a dose of 150mg/kg body weight, intraperitoneally. Since Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 10% glucose solution (8-10ml) intraperitoneally after 6hrs. The rats were then kept for the next 24hrs on 5% glucose solution bottles in their cages to prevent hypoglycemia [10,11,12].

2.4.2 Treatment:

Diabetic rats were randomly divided into four groups with 5rats in each group and all the drugs were given orally as follows.

Group – I: This group was given aqueous suspension of 2% gum acacia as control

Group – II: This group was given aqueous suspension of NIDDWIN 50mg/kg

Group – III: This group was given aqueous suspension of NIDDWIN 100mg/kg

Group – IV: This group was given Glibenclamide 10mg/kg.

Two hours after administration of drugs into different groups they were scarified by decapitation. The liver, skeletal muscle , cardiac muscle were isolated and taken in a 5%trichloroacetic acid in 15ml centrifuge tube with 5ml ethanol and allowed to stand for overnight at room temperature. (Alternatively, placing the tubes in a water bath at 37-40° for 3hours may be carried out.) After precipitation is complete, the tubes are centrifuged at 3000r.p.m. for 15 minutes. The clear liquid is gently decanted from the packed glycogen and the tubes are allowed to ^[13] drain in an inverted position for 10 minutes.

2.5 Reagents

- 1) Anthrone reagent: A solution containing 0.05 per cent anthrone, 1per cent thiourea, and 72 per cent by volume H₂SO₄ is used. For each liter of reagent, place in a suitable flask 280 ml. of distilled water and add cautiously 720 ml. of concentrated H₂SO₄, sp. gr. 1.84, of highest purity. Place in a flask 500mg. of purified anthrone, 10 gm of highest purity thiourea, and 1 liter of the 72 per cent H₂SO₄. Warm the mixture to 80-90°, occasionally shaking the flask to mix the contents. Do not overheat the mixture. Cool and store in a refrigerator. This reagent will keep for at least 2 weeks in a refrigerator.
- 2) 5% Trichloroacetic acid.
- 3) 95 % ethanol.
- 4) Glucose standard:
 - a) Stock solution: Dissolve 100 mg. of dry, highest purity glucose in 100ml. of saturated benzoic acid solution.
 - b) Working standard: Place 5 ml. of the stock solution in a 100 ml. volumetric flask and make up to volume with saturated benzoic acid solution. 2 ml of this solution, containing 0.1 mg. of glucose, are used as a standard.

The glycogen is dissolved by addition of 2 ml. of distilled water, the water being added in a manner that will wash down the sides of the tube. If the glycogen does not dissolve instantly, agitate the tube until solution is complete. A reagent blank is prepared by pipetting 2 ml. of water into a clean centrifuge tube. A standard is prepared by pipetting 2 ml. of standard glucose solution, containing 0.1 mg of glucose, into a similar tube. At this point 10 ml. of anthrone reagent are delivered into each tube gent is directed into the center of the tube ^[14] and should be sufficient to insure good mixing. As each tube receives anthrone reagent, it is tightly capped with an air condenser and placed in a cold tap water bath. The air condenser is prepared by cutting off the small end of a size 0 rubber stoppers and inserting a 4 inch length of glass tubing, 3 to 4 mm. in diameter. This serves to prevent water from entering the tube from the water bath.

After all tubes have reached the temperature of the cold water, they are immersed in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 minutes and then removed to a cold water bath and cooled to room temperature. The tubes and stoppers are wiped dry and the contents of each tube are transferred to a calorimeter tube and read at 620 nm after adjusting the calorimeter ^[15] with the reagent blank. Care is taken to avoid introduction of lint or contaminating carbohydrate into the anthrone reagent.

The calculation of glycogen is as follows:

$$\frac{DU/DS \times \text{Volume of the extract} \times 100 \times 0.9 \times 0.1}{\text{Gram of the tissue}}$$

Where,

DU = optical density of the unknown.

DS = optical density of the standard.

0.9= factor for converting glucose value to glycogen value.

0.1= mg of glucose in 2 ml. of standard solution.

Glycogen content of each tissue in different groups were calculated and expressed as milligrams for 100grams of tissue and results were given in Tables 3.2.1-3.2.3.

2.6 Statistical Analysis:

Results were analyzed by applying unpaired student's t-test by using one way ANOVA using InStat3 software, followed by Dunnet's test. The percentage reduction values were expressed in Mean±SEM.

3. Results

3.1 Acute toxicity studies

Acute toxicity studies indicated that there is mild toxicity with 1000mg/kg after 24hrs of treatment.

3.2 Estimation of glycogen content in liver, skeletal muscle and cardiac muscle by using different doses.

Table 3.2.1: Glycogen content of liver, skeletal muscle, and cardiac muscle in Alloxan induced diabetic rats with NIDDWIN 50mg/kg

Groups	Liver	Skeletal Muscle	Cardiac Muscle
NIDDWIN 50mg/kg	123.45 ± 11.475**	16.00 ± 2.120*	16.31 ± 2.007*

Each value is SEM of 5animals: *P<0.05, **P<0.01. Comparison made between diabetic control rats and NIDDWIN 50mg/kg treated group rats

The glycogen content of liver with NIDDWIN 50mg/kg 123.45mg, skeletal muscle with NIDDWIN 50mg/kg 16.00mg, and cardiac muscle with NIDDWIN 50mg/kg 16.31mg.

Table 3.2.2: Glycogen content of liver, skeletal muscle, and cardiac muscle in Alloxan induced diabetic rats with NIDDWIN 100mg/kg

Groups	Liver	Skeletal Muscle	Cardiac Muscle
NIDDWIN 100mg/kg	190.64 ± 9.394***	19.102 ± 1.814**	20.204 ± 1.29**

Each value is SEM of 5animals: **P<0.01, ***P<0.001. Comparison made between diabetic control rats and NIDDWIN 100mg/kg treated group rats

The glycogen content of liver with NIDDWIN 100mg/kg 190.64, skeletal muscle with NIDDWIN 100mg/kg 19.102mg, and cardiac muscle with NIDDWIN 100mg/kg 20.204mg.

Table 3.2.3: Glycogen content of liver, skeletal muscle, and cardiac muscle in Alloxan induced diabetic rats with Glibenclamide 10mg/kg

Groups	Liver	Skeletal Muscle	Cardiac Muscle
Glibenclamide 10mg/kg	200.904 ± 9.181***	20.598 ± 0.853**	23.06 ± 3.338**

Each value is SEM of 5animals: **P<0.01, ***P<0.001. Comparison made between diabetic control rats and Glibenclamide 10mg/kg treated group rats.

The glycogen content of liver with Glibenclamide10mg/kg 200.904 mg, skeletal muscle with Glibenclamide 10mg/kg 20.598mg, and cardiac muscle with Glibenclamide 10mg/kg 23.06mg.

4. Discussion:

In Alloxan induced diabetic rats increase in glycogen content in the tissues may be due to increasing conversion of glucose to glycogen i.e., by increasing glycogen synthesis in the liver due to release of insulin. Similarly NIDDWIN showed same and less effect than Glibenclamide in increasing the glycogen content in muscles mainly liver [16]. So, the reduction in blood glucose levels shown by NIDDWIN is partly attributed to increase in the glycogen content of the muscles mainly in liver by increasing glycogen synthesis.

It seems that the overall mechanism attributed to anti-diabetic activity of NIDDWIN is partly due to increased glycogen content in muscles like Glibenclamide. As NIDDWIN is a herbo-mineral formulation the observed pharmacological activity produced may be due to presence of the components acting by increasing glycogen content in muscles.

5. Conclusion

The present study suggested that the polyherbal formulation NIDDWIN possess a potent increase in the glycogen content in various muscles especially in liver as same as Glibenclamide.

Therefore further studies are planned to conduct glucose uptake method in diabetic rats and to establish the probable mechanism of NIDDWIN.

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