

Antidiabetic Activity of Ethanolic Extract of *Hydnocarpus Wightiana* Blume Using Stz Induced Diabetes in Sd Rats

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Abstract: *Hydnocarpus Wightiana* Blume is an Indian traditional plant used in treating diabetes. *Chaulmoogra* Oil is obtained from the seeds and has been used in treating leprosy from last twenty years. *Hydnocarpus Wightiana* Blume posses strong antioxidant, α -glucosidase inhibitory activity. And the ethanolic extract of the seed hull also have the chemical constituents like luteolin, *hydnocarpin* which are responsible for having free radical scavenging nature. The Phytochemical screening of the ethanolic extract of seed hull of *Hydnocarpus Wightiana* Blume showed the positive results for Flavonoids, Glycosides, Carbohydrates and Amino Acids. From The Phytochemical study we found that the extract has the antioxidant properties. By this Result we extended our work by administering in diabetes induced SD Rats to check whether it has any antidiabetic activity or not. So, after treating the diabetic rats for 28days with our extract we found that the blood glucose levels got decreased when we compared it with the first day of glucose levels. Hence, we confirmed that the ethanolic extract of the seed hull of *Hydnocarpus Wightiana* Blume has the antidiabetic activity.

Key Words:- *Hydnocarpus Wightiana* Blume; Antidiabetic; GOD POD; OGTT; STZ induced diabetes.

I. Introduction

According to the recent estimates, the human population worldwide appears to be in the midst of an epidemic of diabetes. Diabetes mellitus, the most common endocrine disease, is not a single disease but a group of disorders of varying aetiology and pathogenesis. The management of diabetes is considered a global problem and a cure has yet to be discovered. Despite many great stride have made in understanding the management of diabetes. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. The searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Concurrently, Phytochemical identified from traditional medicinal plants are presenting exciting opportunities for development of new drug therapies. Currently available drug regimes for management of diabetes mellitus have certain drawbacks .In addition, the cost of administering modern antidiabetic drugs is beyond the reach of people in the low income group and those living in the rural areas. The ethanobotanical information reports about 800 plant species that may possess the antidiabetic activity. In that about maximum species have possessed antioxidant property, and multi therapeutic properties. According to ayurveda there are 20 forms of diabetes (prameha), 4 are due to Vata, 6 results from Pitta and 10 are resulted due to Kapha. But the diabetes (Prameha) is Kapha doshaja disease. Ayurvedic practioners first approached diet modification, eliminating sugar and simple carbohydrates. They used herbal preparations; Exercise is another cornerstone of Ayurvedic treatment of diabetes. Yoga and breathing exercises are traditionally used. The most important herbs for all doshas are shilajit, gudmar turmeric, neem, amalaki, guggul, and Arjuna. Turmeric with aloe vera gel (1 to 3 gm) is best used during the early stages of diabetes for regulating pancreas and liver functions. Also include decoctions of triphala, fenugreek, musta, Arjuna, sandalwood, lodhra, ajwan, gokshura, vidanga, guduchi, haritaki, and chitrak. These may be taken with a small amount of ghee. Gudmar and shilajit are excellent. In the classical treatments of Indian medicine system

1.1 *Hydnocarpus Wightiana*: Blume has been advocated to possess the antidiabetic and antileprotic property. Mostly the seeds are used as remedy for leprosy in south Indian region. The belief that natural medicines are much safer than synthetic drugs has gained popularity in recent years lead to tremendous growth of phytopharmaceutical usage. The searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Bearing the above points in mind,

1.2 Hydnocarpus Wightiana: Blume was selected which was traditionally used for various ailments like leprosy, anti-oxidant, secondary syphilis, rheumatism ophthalmic, the folklore claim of *Hydnocarpus Wightiana* Blume shows that it is having antioxidant properties and antidiabetic activity. Hence to identify and prove an excellent drug, the present investigation aims to carry out preliminary Phytochemical studies and pharmacological screening to support the folklore claim on basis of scientific background. However, no systematic study is available in the literature providing scientific justification for its use in diabetes. So, basing on the Venkat reddy et al (2005), I carried out the work on STZ induced diabetic rats. Venkat reddy et al carried out their work on the

1.3 Hydnocarpus Wightiana: Blume seed hulls and proved to have the chemical constituents like hydnocarpin, luteolin, and isohydnocarpin. Which are having the free radical scavenging activity and α -glucosidase activity. The aim of our project is to carry out the preliminary phytochemical studies and pharmacological screening to support the folklore assert on basis of scientific background.

II. Materials And Methods

2.1 Animal selection: About 36 Sprague Dawley (male) rats were taken and divided into 6 groups each of having 6 animals. This is divided based on their body weights. All the S.D rats were feed a normal laboratory chew diet (Nutrilab Rodent Feed, PROVIMI) containing (W/W) of 21.88% crude proteins, 52.15% carbohydrates and 5.97% crude fat and they were housed under a 12:12hour light: dark cycle with 22-25°C. The average body weights of the rats were 240-290 \pm 2. Before injecting the single dose of the Streptozotocin (STZ) to animals they were kept overnight fasting and immediately in next day the STZ mixed in 0.1M citrate buffer is administered to the animals with in the 30min and cold condition to be maintained while using the STZ. About 442.48mg of STZ (SIGMA) is dissolved in the 26ml of citrate buffer this dose is prepared as 45mg/kg bodyweight of the animals.

2.2 Collection of seeds: The *Hydnocarpus Wightiana* Blume seeds were collected from the local market in Hyderabad, (Munna Lal Dawasaz, and Afzalgunj) and were authenticated by Amarendar Reddy (Associate.Prof, Department of Pharmacognosy, Vikas college of Pharmacy, Jangaon, Warangal, AP). About 3kgs of the seeds were bought and they were washed to remove the dirt and then dried for 2-3days. After drying the hulls were separated from the seeds and they were pulverized. The seeds are made into powder using electric grinder. And obtained powder is dried again and sieved to get the fine powder. Then used for the Ethanolic extraction.

2.3 Extraction Procedure: The collection of the *Hydnocarpus Wightiana* Blume seeds and separation of hulls and pulverisation of hulls is collected using Venkat reddy et al., The extraction procedure is carried out with slight modifications. After the pulverization the fine powder is taken and extracted by using the solvent Ethanol. First a thimble is prepared with blotting paper and about 150-200gms of *Hydnocarpus Wightiana* Blume powder is filled in it. After filling thimble it is placed in the soxhlet apparatus. After filling the thimble, 1000 ml of absolute ethanol is taken and from that 750ml of absolute ethanol is filled in the round bottomed flask (RBF) and the remaining is poured into the thimble which is placed in Soxhlet apparatus. This is done to saturate the powder and it is done till the siphon gets filled and acquires free flow of the solvent. In the next step the reflex condenser is placed over the soxhlet and which is made connected to the inlet and outlet. The inlet carries water flow and gets passed into the condenser and leaves through the outlet. And some porcelain chips are added into the RBF to avoid the vigour's boiling and the temperature is adjusted to 58°C. This temperature is adjusted based on the ethanol boiling point. Once the ethanol boils in RBF the vapours enters the reflex condenser and gets condensed and falls onto the thimble in soxhlet apparatus this continues until the ethanol vapours passes through the siphon and falls into the RBF. This cycle is continued until the solvent in soxhlet gets colourless this is checked by the observing the siphon. The extraction is carried out continuously for 16 hours and the extraction mixture in RBF is taken and distilled to collect the extract. The extract is distilled by Rotavapour apparatus by maintain the 55°C temperature and at a speed of 125RPM and also by creating some pressure and carried out for 30min. after that the extract is collected one end and Acetone which is present gets separated at another end. The distillation process is used for separation of any presence of ethanol.

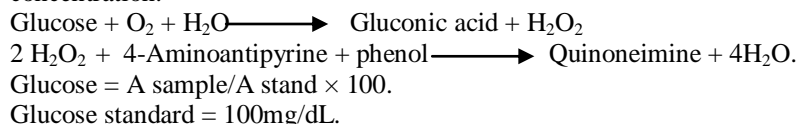
2.4 Preliminary Phytochemicals screening: After the completion of the extraction process the obtained ethanolic extract is to be identified for the presence of the Phytochemicals. The known quantity of ethanolic extract of *Hydnocarpus Wightiana* Blume (EEHWB) is taken and used for the preliminary studies. The freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents. Phytochemicals screening of the extracts was performed using the following reagents and chemicals; Alkaloids and Dragendroff's reagent, Flavonoids with the use of Mg, HCL, and Tannins with ferric chloride and potassium dichromate solutions and Saponins, with ability to produce stable foam and steroids with Libermann-Burchard reagent. These were identified by characteristic colour changes.

2.5 Acute toxicity study: An acute oral toxicity study was performed as per OECD guidelines 423. By Acute toxic class method Swiss albino mice of female sex weighing 20-25gms were used for the Study. Acute toxic class method is a stepwise procedure with use of three animals of a Single sex per step. Depending on mortality or morbidity status of the animals. Average 2-4 steps may be necessary to allow judgement on the acute toxicity of the substance. Three animals were used for each step. The animal were placed individually and observed for any sign of toxicity, morbidity or mortality during the first 24hrs, with special given attention during the first 4 hours and daily thereafter for a total of 14 days. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300 and 2000 mg/kg body weight. From the acute toxicity studies the extract dose was fixed and continued for the next preclinical studies on SD rats by making following groups. After the grouping the experiment is carried out for 28days. With daily administration of standard drug and the EEHWB test drugs. And on the 7, 14, 21, and on 28th day the OGTT is done to check the glucose levels.

GROUP	TREATMENT
Group I	Control rats given only Citrate buffer (p.o.)
Group II	Diabetic rats treated with STZ (45mg/kg/i.p)
Group III	Standard rats treated with STZ (45mg/kg/i.p)+Acarbose (180µg / kg /day p.o.)
Group IV	Test rats treated with STZ (45mg/kg/i.p)+ EEHWB (100mg / kg / day p.o.)
Group V	Test rats treated with STZ (45mg/kg/i.p)+ EEHWB (200mg / kg / day p.o.)
Group VI	Test rats treated with STZ (45mg/kg/i.p)+ EEHWB (400mg / kg / day p.o.)

2.6 OGTT (ORAL GLUCOSE TOLERANCE TEST): The oral glucose tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose, which is the body's main source of energy. An OGTT can be used to diagnose Prediabetes and diabetes. Before doing OGTT animals are fasted over night, and then the blood was collected by retro-orbital method. The blood is collected in the effendrof tubes which contains the EDTA (20%), mix the blood. This is done to avoid the blood coagulation. The collected blood is centrifuged for plasma. The blood is centrifuged for 5-10min at an rpm of 4000 (REMI RM-12C). Then the basal reading of the blood glucose was taken and then the oral glucose is given to the animals basing on their bodyweights. And the blood glucose levels are checked for 30min, 60min, 120min, 240min, and 480min respectively. The glucose levels are compared with the fasting glucose.

2.7 GOD POD: The blood glucose levels are checked using the GOD POD test. After taking the blood samples they are centrifuged at 2000rpm for about 5min. The plasma is separated and used for the test. In 96 well plate about 300µl of the GOD POD reagent was added in the all the wells. The first two wells are left as the blank (only GOD POD) and the next two are used as the standard (GOD POD + 5µl of standard). And from the fifth well the plasma was added about 5µ each well. For each plasma is taken in two wells of the plate. The 96 well plates is kept in the micro well plate reader (Biotek Power Wave XS₂) and using the Gen 5 software it is adjusted to incubate for 15min at 37^oc temperature. The readings were obtained Spectrophotometrically at 546nm. Principle behind GOD POD: Glucose is oxidised by glucose oxidase to gluconic acid and H₂O₂ is liberated. The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrine and phenol by H₂O₂ under the catalytic action of peroxidase; intensity of colour generated is directly proportional to glucose concentration.



2.8 Statistical Analysis: Data obtained from our study were statistically analysed by using Graphpad Instant Demo version 3.0. The values were expressed as mean ± SEM for each group. Significant difference between groups was determined using one-way ANOVA followed by Dunnet multiple comparison test. A p value less than 0.05 were considered significant.

III. Results And Discussion:

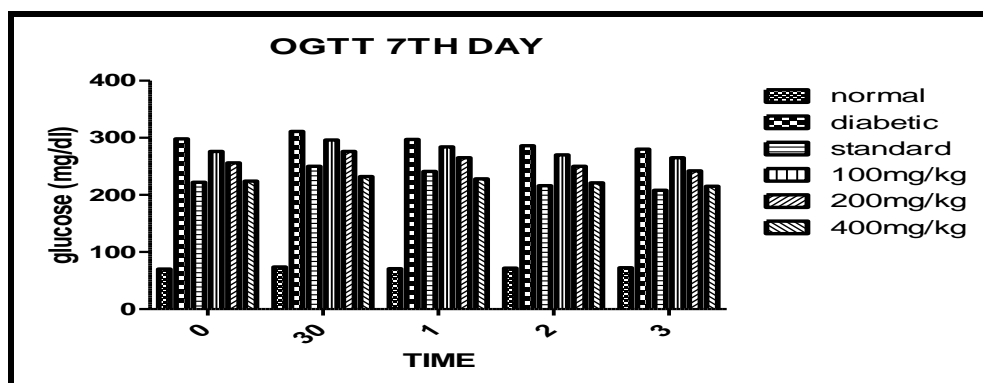
Evaluation of the antioxidant and antidiabetic activity of Ethanolic extract of *Hydnocarpus Wightiana* Blume on Streptozotocin induced diabetic rat was carried out in two phases. Preclinical Phytochemicals screening: Qualitative Phytochemicals analysis of Ethanolic extract of *Hydnocarpus Wightiana* Blume showed the presence of majority of the compounds including alkaloids, glycosides, carbohydrates, steroids, sterols, Flavonoids, tannins, proteins and amino acids. By the qualitative tests we found the positive results glycosides, carbohydrates, Flavonoids and amino acids and proteins. Hence the ethanolic extract of the *Hydnocarpus Wightiana* Blume contains Glycosides, carbohydrates, Flavonoids and amino acids.

In-Vivo studies: The in vivo study is carried out for 28 days after the start of administering the extract and the standard drug (Acarbose). For every seven the OGTT was done by collecting the blood from the rats and the blood glucose levels were recorded. And after 28 days of study the percentage inhibition is calculated and compared with the standard drug inhibition. On the seventh day after drug administration the OGTT was done. Effect of Ethanolic extract of *Hydnocarpus Wightiana* Blume seed hull on blood glucose levels of normal and diabetic fasted rats after a glucose load (2 g/kg). The blood glucose level was increased rapidly 1/2 h after administration of glucose, and thereafter decreased gradually. Standard drug Acarbose and three different doses of the extract (100mg/kg, 200 mg/kg & 400 mg/kg) were given orally 30 min before the glucose administration. In normal animals gradual rise in blood glucose level after the glucose load was observed and maximum at 2 hrs. Diabetic control animals showed a gradual rise in blood glucose level and stabilized at 2hr. 200 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed minimum fall in blood glucose concentration (232 mg/dl) but in 400 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed maximum fall in blood glucose concentration (215mg/dl, $p < 0.0001$). The values were expressed as Mean \pm S.E.M and statistically analysed with the help of Graph pad prism software and values are compared using one way ANOVA OGTT on 14th day of treatment: Effect of *Hydnocarpus Wightiana* Blume seed hull extract on blood glucose levels of normal and diabetic fasted rats after a glucose load (2 g/kg). The blood glucose level was increased rapidly 1/2 h after administration of glucose, and thereafter decreased gradually. Standard drug Acarbose and three different doses of the extract (100mg/kg, 200 mg/kg & 400 mg/kg) were given orally 30 min before the glucose administration. In normal animals gradual rise in blood glucose level after the glucose load was observed and maximum at 2 hrs. 200 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed minimum fall in blood glucose concentration (225 mg/dl) but in 400 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed maximum fall in blood glucose concentration (201mg/dl, $p < 0.0001$). The values were expressed as Mean \pm S.E.M and statistically analysed with the help of Graph pad prism software and values are compared using one way ANOVAs.

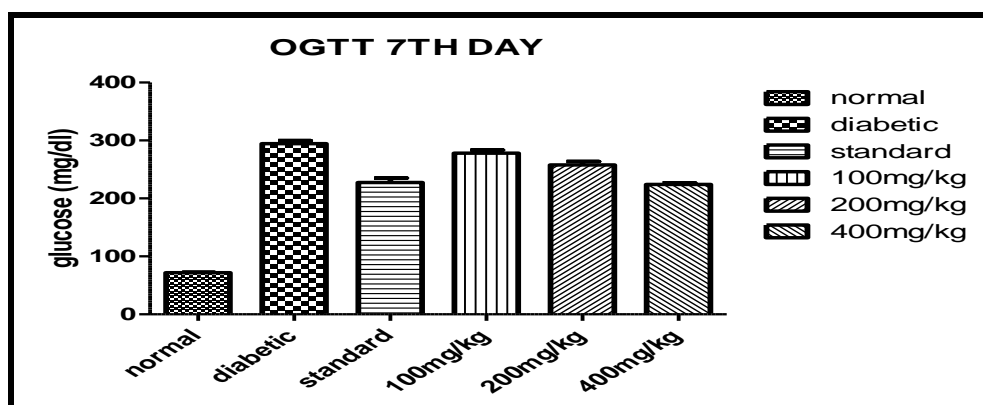
OGTT on 21st Day: Effect of *Hydnocarpus Wightiana* Blume seed hull extract on blood glucose levels of normal and diabetic fasted rats after a glucose load (2 g/kg). The blood glucose level was increased rapidly 1/2 h after administration of glucose, and thereafter decreased gradually. Standard drug Acarbose and three different doses of the extract (100mg/kg, 200 mg/kg & 400 mg/kg) were given orally 30 min before the glucose administration. In normal animals gradual rise in blood glucose level after the glucose load was observed and maximum at 2 hrs. 200 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed minimum fall in blood glucose concentration (179 mg/dl) but in 400 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed maximum fall in blood glucose concentration (136 mg/dl, $p < 0.0001$). The values were expressed as Mean \pm S.E.M and statistically analysed with the help of Graph pad prism software and values are compared using one way ANOVAs. OGTT on 28th Day: Effect of *Hydnocarpus Wightiana* Blume seed hull extract on blood glucose levels of normal and diabetic fasted rats after a glucose load (2 g/kg). The blood glucose level was increased rapidly 1/2 h after administration of glucose, and thereafter decreased gradually. Standard drug Acarbose and three different doses of the extract (100mg/kg, 200 mg/kg & 400 mg/kg) were given orally 30 min before the glucose administration. In normal animals gradual rise in blood glucose level after the glucose load was observed and maximum at 2 hrs. 200 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed minimum fall in blood glucose concentration (150 mg/dl) but in 400 mg/kg of ethanolic extract of *Hydnocarpus Wightiana* Blume showed maximum fall in blood glucose concentration (120 mg/dl, $p < 0.0001$). The values were expressed as Mean \pm S.E.M and statistically analysed with the help of Graph pad prism software and values are compared using one way ANOVAs.

CONDITION	0hr	30min	1hr	2hr	3hr
Normal	70±2	73±2	70±2	71±2	72±2
Diabetic	298±2	311±2	297±2	286±2	280±2
standard	222±2	250±2	241±2	216±2**	208±2**
100mg/kg	276±2	296±2	284±2	270±2	265±2
200mg/kg	256±2	276±2	265±2	250±2*	232±2*
400mg/kg	224±2	232±2	228±2	221±2**	215±2**

The plasma glucose (mg/dl) values done on the 7th day after the treatment by OGTT.



The graph plotted between the time and plasma glucose (mg/dl), after zero hour immediate oral glucose is given so, at 30min slight increase in plasma glucose levels and later decreased because of the elimination of glucose.

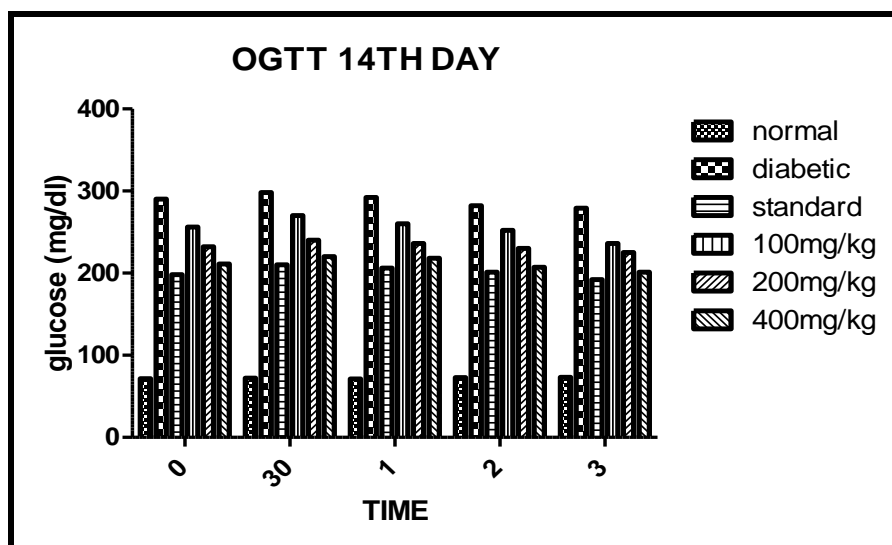


The graph showing the comparison between the normal, diabetic, standard, 100mg/kg extract, 200mg/kg extract and 400mg/kg extract doses.

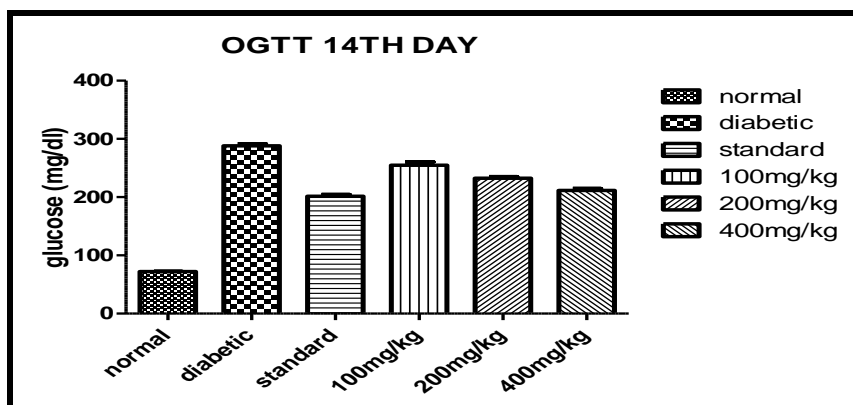
BLOOD GLUCOSE LEVELS ON 14TH DAY:

CONDITION	0hr	30min	1hr	2hr	3hr
Normal	71±1	72±1	70±1	71±1	72±1
Diabetic	290±2	298±2	292±2	282±2	279±2
standard	198±1	210±1	206±1	201±1**	192±1**
100mg/kg	256±1	270±1	260±1	252±1	236±1
200mg/kg	232±2	240±	236±2	230±2*	225±2*
400mg/kg	211±1	220±1	218±1	207±1**	201±1**

The plasma glucose (mg/dl) values collected on the 14th day after the treatment by OGTT.



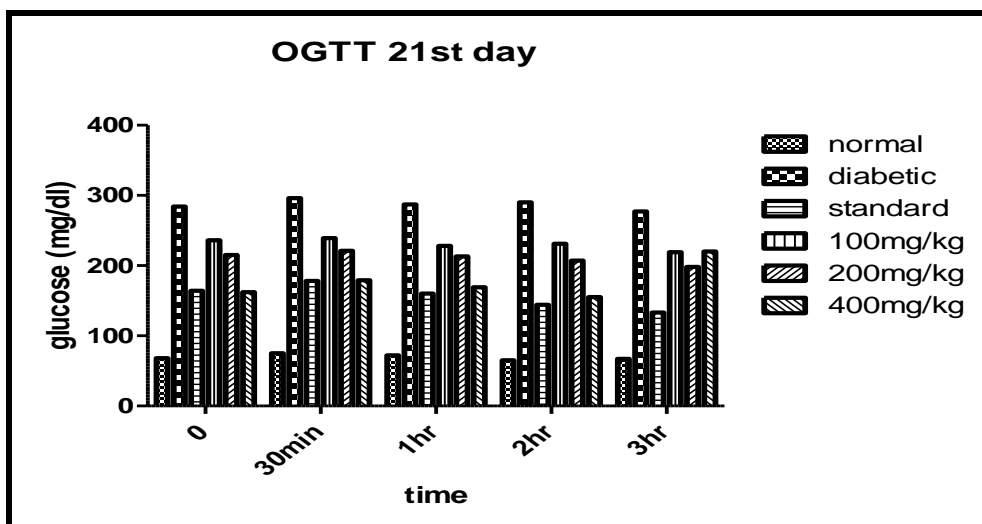
The graph plotted between the time and plasma glucose (mg/dl), after zero hour immediate oral glucose is given so, at 30min slight increase in plasma glucose levels and later decreased because of the elimination of glucose.



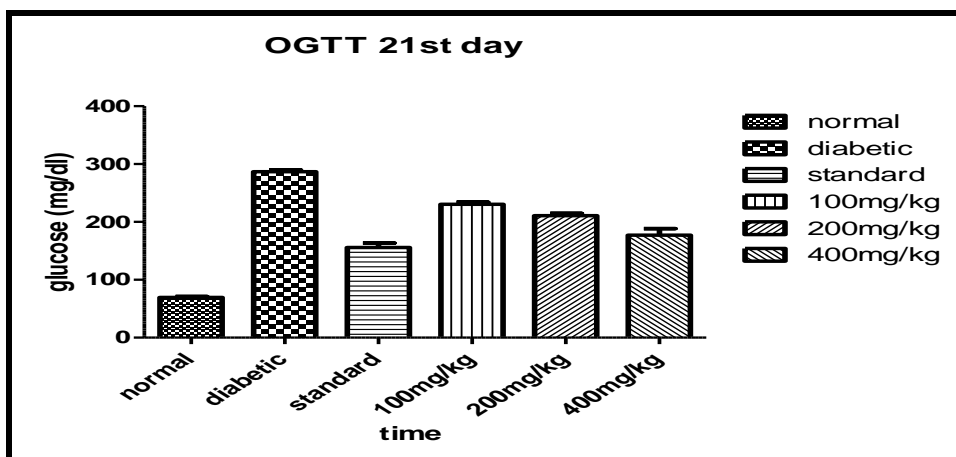
The graph showing the comparison between the normal, diabetic, standard, 100mg/kg extract, 200mg/kg extract and 400mg/kg extract doses.

CONDITION	0hr	30min	1hr	2hr	3hr
Normal	68±1	75±1	72±1	65±1	67±1
Diabetic	284±2	296±2	287±2	290±2	277±2
Standard	164±1	178±1	160±1	144±1**	133±1
100mg/kg	236±2	239±2	228±2	231±2	219±2
200mg/kg	215±2	221±2	213±2	207±2*	198±2*
400mg/kg	162±1	179±1	169±1	155±1**	136±1**

The plasma glucose (mg/dl) values collected on the 21st day after the treatment by OGTT



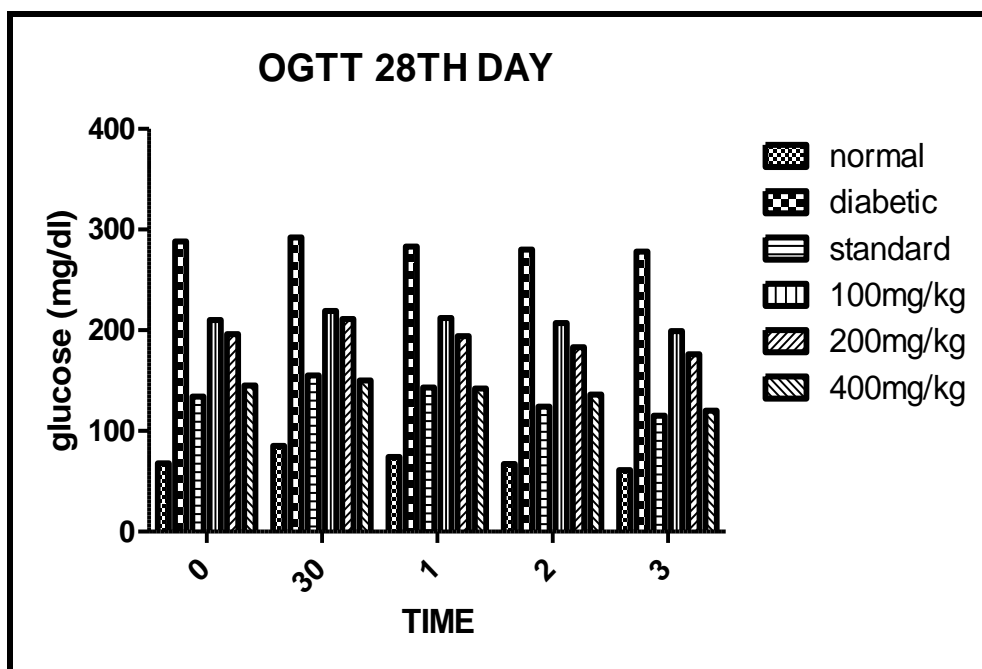
The graph plotted between the time and plasma glucose (mg/dl), after zero hour immediate oral glucose is given so, at 30min slight increase in plasma glucose levels and later decreased because of the elimination of glucose.



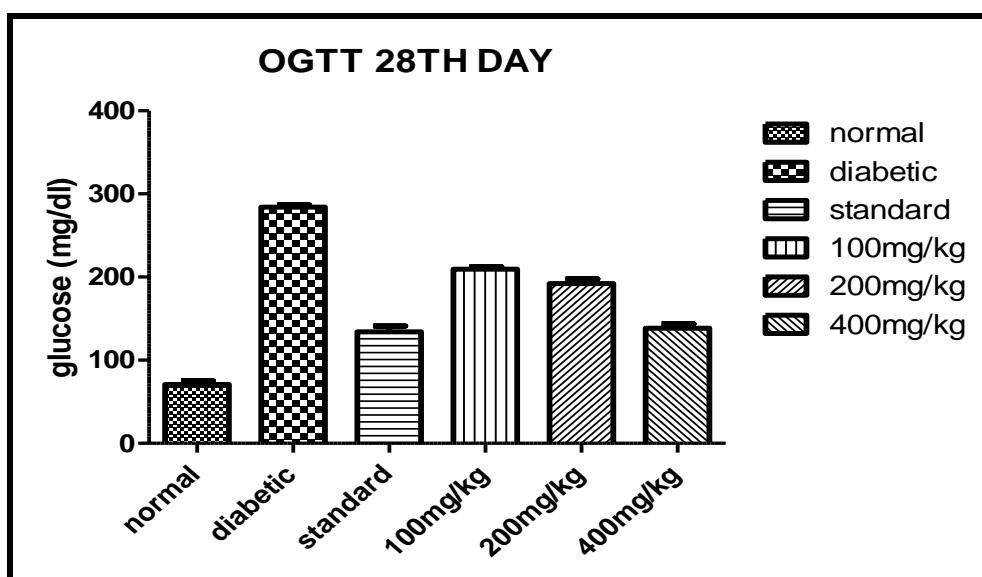
The graph showing the comparison between the normal, diabetic, standard, 100mg/kg extract, 200mg/kg extract and 400mg/kg extract doses.

CONDITION	0hr	30min	1hr	2hr	3hr
Normal	67±1	85±1	74±1	67±1	61±1
Diabetic	288±2	292±2	283±2	280±2	278±2
Standard	134±1	155±1	143±1	124±1**	115±1
100mg/kg	210±2	219±2	212±2	207±2	199±2
200mg/kg	196±2	211±2	194±2	183±2*	176±2*
400mg/kg	145±1	150±1	142±1	136±1**	120±1**

The plasma glucose (mg/dl) values collected on the 28th day after the treatment by OGTT.



The graph plotted between the time and plasma glucose (mg/dl), after zero hour immediate oral glucose is given so, at 30min slight increase in plasma glucose levels and later decreased because of the elimination of glucose.

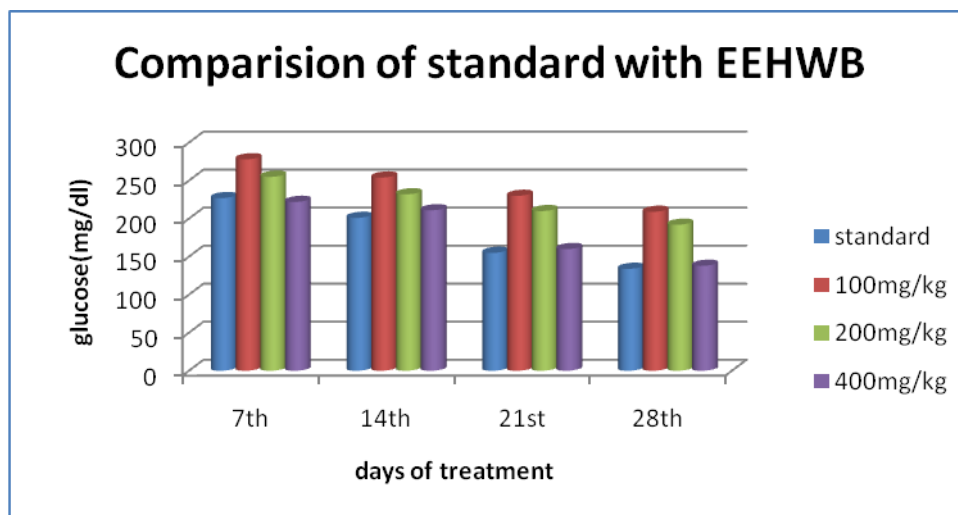


The graph showing the comparison between the normal, diabetic, standard, 100mg/kg extract, 200mg/kg extract and 400mg/kg extract doses.

Comparison of extract with standard:

	STANDARD	100MG/KG	200MG/KG	400MG/KG
7 TH DAY	227±2	278±1	255±2	222±1
14 TH DAY	201±1	254±2	232±2	211±1
21 ST DAY	155±2	230±2	210±2	160±1
28 TH DAY	134±1	209±1	192±1	138±2

The plasma glucose (mg/dl) values collected on 7th, 14th, 21st, and 28th respectively and compared with the standard values.

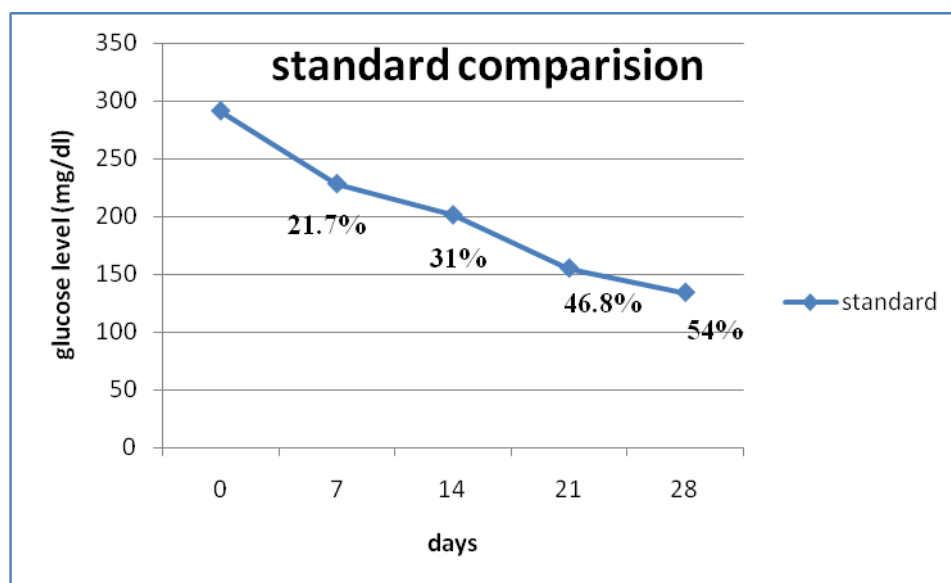


The graph showing the difference in the plasma glucose (mg/dl) from the starting day of treatment to the 28 days after the treatment.

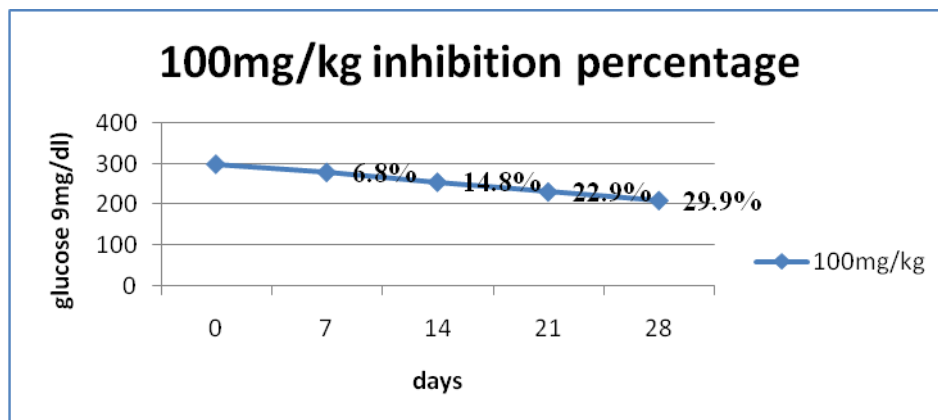
Percentage Inhibition: The percentage inhibition is calculated by taking the results from the day of treatment to the end of treatment. Comparing the standard with the different doses of extracts like 100mg/kg, 200mg/kg, and 400mg/kg.

	Days	STAND	100mg/kg	200mg/kg	400mg/kg
STZ	0	291±2	298±2	287±2	284±2
7 th	7	228±1*	278±1	255±2	226±2*
14 th	14	201±1*	254±2	232±2	201±2**
21 st	21	155±2*	230±2	210±2	160±1
28 th	28	134±1**	209±1	192±1*	138±2**

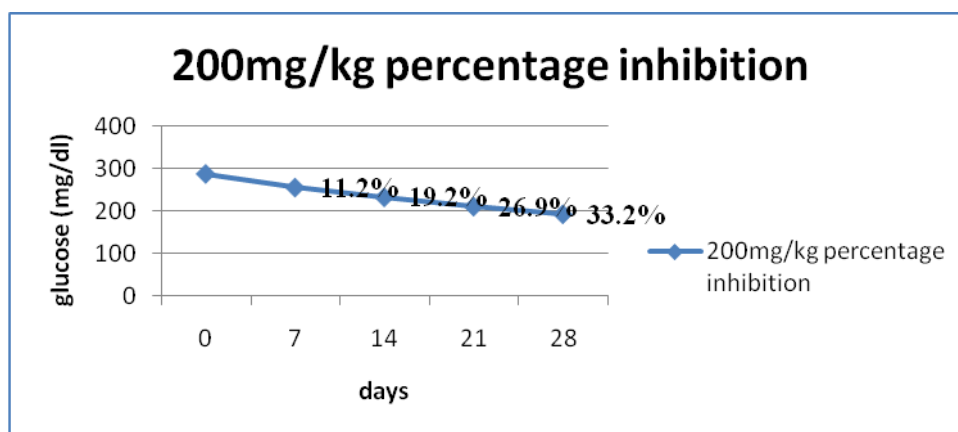
The table showing the glucose levels from day one to the end of the treatment, comparing with the standard.



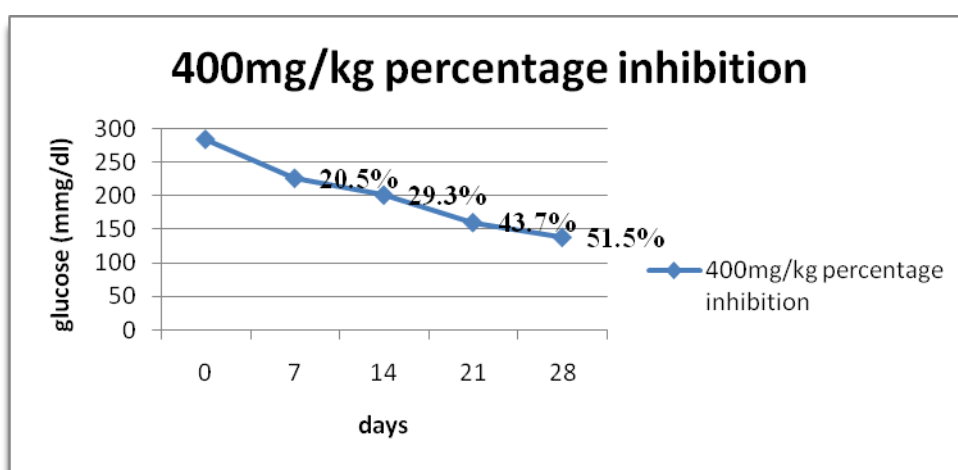
The graph between the days and the plasma glucose levels from the day from the treatment to the end of the treatment showing the 54% of the inhibition on the 28th day, 31% on the 14th day and 21% on the 7th day after the treatment.



The graph between the days and the plasma glucose levels from the day from the treatment to the end of the treatment showing the 29% of the inhibition on the 28th day, 14% on the 14th day and 6.7% on the 7th day after the treatment.



The graph between the days and the plasma glucose levels from the day from the treatment to the end of the treatment showing the 33% of the inhibition on the 28th day, 19% on the 14th day and 11% on the 7th day after the treatment.



The graph between the days and the plasma glucose levels from the day from the treatment to the end of the treatment showing the 51% of the inhibition on the 28th day, 29% on the 14th day and 20% on the 7th day after the treatment.

By comparing the glucose levels of the ethanolic extract and standard drug found some inhibition from day 7 to the 28 day. The standard drug showed the 54% inhibition. 100mg/kg of EEHWB showed 29.9% and 200mg/kg of EEHWB showed 33.2% and 400mg/kg showed 51.5% inhibition.

IV. CONCLUSION

From the present study, It supports that therapy with alpha-glucosidase inhibitors could be an interesting adjunctive pharmacological approach to the treatment of type 2 diabetes. In this regard, the protection determined by EEHWB clearly suggests the potential usefulness of this plant. We concluded that the Ethanolic extract of *Hydnocarpus Wightiana* Blume produced a significant decrease of plasma glucose level. These results suggest that the effectiveness of the drugs depend probably, on the accumulative effect of active principles. In contrast, the significant increase in plasma glucose levels of untreated diabetic rats may be due to a progressive severity of untreated diabetes. Summarizing, it could be proofed that the traditional use of *Hydnocarpus Wightiana* Blume antidiabetic agent is justified and that extracts from this plant show a dose-dependent activity which is comparable to the standard antidiabetic drug Acarbose. In conclusion, *Hydnocarpus Wightiana* Blume showed strong inhibitory activity against α -glucosidase in vitro and in vivo. Thus, chronic consumption of *Hydnocarpus Wightiana* could be helpful in improving hyperglycemia and preventing diabetic complications. Further study to identify the active component responsible for the inhibition of α -glucosidase is strongly recommended. Further, studies with purified isolated Phytochemicals constituents are needed to understand the complete mechanism of antidiabetic activity of *Hydnocarpus Wightiana* Blume.

V. Acknowledgements

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