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HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC ACTIVITY OF *SYZYGIUM ALTERNIFOLIUM (WT.) WALP.* LEAF EXTRACTS IN NORMAL AND DIABETIC RATS.

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ABSTRACT

Aqueous, ethanolic and chloroform fraction of Syzygium alternifolium leafs were prepared and given different doses of these extracts individually to different batches of rats (both normal and alloxan induced diabetic rats) after an overnight fast. The blood glucose levels were measured at 0, 1, 3, 5 and 7 hours after the treatment. The ethanol extract of Syzygium alternifolium at a dosage of 0.75 g/kg b.w. is showing maximum blood glucose lowering effect in both normal and alloxan induced diabetic rats. The aqueous and chloroform fractions are also showing hypoglycemic and antihyperglycemic activity, but the effect is significantly less than that of ethanol extract. The antihyperglycemic activity of Syzygium alternifolium leafs was compared with the treatment of Glibenclamide.

Key words: *Hypoglycemic, syzygium alternifolium, alloxan, glibenclamide*

Introduction

All over the world, plants are put to medicinal use from very ancient times in addition to the other benefits derived from them. Today we find in every country the folklore living in the nooks and corners are habituated to use their plant resources. They have developed ethnic systems of medicine. Some of them are developed or prolonged experience.In modern era, the medicinal plants have been gradually replaced by synthetic drugs. But of late, it is being realized that several synthetic drugs produce receptor up regulation. Drug tolerance are also responsible for many of adverse effects.

25% of the prescriptions in the west relate to traditional medicines, necessitating the need to conduct research on isolated bio-active principle of plant drugs. This has lead to the revival of interest in the herbal medicine and the investigations in this field have gained great importance.

Diabetes mellitus is the most common metabolic disorder known to the ancient indian physician, some 3000 years ago, as can be seen, from the medical texts such as charta samhita and sushruta sumahita. They have discussed the honey urine in detail. However, the name diabetes was given by two Roman Physicians celsius and Aretaeus in 1st A.D. in 1921 Banting and best solved the problem of diabetes to a great extent by discovering insulin as a therapeutic agent in insulin dependent diabetes mellitus (IDDM). The introduction of oral hypoglycemic agents was another advancement to convey better management of non-insulin dependent diabetes mellitus (NIDDM). But none of them unequivocally was successful in maintaining the normal glucose levels and avoiding the late stage complications of complications of diabetes (neuropathy nephropathy, blindness, infection, Vescular insufficiency). About 15-20% of patients with newly diagnosed NIDDM has little or no response to sulphonyl ureas and with each year of treatment about 3-5% of the patients who have achieved better acceptable glycemic control loose their responsiveness. Therapy with

biguanides is associated with lactic blood disorders, water retention and several others.

Inspite of all the advances in therapeutics, diabetes still remain a major cause of morbidity and mortality in the world. As per the available data there are at least 20 million diabetics in India.

The most ancient medical systems of India provides information that madhumeha (diabetes) has long been treated with various herbs and herbomineral drugs. It is observed that some are used as antidiabetic medicines by folklore. Notable among them are the use of fresh juice of bael, onions, garlic.

Analytical methods:

Blood glucose Estimation: Several methods are used for the estimation of blood glucose. In my study im going to use orthotoludine method. This method estimates only glucose but no other reducing substances like glucuronic acid and glutathione. But we have decided to use "Semi Auto Analyses" for my study.

Principle:

"Semi Auto Analyser" with which we can find out serum glucose level for a series of samples collected from experimental animals or patients per clinicial study.

Study of Hypoglycemic effects of Syzygium Alternifolium in Diabetic Rabbits

The different leat extracts of Syzygium alternifolium were shown to lower blood glucose in laboratory animals. Syzygium alternifolium seeds powder being used with positive results by some local people suffering from diabetes. Fruits are also used to control diabetes. Based on this to know the leaves of Syzygium alternifolium either have any activity are not we carried out our scientific study. It was planned to find the response of the substances in subsists on blood glucose reduction individually.

Plant Material:

The fresh leaves of Syzygium alternifolium were collected from Tirumala Hills, TPT, Chittoor District. Then the leaves were subjected for air-drying. After drying, leaves were ground by mixes. The coarse powder was stored separately in airtight container until the time of use.

Selection of Soluents:

The solvents were selected bussed on polarity. The polarity of solvents decreases in order to Water, Alcohol, Chloroform, respectively.

a). Preparation of Leaf Extracts of Syzygium Alternifolium

Soxhalet apparatus was used for extraction procedure. The extract was filtered and evaporated in water both until to get the residue. The residue was dried, and stored in well-closed container.

(b) Experimental Diabetes in Rabbits :

Several methods are being used to produce experimental diabetes in animals. These include pituitary diabetes, pancreatectomy, glucose diabetes and alloxan induced diabetes. In the present study, the author used alloxan for producing experimental diabetes in rabbits as followed by Bhimji et al., (1985).

Procedure for inducing alloxan diabetes in rabbits:

Albino rabbits of either sex were fasted overnight before injection with alloxan. Alloxan monohydrate (Aldrich & Thomas Laboratories, Wal Mum) was dissolved in sterile saline immediately before use and was injected (100mg /kg body weight) into the marginal ear vein of rabbits lightly anesthetised with anesthetic either.

Since alloxan is capable of producing fatal hypoglycemia as a result of massive insulin release from the pancreas, animals were treated with 20% glucose (15 to 20 ml) sub-cutaneously every 4 hours for the first 24 hours following alloxan administration. To prevent dehydration from the severe polyuria, intravenous saline (10 ml/ kg) was also administered.

Rabbits, which did not become diabetic, were given another injection of alloxan. Most of the rabbits became diabetic within 24 to 48 hours after a single alloxan injection. The blood glucose levels ranged from 250 to 500 mg %. Experimental diabetes was produced in rabbits as and when required for the study.

(c) Experimental Design:

*Oral administration of drugs: F*or administering the drugs orally, a mouth gag was introduced in between two jaws and held in position by holding the upper and lower jaws with the left hand. Oral feeding tube was moistened with glycerin and introduced into the mouth through the central hole of the mouth gag with the other hand. The tube was pushed slowly such that it enters the oesophagus and reaches the stomach. To the other end of the tube a funnel was attached.

After administering 2 ml of distilled water, to ensure free flow into the stomach, the required quantity of drug in the form of solution or suspension was administered through the funnel and the stomach tube. This was followed by the administration of 3 ml of distilled water to carry any solution or particles adhering to the funnel and the walls of the tube into the stomach and to ensure the administration of the correct dose of the drugs. Afterwards the funnels and the oral feeding tube were removed gently.

Procedure :

Alloxan induced diabetic rabbits of either sex weighing 1.4 to 2 kg were used in the study. Animals were divided into 4 groups of five each and ere provided with standard diet and water *ad libitum*. All the rabbits were kept in cages with wide squares of bottom mesh to avoid coprophagy. They were fasted for 18 hours prior to the experiment, giving access to water. Rabbits were fixed in a wooden stall with their head protruding out for either administering the drugs or for withdrawing blood samples.

1) Group I served as control.
2) Group II were given orally, with suspension of chloroform extract of syzygium alternifolium at dose level of 150 mg / kg body weight.
3) Group III were given orally, with suspension of alcohol extract of syzygium alternifolium at dose level of 150 mg / kg body weight.
4) Group IV were given orally, with suspension of water extract of syzygium alternifolium at dose level of 150 mg / kg body weight.
5) Group V were given orally, with suspension of glibenclamide at the dose level of 500 micrograms / kg body weight.

Withdrawing of blood samples:

The blood samples were withdrawn initially and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20 and 24 hours after treatments, from the marginal ear vein of each rabbit and were analysed for glucose content. The ears were shaved and the blood vessels were made to dilate either by warming the ear on a low voltage electric lamp or by rubbing with a cotton swab. The dilated vein was punctured by means of a sharp syringe needle in the direction of venous blood flow. The blood flowing outside was collected in vials containing a suitable anticoagulant (1:3 mixtures of sodium fluoride and potassium oxalate).

Methodology:

As per preliminary pharmacological screening of syzygium alternifolium. Determination of Toxicity Studies (Kulkarni S.K. (1999 Ed.) 165 – 71)[3]

A. Acute toxicity, the LD50 is determined in mice using the method of Horn et al. (1956). Doses of the compound are given in 4 groups of 3 mice each in a geometrical progression starting with a dose of 464 mg/kg ip and mortality in 24 hours recorded. The LD50 with fiducial limits is read out from the table given in the method.

Table 4.4 (S. K. Kulkarni, Hand book of Experimental Pharmacology) Transformation of Percentages of Probits

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.83	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Correction for 0% dead = 100 (0.25/n) and 100% dead =100× [(1 – 0.25)/n]

- **Gross behavioural studies** (Kulkarni S.K, 1999 Ed., 119)[3]
- After ip Administration of the compound to 2 groups of 3 mice each the animals are observed for gross behavioural effects. The animals are observed continuously for 3 hours after administration of the compound, then every 30 minutes for next three hours and finally after 24 hours. CNS stimulation is judged by increased spontaneous motor activity (SMA), piloerection, exophthalmos, clonic and / or tonic convulsions; CNS depression is judge by reduced SMA, sedation, ptosis, crouching, catalepsy and autonomic effects like piloerection, urination, defaecation, salivation, lachrymation etc. At ½ the LD50 these effects are recorded using 2 groups of 3 mice each and effect on the body temperature is also recorded with a telethermometer suing YSI type 402 physiological probe.
- **Antidiabetic activity** (Phytother Res 2002 Sep, 16 (6): 590-2)[7]

  Animals : Rabbit (2.0 to 3.0 kg)
  Species : Chinchilla (Either sex)
  Drugs : Alloxan (150 mg/kg)
  Standard drug : Glibenclamide (1.4 mg/kg)

Procedure

Rabbit weighing 2.0 to 3.0 kg are used, divided into 3 groups. Test (3), standard (3) and control (2), Alloxan is used to induced the diabetes, Blood samples are collected and estimated by colorimetically or using semi auto analyzer.

- **Anti inflammatory** (J Ethno Pharmacol 2000 Sep. 72(1-2): 87-92)[8]

  Animals : Albino Rat (150 to 200 g)
  Species : Wistar strain (Either sex)
  Drugs : Carrageenan (1% w/v solution, i.e. 0.1ml)
  Standard drug : Indomethacin (20 mg/kg)

Procedure

Rats weight 150 to 200 g used, divided into 2 groups. Test(3) standard (3) and control (2). Paw oedema is induced by chronic inflammatory agent carrageenan, the extent of inflammation and the percentage decrease in inflammation is determine by use of plethysmograph.

- **Antipyretic activity** (Kulkarni S.K, 1999 Ed :165)[3]

  Animals : Albino Rat (150 to 200 g)

Species : Wistar strain (Either sex)
Drugs : Brewer's yeast (10ml/kg g)
Standard drug : Paracetamol (1.4 mg/kg)

Procedure

Rats weight 150 to 200 g used, divided into 3 groups control (4), test (3) standard (5), Pyrexia is induced by Brewer's yeast. Pyrexia is measured by telethermameter, results are evaluated by comparing with the control and standard.

- **Analgesic activity** (Vogel (1997) 368)[4]

Animals : Albino Mice (20 to 30 gms)
Species : Swiss strain (Either sex)
Equipment : Analgesiometer
Standard drug : Brefenorphen (1.4 mg/kg)

Procedure

Mice weight 22-30 g are used, divided into 3 groups control (3) standard (4) test (4). Pain is induced by analgesiometer. Pain threshold measured by comparing with the control and standard.

- **Diuretic activity** (Vogel, 1997 Ed.)[4]

Animals : Albino Rat (100 to 200 g)
Species : Wistar strain (Either sex)
Standard drug : Frusemide (1 to 2mg/kg)

Procedure

Rats weighing 100-200 gms are used, divided into 3 groups, Test (4), Standard (4) and control (3). The test is based on water, sodium excretion in test animals and compare to rats treated with a high dose of urea.

- **Rota Rod Experiment** (Kulkarni, S.K. 1999 Ed: 122)[3]

Animals : Albino Mice (20 to 25 gs)
Species : Swiss strain (Either sex)
Equipment : Rota Rod
Standard drug : Diazepam (4 mg/kg)

Procedure

Mice weighting 22-24g are used divided into 3 groups Standard (4), Test (4), Control (3). Note down the fall off time. When the mouse falls from the rotating rod and evaluated by comparing with the standard.

- **Anti-histaminic activity** (Kulkarni S.K, 1999 Ed)[3]

Animals : Guinea pig (300 to 400 gs)
Species : Inbred (Either sex)
Equipments : Histamine Chamber
Standard drug : Mepyremine Maleate (1.4 mg/kg)

Procedure

Guinea pigs or weighing 300 to 400 gs for divided into 3 groups, test (2), standard (2) and control (2) finally atomized mist of 0.2% solution of histamine acid phosphate will be blown in to chamber containing Guinea pig. 2 ½ hours later the animals will be injected ip with a dose of antihistamine. After 30 min. they will be again exposed to histamine.

The animals who with standard exposure to histamine for 10 min. will be considered completely protected. Each antihistamine drug will be tested at 4 dose levels.

conclusion

To compare the pharmacological activity of crude drug extract (**syzygium alternifolium**.) with presently available standard drug as per project methodology in Phase II.

- Determination of toxicity studies
- Gross behavioral studies
- Antidiabetic activity
- Anti inflammatory activity
- Anti pyretic activity
- Analgesic activity
- Diuretic activity
- Rota Rod experiment
- Anti – Histarminic activity

syzygium alternifolium is one of the ancient plant which is used in traditional system of medicine ayurveda, siddha etc.It may be the plant have some activity improved to evaluated & discover the activity of the plant would thus prove a important contribution to science.The research with this plant is most suitable to evaluate it's efficacy.

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