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RESEARCH OF HERBAL CAPSULE FOR DIABETES TYPE-2

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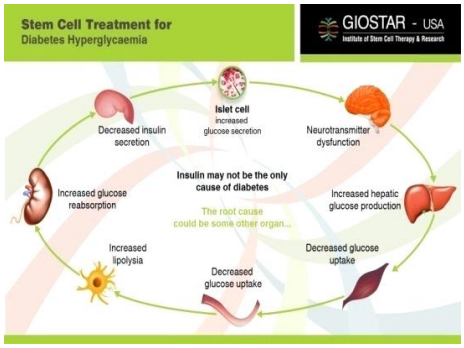
- **ABSTRACT:-**

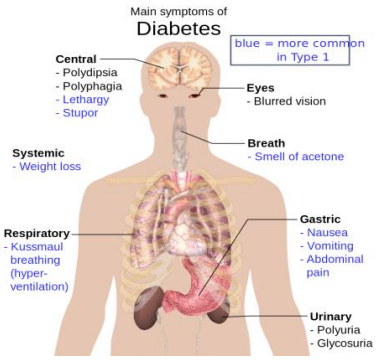
A research work of herbal medicines viz. Neem leaf extract (Azadirachtaondica) , Nayantara leaf extract (cathantharoseas) and Bitter Melon fruit (monodica charantia)) powder dry and Syzygium Cumini dry powder with the patent dry gliciazide were studied on blood glucose level, hematological parameters heathy adult rats were randomly divided into 5 equal groups namely A,B,C,D and E. One group (groupA) was kept as control .The rest four groups (B,C,D,andE) of rats were treated with gliclazide 4.5mg/kg.bd.wt/day. Neem leaf extract (NtLE)1gm/kg.bd.wt/day. Nayantara leaf (NtLE) 1gm/kg.bd.wt/day.bitter melon fruit dry powder (NtLE) 1gm/ kg.bd.wt/day. Syzygium cumini (NtLE)1gm/kg.bd.wt/day. The herbal drugs used in the study bitter melon fruit powder was more effective in increasing the body Wt. in comparison with other herbal preparation (NtLE) and (NLE) and Syzygium Cumini from the present study it may be further revealed that although the patent drug Gliclazide was found to be highly effective, as blood glucose agent, but the efficacy of their different combined froms of herbal preparation was also seemed to be encouraging.

- **KEY WORD** :- Herbal medicine, diabetic patients ,neem leaf ,nayantara leaf ,momordica charantia, syzygium cumini.

- **INTRODUCTION:-** Diabetes is a metabolic disorder of carbohydrate fat and protein metabolism which is considered as one of the major health problem in the world today. In the year 2000 about 17% million people had diabetes and it is estimated that it will increasing up to 366 million . Bitter melon (momordica charantia)is a common plant known for its medicinal properties such as anti –inflammatory ,antidiabetic. Cantharanthus roseas linn.is a species of the genus catharanthus in the family Apocynaceae that is used in many herbal medicine preparations. Neem is widely used as amdicinal plant for thousand of years. Different parts for neem (Azadirachta indica) like neem leaf extract have been shown to posses hypoglycemic effect.

- **PHARMACOLOGY:-** Hypoglycemic activity:- Charantin isolated from fruits M.charantia was tested for its hypoglycemic activity. Charantin was found to be more potent then to lbutamide however both compounds produced similar pattern of blood sugar change . The Hypoglycemic Nayantara leaf :- 2 fresh leaves ,flower ,leaf powder suspension methanolic extract ,aq.extract .





(b)

- **DOSE:-** 0.5-1.0ml/kg or 1gm/kg bd .wt.oraganisms:-Normal and alloxoan diabetic rabbit ,noramal and streptozotocin – induced pharmacological activity :- antidiabetic.

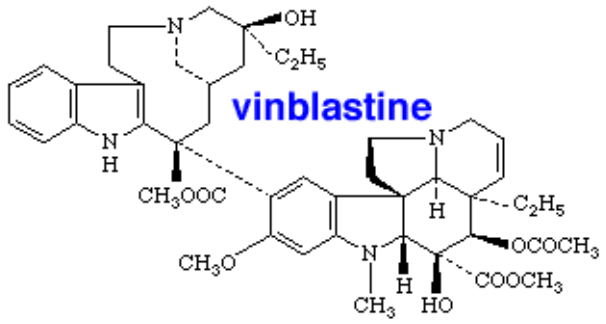
- **MONOGRAPH :-**1) Bangali name :- nayantara 2) englih name :- capeperiwinkle , old –maid.

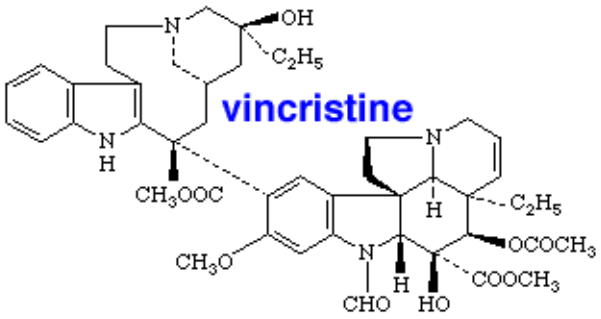
3) scientific name :- catharanthus roseas.4) family :- Apocynaceae. 5) duration :- perenninal or annual. 6)growth habit:- Herb 7) leaf :- alkaloid indole category. 8) vinca :- catharanthus rosea . 9) chemicals :- vinblastine sulphate, vincristine sulphate.

- **STRUCTURE:-** vinblasting R= CH3
  vincristind- R= CHO

(c)

(d)



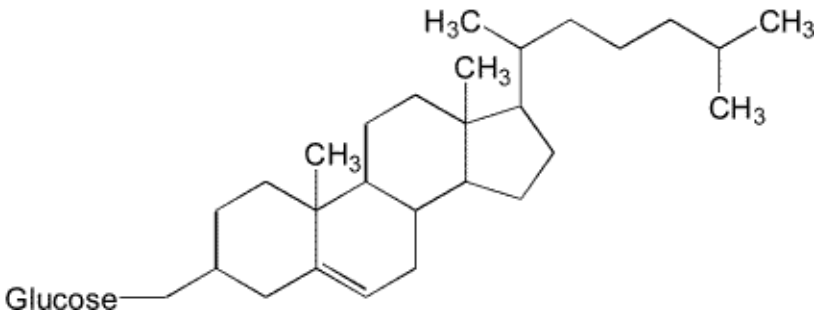


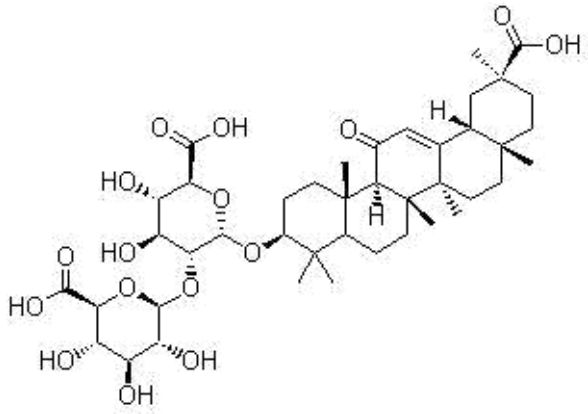
2) charantin :- chemical :- charantin ,stearoidal saponine.

Category :- alkoids

(e)

Momorcharin





Charantin

(f)

- Neem:- synonym:- margosa .
- Biological source:- Azadirachta indica .
- family:- meliaceae .
- macroscopic characters :- leaves :- alternate, exptipulate, impairipinnete leaf lets 20-25 cmin length lanceolata closely clustered towards the ends of bronches . serrate margin, green ,bitter.
- Chemical:- Azadirachtin , meliantriol, salanin ,nimbin.
- Use:- reducing sugars , saponins insecticide, antifeedant, nematocide and antimicrobial properties
- **METHOD :- Soxhlet extraction :-** Bitter Melon fruit was dried powder (1g) and extracted with 200 ml of ethanol and added (1g) neem leaves and nayantara leaves juice and Syzysium Cumini (1g) and for15min.extracted. Neem leaf and Nayantara leaf extract :- Procedure :- Fresh Neem leaf and Nayantara leaves were collected 1gm /kg bd.wt./day and then ground with mortar and pestle . Finally only the leaf extract was mixed with 10ml distilled water and stirred for homogenous mixture ,kept 6 hr and then filtered with silk cloth added in Soxhlet extraction method. Recorded and during treatment of different herbal preparations of patent drug. Statistical analysis :- The data were analyzed statistically between treatment and control values by well known student's test ("t"test) Dosages schedule :-

Group A:- control (normal rat without any treatment) Group B:-normal rat treated with comprid (gliclazide) 4.5 mg/kg bd.wt/ day orally for 14 days.

Group C:- normal rat treated with NLE 1gm/kg.bd.wt/day orally for 14 day .

GroupD:- normal rat treated with nayantara 1 gm/kg bd.wt/day orally for 14 day .

Group E:- normal rat treated with bitter melon 1 gm/kg bd.wt/day orally for 14 day

Detailed phytochemical examination were corried out for 4 ingredient extracts as per the std. method .

A) Tests for Alkaloids :- To the extracy ,dilute hydrochloric was added . shaken well and filtered with the filterate,the following test were performed .
1) Mayer's reagent test :- To 3ml of filterate ,few drops of mayer's reagent were added along sides of tube .formation of creamy precipitate indicates the presence of alkaloids . B) Tests for carbohydrates :- Molisch test :- 1) 2ml of aqueous extract was treated with 2 drops of alcoholic a-naphthol solution in a test tube and then 1ml of concentration sulphuric acid was added carefully along the sides of the test tube .formation of violet ring at the junction indicates the presence of carbohydrates . C) Tests for Reducing sugars :- fehling's test :- To 1ml of aqueous extract ,1ml of fehling's A and 1ml of fehling 's B solution were added in at test tube and heated on awater bath for 10 min. formation of red precipitated indicates the presence of reducing sugar . D) Tests for flavonoids :- Alkaline reagent test :- the extract was treated with few drops of sodium hydroxide solution separately in a test tube . formation of intense yellow color, which become colorless an addition of few drops of dilute acid indicates the presence of flavonoids . E) Tests for Glycosides :- Test legal's :- 1ml of test solution was dissolved in pyridine . 1ml of sodium nitropruside solution was added and made . alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of cardiac .

EXPERIMENTAL ANIMAL :- Wistar albino rats weighing 200-250g of either sex was maintained in the department of animal house for experimental purpose. Then all the animals were acclimatized for two weeks under standard husbandry conditions i.e. room temperature of 25±1°C; relative humidity 45-55% and a 12:12h light/dark cycle. The animals had free access to standard diet with water supplied ad libitum under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of nonspecific stress. All the protocols and the experiments were conducted in strict compliance according to Institutional Animal ethical Committee guidelines. The extracts was evaluated for the antidiabetic activity using following experimental model. All the animals were divided into 7 groups each containing 6 rats as follows; Group I- Control group: 2ml Saline p.o.; Group II- Diabetic Control: 2ml Saline p.o.; Group III- Petroleum Ether Extract: 160 mg/kg p.o.; Group IV- Chloroform Extract: 200 mg/kg p.o.; Group V- Ethanol Extract: 240 mg/kg p.o.; Group VI- Aqueous Extract: 180 mg/kg p.o.; Group VIIGlibenclamide: 10 mg/kg p.o. All the animals were fasted over night and then blood glucose level was determined immediately before treatment and then 7 hrs after treatment[14].

Oral glucose tolerance test (OGTT) Animals were fasted for 12 h before the OGTT. Glucose (1g/kg) was administered by gavages 30min after oral administration of 250mg/kg of Girardinia heterophylla leave extracts. Glibenclamide at dose of 10mg/kg was used as standard drug.[15] Blood glucose level was measured each hour after glucose loading in rats under light ether anesthesia. Blood was obtained from retro orbital puncture by using heparinised capillary tube and immediately centrifuged for 5 min. Plasma was analyzed for glucose content using a glucose oxygenase method (Sigma diagnostics centre, Dehradun)[16].

2.9 Statistical Analysis Results of Anti-diabetic activity were reported as Mean ± SEM. Significant intergroup difference of each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. The calculated mean tabulated along with the Dunnet's't' test was used for individual comparison.

3.2 Antidiabetic activity :- The petroleum ether (40-600C), chloroform, ethanol and aqueous extracts were given orally at a dose of 200 mg/kg b.w. with the help of gastric livages tube in alloxan induced diabetic rats. Further the blood glucose level was analyzed initially (0 hr), 1st hr, 3rd hr, 5th hr and 7th hr after single dose and 7th day after prolonged treatment of leave extracts. Normal control and diabetic control animals received equal volume of

normal saline and Glibenclamide (10 mg/kg b.w.) served as standard control. Blood glucose level was measured in all groups by using glucometer (Pulsatum, Pulsatum Health Care Pvt. Ltd., Bangalore) and glucose test kit. Random blood glucose level after single dose administration of reference and test drug in Alloxan induced diabetes rats indicating that chloroform, ethanol and water extract showed significant ($p \leq 0.005$) reduction in blood glucose in comparison with diabetic control group at every alternative hour for 7 hour observation. The results were comparable with standard drug Glibenclamide and Ethanolic extract of G. heterophylla, which shows statistically significant ($p \leq 0.005$) improvement in antidiabetic activity on single dose treatment at 7th hour compared to diabetic control group (table 1).

3.4 Histopathology:-The Histopathological report of the kidney (figure 1) showing the normal tubular structure, tubular epithelium attached with base line of the normal control animals (A). The diabetic animals showing induced nephropathy in which cell cytoplasm completely warned out & the nucleolus is pyknotic & lumen of tubule shown cell debris (B). The animals treated with Petrolium ether extract showing the partial recovery with few damaged tubules, damaged cells with pyknotic nucleolus (C). The animals treated with Chloroform Extract showing less recovery, damaged tubules still present with cell debris in lumen of tubule (D). The group treated with Ethanol extracts has observing good recovery from the damaged tubules and new cells were replacing the damaged ones of the tubules (E). The group administered with Aqueous Extract showing ongoing partial recovery from damage (F). The Glibenclamide treated group was showing the good recovery with apparently normal tubules (G).

(a)rat for diabetics **(b)**

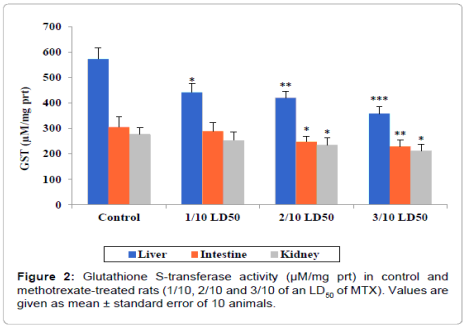
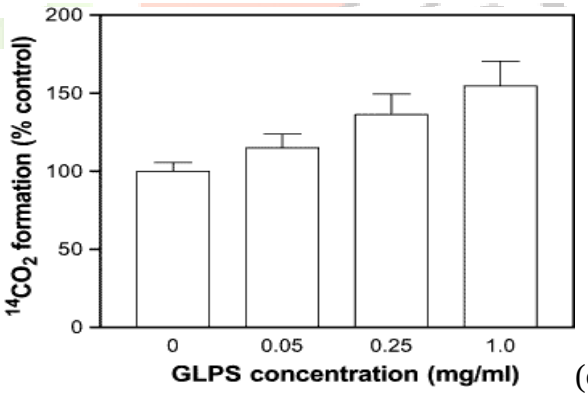
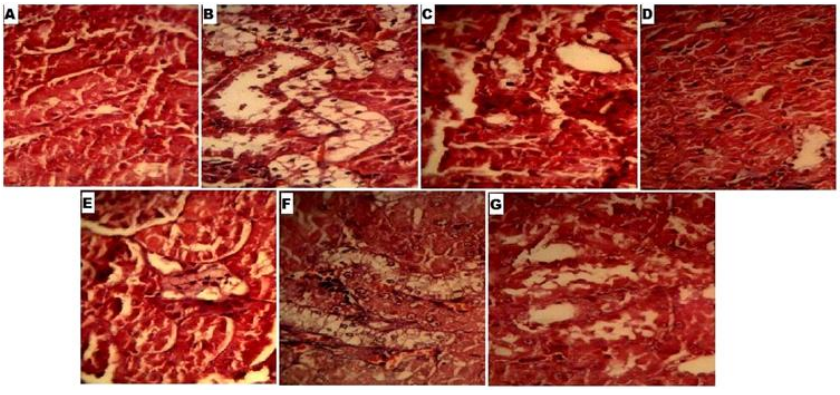


Figure 2: Glutathione S-transferase activity (μM/mg prt) in control and methotrexate-treated rats (1/10, 2/10 and 3/10 of an LD_{50} of MTX). Values are given as mean ± standard error of 10 animals.



(c)



(a)

SOFT CAPSULE FORM COMPSION :- HERBAL MEDICINAL LIQUID .

Ingredients/Materials	Amount (mg)	Function
Glycerin	52	Plasticizer
Propylmethyl Paraben	0.512 mg	Preservative
Methyl Parabe	0.128 mg	Preservative
Gelatin 120 Bloom	152 mg	Basis of gelatin
Briliant Blue Ponceau	0.3 mg	Coloring agent
Sunset Yellow	0.3 mg	Coloring agent
Titanium Dioxide	16 mg	Opacifier
Water	120ml	Solvent
Sorbitol Liquid	16 mg	Plasticizer
Herbal product solution	42.30 mg	

		40 mg	120 mg
Ingredient	Function	mg/capsule	mg/capsule
Compound (1) herbal product	Drug	42.30 [1]	126.90[2]
Liquid.	substance		
Mono/Diglycerides of	Lipid phase	130.57	391.70
Caprylic/Capric Acid			
Polyoxyl 35 Castor Oil	Surfactant	86.86	260.57
(NF)			
Macrogolglycerol			
Ricinoleate (Ph. Eur.)			
Propylene Glycol	Solvent	21.71	65.14
Vitamin E (dl-alpha	Anti-	0.56	1.69
tocopherol) (USP)	oxidant		
All-rac-alpha-tocopherol			
(Ph. Eur.)			
Nitrogen[3]	Processing	q.s.	q.s.
	aid		
Total Fill Weight		282.00	846.00
Soft Gelatin Capsule	Shell	280 [4]	590 [5]
Shell			
Wet Total Capsule		562	1436
Weight			
Dry Total Capsule		480	1250
Weight			

- 142.30 mg of Compound (1) herbal product liq. is equivalent to 40.0 mg of the active moiety.
- 126.90 mg of Compound (1) herbal product liq. is equivalent to 120.0 mg of the active moiety.
- Nitrogen is used as a processing aid and does not appear in the final product.

• The approximate weight of the capsule shell before drying and finishing is 280 mg. The approximate weight of the capsule shell after drying and finishing is 198 mg.

Two specific soft-gel capsule drug product formulations were prepared according to the above general Formulation #1, a 40 mg product and a 120 mg product

CONCLUSION-

Natural product are acquiring substantial importance for the treatment of diabeties .Recent advances in scientific research heve proved the role of M.charantia,neem(Azadirachtaondica), nayantara(cathantharoseas) , syzygium cumini ,for management of diabetes . charatin is also being investigated as bioactivity principle for hypoglycemic activity . There is need to evaluate this natural compound clinically . Substantial clinical data need to be generated to establish hypoglycemic potential of this compound and to support emergence of safe and effective antidiabetic agent . Cost effective synthesis procedures and purification process needs to be developed. Effective isolation techniques which can isolate Stigmasterol Gluside and B- Sitosterol Glucoside distinctly need to be established . It is seen from the literature that C.roseus is a medicinal plant used a phytomedicine to treat a wide range of health complications like diabetics as well as medicinally important chemical like saponin, flavonoid , vinblastine and vincristine have been reported to be present in various part its diversity of traditional uses. The present research reveals that the herb nayantara is used in treating various aliments . Neem leaf, nayantara leaves and bitter melon fruits dry powder and syzygium cumi seeds powder extract decreased blood glucose level with the highest reduction by gliclazide (45%). The exact mechanism in reducing blood level is not well understood . The mechanism of reducing blood glucose might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor .The activity constituents of these herbal products might be responsible for antihyperglycemic activity .

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