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Determination of Anti Diabetic Potency of Leaves of Costus Igneus and Roots of Naregamia Alata on Streptozotocic Induced Diabetic Rats

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Abstract:
The Study involved determination of anti diabetic properties of leaves of Costus igneus and roots of Naregamia alata extracted using ethanol as solvent by maceration technique. The extracts were screened for standard phytochemical screening. The rats were made diabetic with 60mg/kg dosage streptozotocin administered intraperitoneally. The study was carried out for 21days, and were administered with dose of 200mg/kg body weight and 400mg/kg body weight both individually and in combination against glibenclamide at 5mg/kg body weight. The blood glucose levels were noted on 3rd day 7th day 14th day using glucometer by application of tail prick method.The extracts On the day 7 blood glucose levels were decreased upto following % with Ci 58% , Na 62% and Ci + Na 66% , On day 14 Ci 67%, Na 69% and Ci + Na 71%, on day 21 Ci 65 , Na 64% and Ci + Na 68% Ci 58% ,effects were produced and was compared to 85% effect of Glibenclamide against normal. The extracts were also exposed to invitro analysis and The IC50 in Alpha amylase inhibition assay for Acarbose 44.3ug/ml conc, Ci 59.2 ug/ml concentration and Na 61.3ug/ml conc. The Ic50 in Alpha Glucosidase inhibition assay was obtained as Acarbose at 45.5ug/ml, Ci at 55.9ug/ml and N.a at 64.2ug/ml.On the last day, pancreas of rats was isolated by exposing the rat to general anesthesia and was stored in 10% formaldehyde solution for evaluation.

Key Words: *C.igneus, N alata, Hyperglycemia, Pancreas, insulin.*

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I. Introduction

As per WHO, health is a complete state of mental, physical and social well being. In other words, health is absence of any disease in an individual's body.[1]Globally one disorder is prevalent now a days that is diabetes. associated with diabetes are all its side effects that come along.

In diabetes, the cells of pancreas loose their ability to produce insulin sufficient for body or in other case, even though the insulin is produced the cells of the body loose their ability to uptake insulin from the blood as a result of which hyper glycemic condition is produced[2]

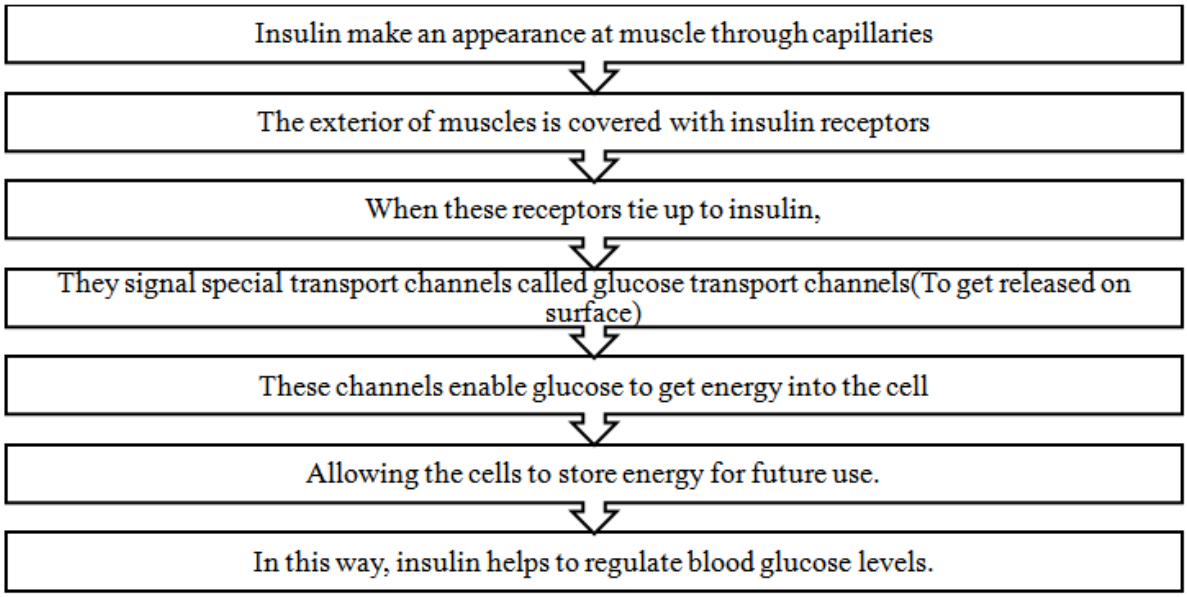
How insulin aids in reducing blood glucose levels: [3]

After meal, glucose reaches pancreas via blood stream with the help of capillaries.

This triggers the release of insulin which is stored inside the β-cells.

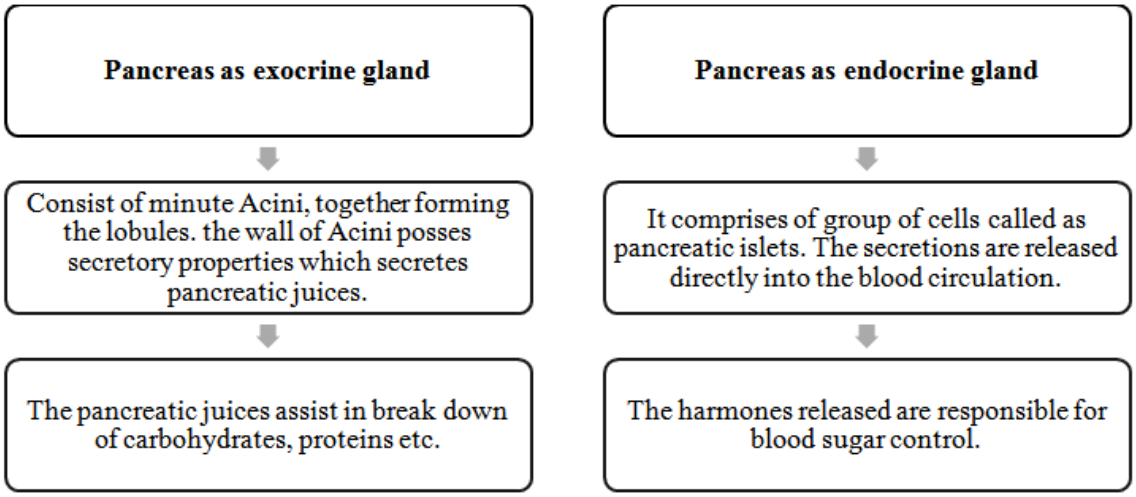
The insulin travels through the blood stream to different parts of body:Muscle, Fat&Liver

Insulin helps these tissues to glucose as an energy source.



Flow chart 1: explaination of insulin action

With the disease there are many drugs available in market that serve as potent drugs to reduce blood glucose levels in the body. One such drug is Glibenclamide. The mechanism of action of the drug consists in the inhibition of the ATP-sensitive K+ channels, which leads to depolarization of the cells and insulin secretion.
Pancreas is one such gland that is responsible to make insulin.
Pancreas is a gland that is characterized under both exocrine and endocrine glands.



Associated with herbal drugs comes a lot of sideeffects potent enough to produce other disorder in body of an individual. To over come this, drugs with high potency should find their way in the treatment pattern.
Herbal drugs until now have been proved to be beneficial and with minimal side effects.
They hold an important place in the heart of Ayurveda and has been used for the treatments from a long run.

II. Materials:

Dried *leaves of plant "**Costus igneus**"* and dried *roots of "**Naregamia alata**"* was identified, collected and authenticated by Dr.K.MADHAVA CHETTY, Assistant professor - dept of Botany – Pharmacognosy. Sri Venkateshwara University, Tripathi

The standard drug was obtained from Soma agencies 4-4-286/2, Inder Bagh, Koti, Hyderabad, Telangana 500095.

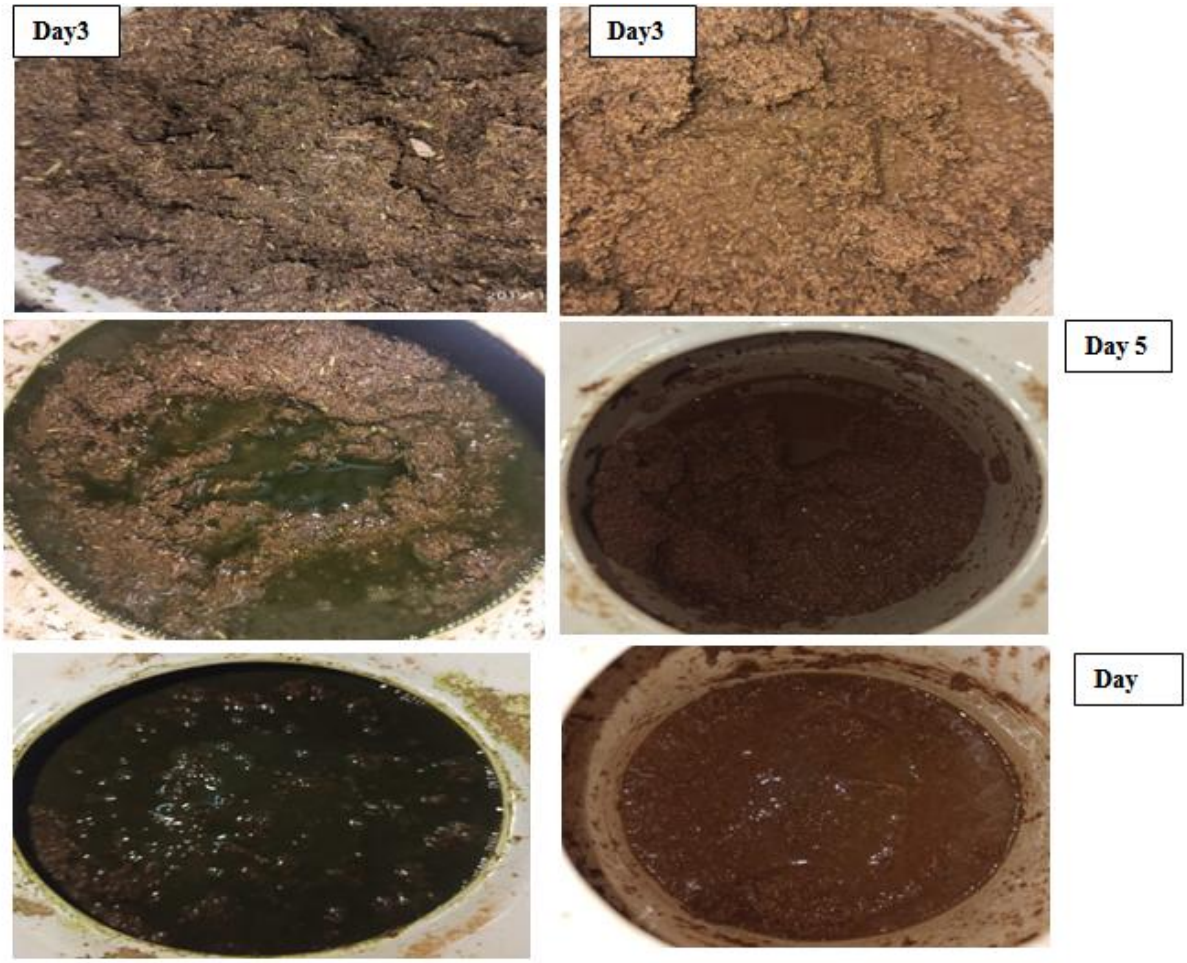
PLANT PROFILE:

BOTANICAL NAME	**Costus igneus**[4]	**Naregamia alata**[5]
FAMILY	Costaceae	Maliaceae
VERNACULAR NAMES	• English: Spiral ginger • Hindi: Jarul • Telugu.: Peddavesiga • Urdu: Bijasar	• Nelakanchi, Nilanarakam • English: Goanase ipecacuahana • Hindi: Pitmari • Telugu: Pagapappu
USES	Used in 1. Microbial injections 2. To reduce oxidative stress 3. Known to posses hepato protective action 4. Acts as antiproliferative 5. Enhances learning and memory	Used in 1. For the treatment of migrane 2. Used as anti purgative in the treatment of asthama and cough 3. It is used to treat rheumatism 4. Also known to possess ulcer protective action.
	 Image 1: plant Costus igneus	 Image 2: Naregamia alata

Table 1: plant profile's

METHOD OF EXTRACTION: [6]

The extraction was carried out by maceration technique using ethanol as solvent. The solvent and the dried powdered drugs were kept in porecelian jar with stirring 3 times per day for 7 days. The mixture was then filtered using a muslin cloth and the extract was exposed for evaporation of excess ethanol for 7 days. The remaining was kept in air tight jar an used through out the study duration.



Images 3,4,5,6,7,8 from left to right) of Costus igneus (right) and Naregamia alata (left)crude drugs at their third, fifth and sevent day of maceration(top to bottom)

PHYTOCHEMICAL SCREENING[6]

The phytohchemical screening was performed by following standadrd tests like Mayers test, wagners test for alkaloids, Molish test and Barfords test for Carbohydrates, fehlings test and Bail's test for reducing sugars, Milions test for Amino acids, Shindona test for Flavanoids, froth formation test for Spaonins, killer kilani test for Glycosides and Liberman Burchad test for steroids and triterpenoids.

EXPERIMENTAL ANIMALS [7]

60 Wistar rats of either sex weighing between 180gm-200gm were taken & the study was carried out for 21 days. Before the experiment, the rats wereacclimatized for 7 days.

The rats were housed under the standard environmental condition and exposed a 12 hour light and dark cycle and were provided standard pellets of rat food with free access to water. The bedding of the rats was changed every day to maintain hygienic conditions.

After 7 days, the rats were divided into the following 9 groups with 6 rats in each group selected on a random basis. And rest other 6 rats were subjected to acute toxicity studies.

Image 9: Marking of rats for grouping

ACUTE TOXICITY STUDIES:[8]

Acute toxicity studies were performed by considering 2000mg/kg as the maximum dose. One tenth of the dose was taken that is 200mg/kg body weight half of the dose of 200mg/kg and twice the one tenth of the dose that is 400mg/kg was administered to rats per oral. The rats did not show any abnormality or any sign of toxicity. The doses were selected.

Protocol no:IAEC-012/SES/2019/008

EXPERIMENTAL DESIGN:

GROUP	GROUP NAME	DOSE	ROUTE
Group 1	Normal saline	10ml/kg b.w	Per oral
Group 2	Toxic control - Streptozotocin 60mg/kg	60mg/kg b.w	i.p
Group 3	Standard - Glibenclamide 5mg/kg	5mg/kg b.w	Per oral
Group 4	Toxic control + Costus igneus - dose 1	200mg/kg b.w	Per oral
Group 5	Toxic control + Costus igneus – dose 2	400mg/kg b.w	Per oral
Group 6	Toxic control + Naregamia alata - dose 1	200mg/kg b.w	Per oral
Group 7	Toxic control + Naregamia alata – dose 2	400mg/kg b.w	Per oral
Group 8	Toxic control +C.igneus + N.alata – dose 1	100+100mg/kg b.w	Per oral
Group 9	Toxic control + C.igneus + N.alata – dose 2	200+200mg/kg b.w	Per oral

Table 2: Experimental design

SCREENING METHOD:[9]the rats were made diabetic with single intraperitoneal injection of streptozotocin at 60mg/kg body weight. Before administration rats were kept fasting was 24hours with free accesses to water. The rats were provided with continuous supply of glucose water for 48hours after administration of STZ to avoid mortality.

COLLECTION OF BLOOD:[10]

The blood was collected by performing retro orbital puncture to rat. Around 2ml of blood was collected in heparinized ampuoles to perfom biochemical estimations. The samples were sent to VIJAYA DIAGNOSTICS was further evaluation.

ISOLATION OF ORGANS:[11]

Rats were put in desicator with ether anesthesia and waited until the consciousness of rats were lost and rat became immobile. The rats were then pinned to wax tray to isolte pancreas.

The organs were sent to shadan medical college- Peerancheru Hyderabad.

Image 9: rat in desiccatorImage 10: of isolated organs.

INVITRO ANALYSIS:[12,13]

The extracts were sent for invitro evaluation for Alpha Amylase Inhibition Assay and Alpha Glucosidase inhibition assay. As these two enzymes are responsible to break down large molecules of starch into simpler molecules of sugar increasing the levels of glucose in the body.

III. Results:

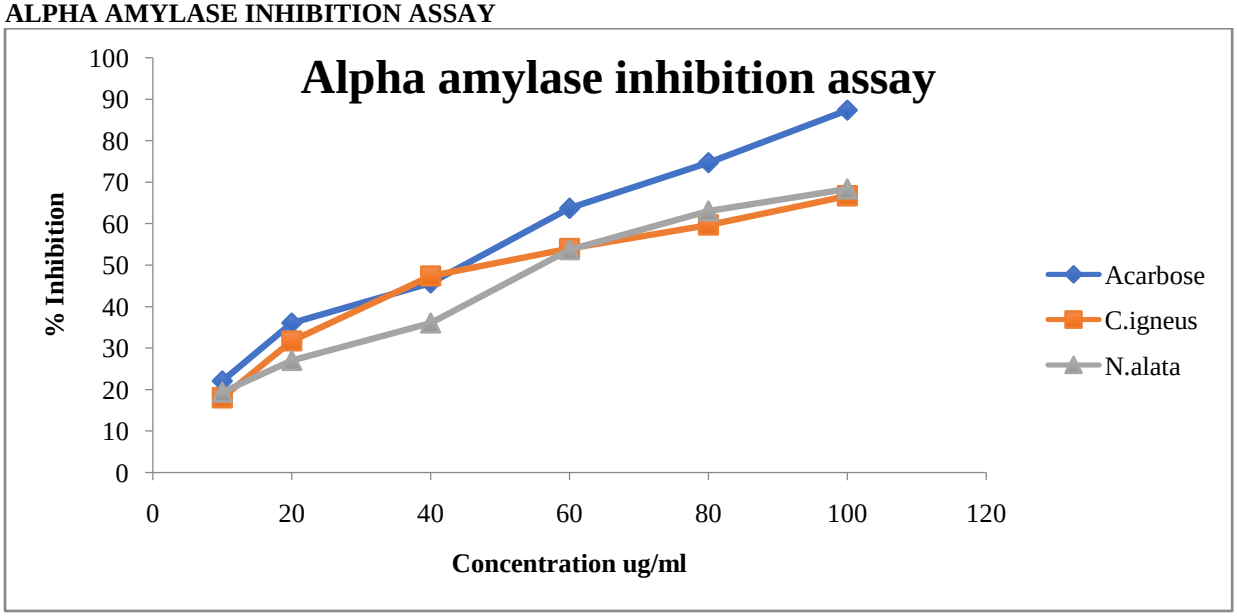
PHYTOCHEMICAL RESULTS:

Constituent	Costus igneus	Naregamia alata
Flavanoids	++	+++
Steroids	+	
Glycosides	++	+
Saponins	++	-
Alkaloids	+	+
Sugar	-	-
Phenol	+	++
Tannins	+	++
Terpenoids	++	++

Table 3: phytoconstituents

INVITRO RESULTS:

ALPHA AMYLASE INHIBITION ASSAY



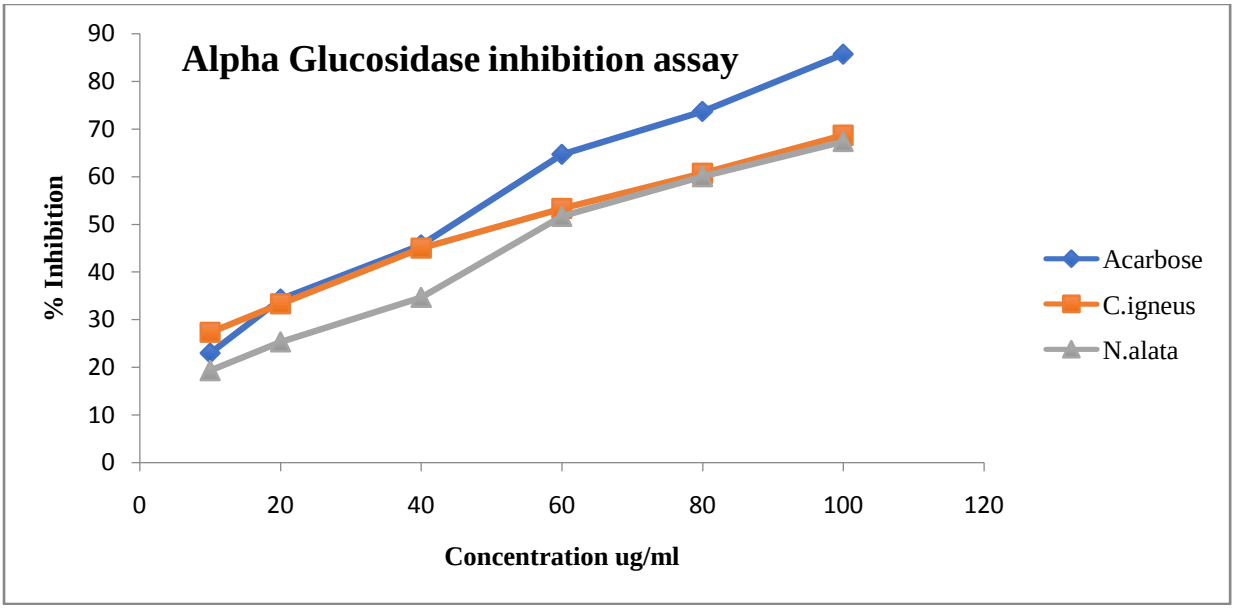
Graph 1: Alpha amylase inhibition assay

IC50 value

Acarbose	44.3ug/ml***
C.igneus	59.2 ug/ml*
N.alata	61.3ug/ml**

Table 4: Ic50 values From Alpha Amylase inhibition assay

ALPHA GLUCOSIDASE INHIBITION ASSAY



Graph 2: Inhibition of Alpha Glucosidase assay

IC50 value	
Acarbose	45.5ug/ml***
C.igneus	55.9ug/ml*
N.alata	64.2ug/ml**

Table 5: Ic50 values from Alpha Glucosidase assay.

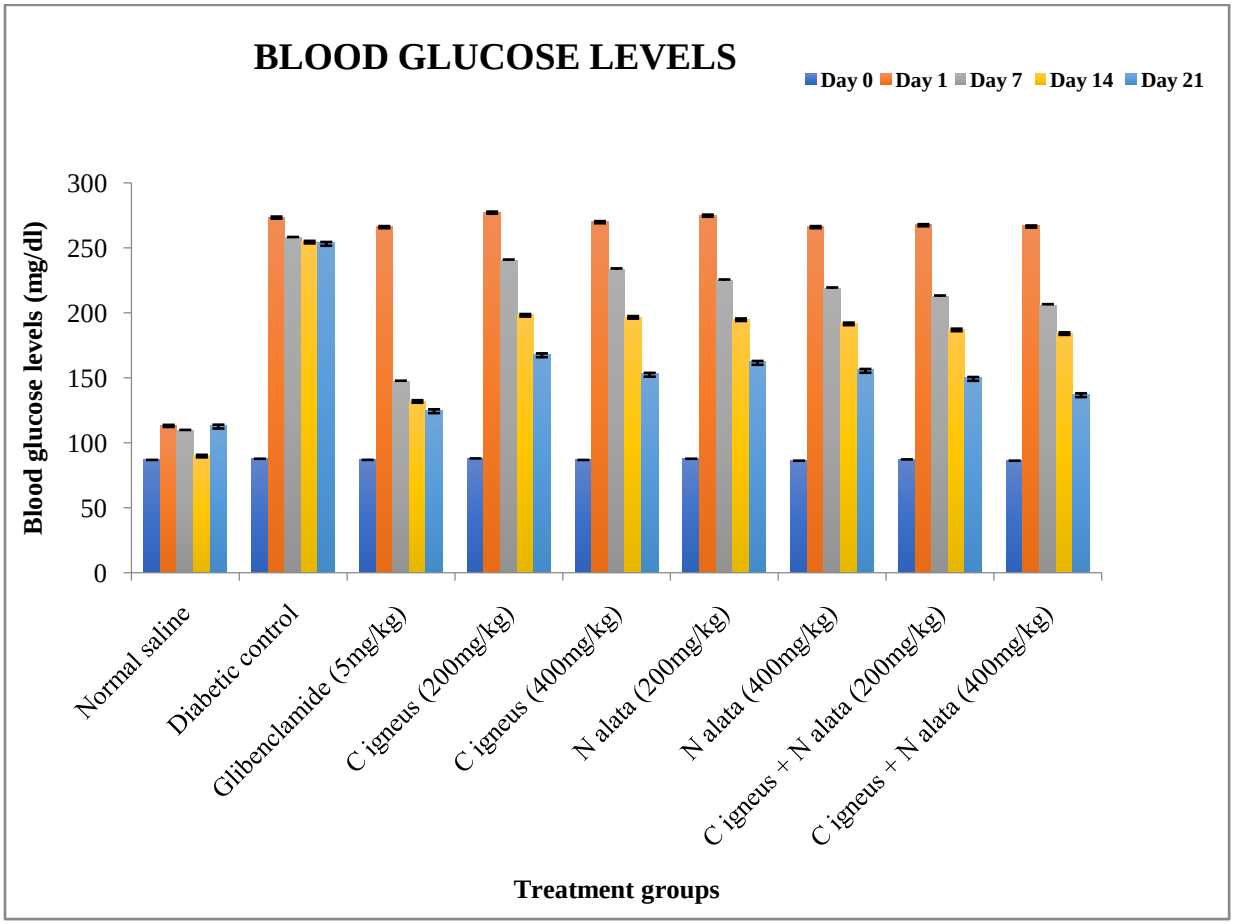
BLOOD GLUCOSE LEVELS

Group	**Day 0**	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal saline	86.78 ± 0.71	113.33 ± 0.55	109.16 ± 2.22	90 ± 0.73	113.5 ± 2.02
Diabetic control	87.66 ± 0.80	273.5 ± 0.76	257.5 ± 1.54	254.5 ± 0.61	253.66 ± 0.74
Glibenclamide (5mg/kg)	86.83 ± 0.30***	266.16 ± 0.87***	136.83 ± 1.72***	132.5 ± 1.31***	100 ± 1.06***
Ci(200mg/kg)	87.91 ± 0.77*	277.33 ± 0.80*	240.16 ± 0.79*	198.0.30 ±0.03 *	168 ± 0.68*
Ci(400mg/kg)	86.73 ± 0.26*	270 ± 1.77*	233.33 ± 1.08*	196.66 ± 0.33*	153 ± 0.86*
Na(200mg/kg)	87.66 ± 0.98**	275 ± 1.36**	224.83 ± 0.79**	194.83 ± 0.30**	161.5 ± 1.30**
Na (400mg/kg)	86.166 ± 0.30**	266.16 ± 0.600**	218.66 ± 0.71**	191.66 ± 0.8**	156 ± 0.93**
Ci +Na(200mg/kg)	87.16 ± 0.16***	267.66 ± 0.61***	212.5 ± 1.02***	187 ± 0.36***	149.83 ± 0.79***
Ci+Na (400mg/kg)	86.16 6 ± 0.60***	266.66 ± 1.4***	205.83 ± 1.37***	184.16 ± 0.30***	147.33 ± 0.71***

Table 6: Blood glucose levels observed on various days

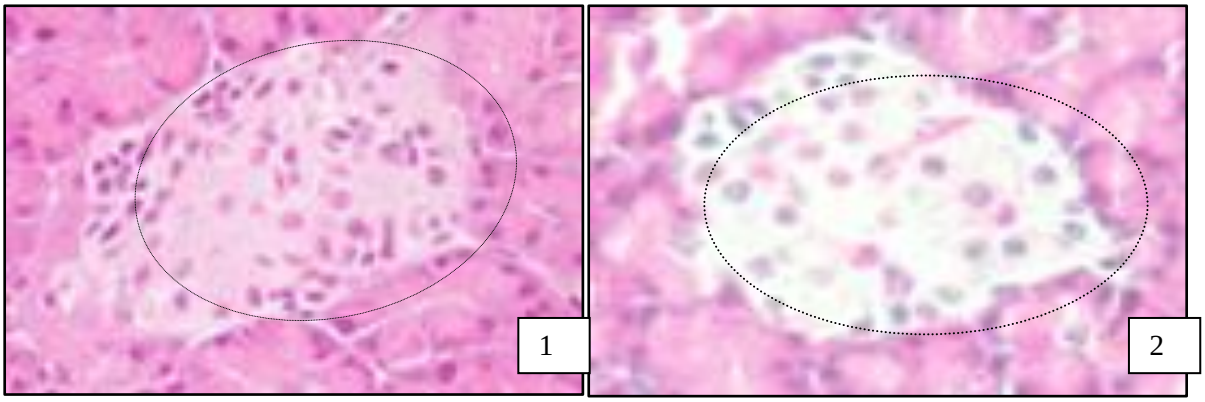
The values in the table are represented as Mean ± SEM

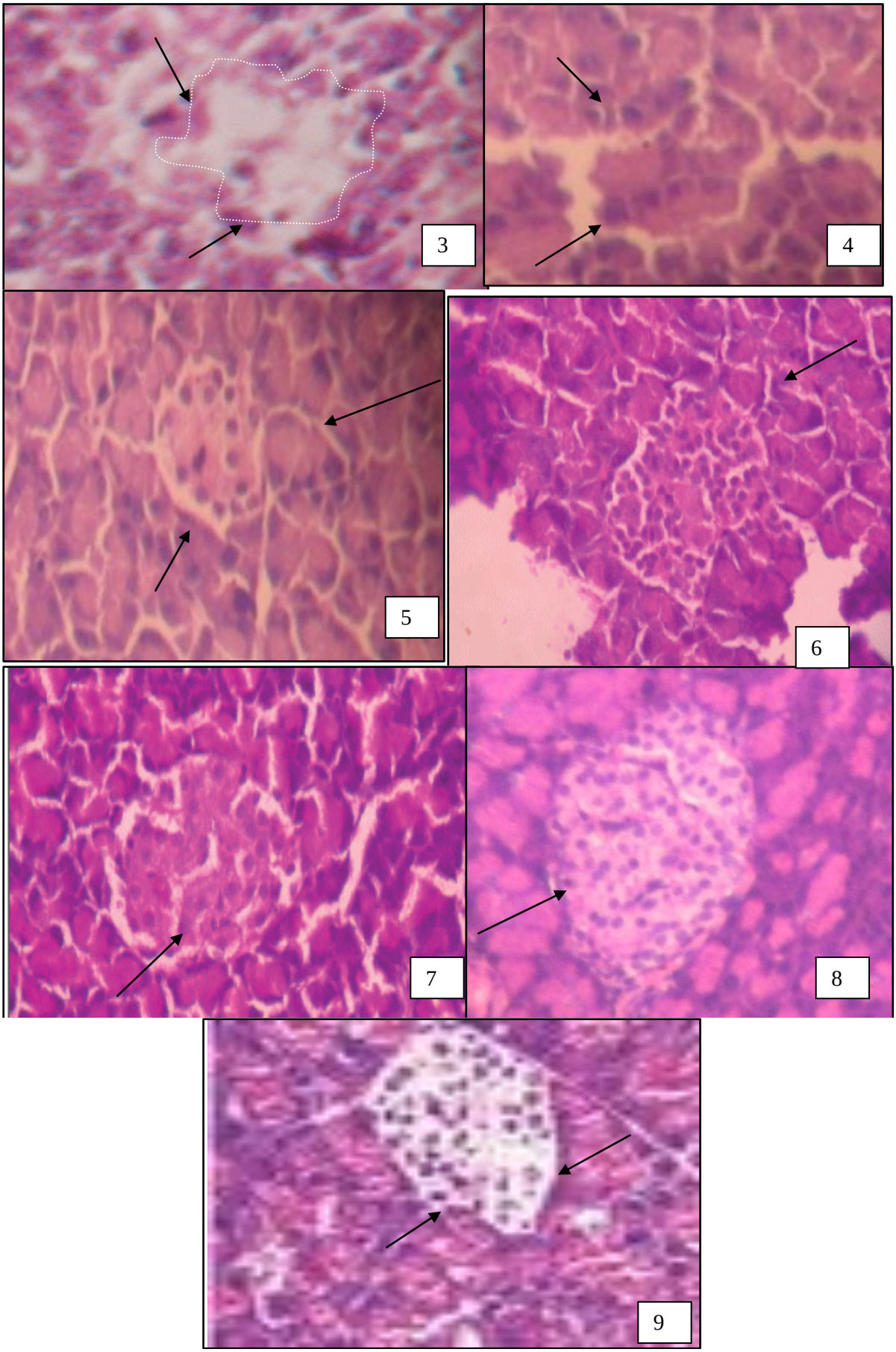
***P<0.05, P<0.01**, P<0.001* was considered significant when compared to diabetic control group



Graph 3: blood glucose levels

PICTOGRAPHS OF PANCREAS





Images of histological slides of pancrease on the whole considered .

1. Normal saline: The slides display normal framing of pancreas with normal amount of acini and islets of langerhans.
2. Diabetic control: With Glibenclamide(5mg/kg) tissues displayed noticeable restoration of tissue of pancreas and islets of langerhans.
3. Diabetic control : The slides of samples that were kept as strptozotocin control displayed severe necrosis to the tissue of pancrase and damage to islets of langerhans. The cell walled disappeared and nucleus is not clearly seen
4. C igneus (200mg/kg): At dose of 200mg/kg of *Costus igneus*, the rats were able to restore the damage to an extent but the cells appared quite irregular in size and less in number
5. C igneus (400mg/kg At the dose 400mg/kg of *Costus igneus* the cells of pancreases exhibited less vacuolization in exocrine tissue and increase in number of acini and islets of langerhans were intact
6. N alata (200mg/kg): At 200mg/kg of *Naregamia alata*, the tissues showed regeneration of wall of islets of langerhans and gradual increase in acini number is also seen.
7. N alata (400mg/kg): At 550mg/kg of *Naregamia alata*, the tissues exhibit equal to no vacuolization in tissue, the islets wall is visible number of increased no acini and improved cells
8. C igneus + N alata (200mg/kg)displayed improvement in Islets of Langerhans shape, size and density. Cells are
9. C igneus +N alata (400mg/kg) displayed restoration of cell population and enlargement of cell size is also seen. Islets of Langerhans are in close proximity with very mild vacuolization in exocrine tissue.

IV. Discussion

In my study I have used leaves of plant *Costus igneus* from the family Costaceae at various doses of 200mg/kg b.w and 400mg/kg b.w (referred as Ci) and roots of plant *Naregamia alata* from the family Maliaceae at various doses of 200mg/kg b.w and 400mg/kg b.w (referred as N.a). The plants were also used in combination of 100mg/kg Ci + 100mg/kg Na and also 200mg/kg Ci + 200mg/kg Na.

Phytochemicals such as alkaloids, steroids, flavonoids, tannins, glycosides and terpenoids were found in significant amounts.

Hyperglycemia is primarily increased glucose in blood. This is seen when the pancrease either loose their ability to produce insulin or when the cells of body do not welcome insulin into them.

One is caused due to change and shrinkage in beta cells of pancreas which produce insulin or when there is reduction in receptors of insulin in body leading to less signaling of insulin molecule towards the cell, making the cell not opening the gates for glucose to enter leading to heavy traffic of glucose in blood.

Studies conducted on *Cucurbita maxima* by Ashok Sharma et al* concluded that alcoholic extracts in stz induced diabetic rats produced 74% effect in reducing blood glucose levels.

The outcome of my study is compared with that of toxic v/s standard and best dose of each plant and combination of both at the highest dosage.

On the day 7 blood glucose levels were decreased upto following % with Ci 58% , Na 62% and Ci + Na 66% , On day 14 Ci 67%, Na 69% and Ci + Na 71%, on day 21 Ci 65 , Na 64% and Ci + Na 68% Ci 58% ,effects were produced and was compared to 85% effect of glibenclamide against normal.

Invitro analysis for anti diabetic action was determined using Alpha amylase and Alpha glucosidase assay which are most important enzymes in metabolism of carbohydrates, the assay was performed using Acarbose as standard. Alpha amylase and Alpha glucosidase inhibitors are utilized to obtain rapid control over increased glucose in blood. Alpha amylase is involved in breaking of long carbohydrate chains and glucosidase breaks down starch and 2 sugar molecules into glucose each, which should be stopped to control hyper glycemia. The IC50 in Alpha amylase inhibition assay for Acarbose is obtained at 44.3ug/ml concentration, for Ci Ic50 is obtained at 59.2 ug/ml conc and for Na Ic50 is obtained at 61.3ug/ml conc. The Ic50 in Alpha Glucosidase inhibition assay was obtained as Acarbose at 45.5ug/ml, Ci at 55.9ug/ml and Na at 64.2ug/ml.

V. Conclusion

The study significantly exhibited anti diabetic potential of Costus igneus and Naregamia alata with the % potency showcased in the discussion part. As a result of positive outcome of preclinical trials more immense research work can be carried out for the combination therapy of Costus igneus and Naregamia alata for the development for potential drug for reducing glucose in blood.

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