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Evaluation Study of Ethanolic Extract of *Gymnema sylvest* and Amitriptyline In Streptozotocin Induced Diabetic Neuropathic Albino Rats

Shabana Kausar Khan*, Dr. Ritu Gilhotra, S. Pathak, Dhirendra Kumar Sharma
School of Pharmacy, SGVU

ABSTRACT:

The object of the study was to investigate the potential effect of Gymnemasylvestre (Gs) in Albino rat. Diabetes was induced via a single intra peritoneal injection of streptozotocin (STZ; 60 mg/kg). Treatment with Gs extract (50 or 100 mg/kg/day) began two weeks following the administration of STZ and was continued for five weeks.
Diabetic neuropathy is defined as the damage of nerve due to high blood glucose level. High blood glucose level leads to many disease such as renal failure, cataract etc. Streptozotocin can selectively damage insulin-producing cell. In conclusion amitriptyline leads to no body weight gain, causes increase in grip strength. In Diabetes neuropathy various organ affects viz, leg, Hand, Eye, Heart etc. Understanding the mechanism behind pain.

Keywords: Diabetic neuropathy, Albino rats, Streptozotocin, Amitriptyline.

1. Introduction:

Peripheral neuropathy is a problem with an individual suffering from diabetes leads to renal failure cataract. Peripheral neuropathy causes nerve damage. It affects many part of our body i.e. leg, hand , Heart, etc. In this study, Albino rats was induced with streptozotocin with the combination of Metformin, Amitriptyline which leads to normal glycemic **Amitriptyline**, sold under the brand name **Elavil** among others, is a medicine used to treat a number of mental illnesses.[2] These include major depressive disorder and anxiety disorder, and less commonly attention deficit hyperactivity disorder and bipolar disorder.[2][3] Other uses include prevention of migraines, treatment of neuropathic pain such as fibromylgia and postherpetic neuralgia, anlesscommonly insomnia.[2][4] It is in the tricyclic antidepressant (TCA) class and its exact mechanism of action is unclear. Amitrityline is taken by mouth.[2] Amitriptyline is used for a number of medical conditions including (MDD).[11][12][13] Some evidence suggests amitriptyline may be more effective than other antidepressants,[14] including selective serotonin reuptake inhibitors (SSRIs),[15] although it is rarely used as a first-line antidepressant due to its higher toxicity in overdose and generally poorer tolerability.[12] It is TGA-labeled for migraine prevention, also in cases of neuropathic

pain disorders,[12] fibromyalgia[4] and nocturnal enuresis.[12][16] Amitriptyline is a popular off-label treatment for irritable bowel syndrome (IBS),[17] although it is most frequently reserved for severe cases of abdominal pain in patients with IBS because it needs to be taken regularly to work and has a generally poor tolerability profile, although a firm evidence base supports its efficacy in this indication.[17] Amitriptyline can also be used as an anticholinergicdrug in the treatment of early-stage Parkinson's disease if depression also needs to be treated[18]. Amitriptyline is the most widely researched agent for prevention of frequent tension headaches.[19]

Amitriptyline acts primarily as a serotonin-norepinephrine reuptake inhibitor, with strong actions on the serotonin transporter and moderate effects on the norepinephrine transporter.[34][35] It has negligible influence on the dopamine transporter and therefore does not affect dopamine reuptake, being nearly 1,000 times weaker on it than on serotonin.[35] It is metabolised to nortriptyline—a more potent and selective norepinephrine reuptake inhibitor—which may complement its effects on norepinephrine reuptake.[30] It promotes the heterodimerization of these proteins in the absence of NGF and has potent neurotrophic activity. Neurotrophin with powerful antidepressant effects, and as such this property may contribute significantly to its therapeutic efficacy against depression.

Advantages:

With regard to pain, Amitrityline is considered as meaningful pain modulator, especially with nerve damage.Amitriptyline is an antidepressant drug but it is used more commonly for pain .It is an excellent result for chronic pain. It is taken before bed time. Amitriptyline is used as first line treatment for chronic pain. Use of antidepressnats for headache prophylaxis has been recommended

Pharmacological Aspects:

- Hypolipidaemic Activity
- Weight Loss
- Sweet Taste Suppression Activity
- Anti-diabetic
- Anti-microbial
- Anti-viral
- Cataract

2. EXTRACTION:

2.1 Successive Solvent Extraction:

Stem and leaves of the herb *Gymnemasylvestre*. were shade dried. The sun dried plant material was coarsely powdered and subjected to extraction with petroleum ether in Soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. Deffated marc of drug was subjected to extraction with chloroform in a Soxhlet apparatus. The extraction was continued for a period of 48 hours. The extract was then concentrated and finally dried to a constant weight. Marc obtained after chloroform extraction was subjected to extraction with ethyl acetate. The extraction was continued for the period of 48 hours. The

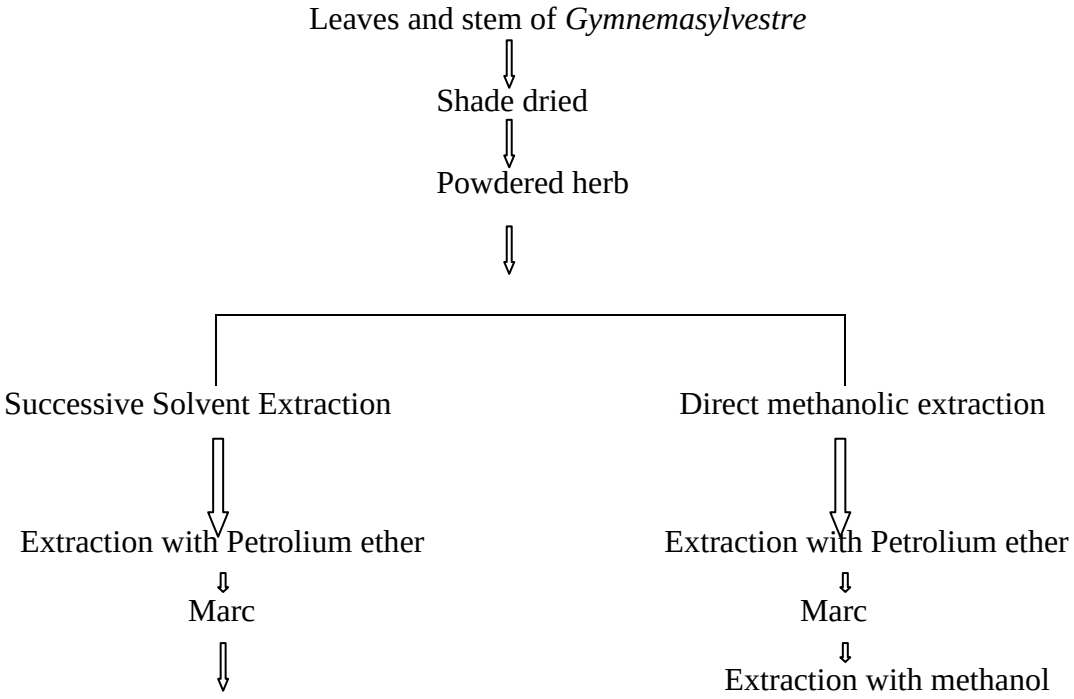
extracted was then concentrated and finally dried. Marc obtained after ethyl acetate extraction was subjected to extraction with methanol in Soxhlet apparatus. The extract was then concentrated and finally dried to a constant weight. Lastly the marc obtained was subjected to hot water maceration. The maceration was continued for 24 hours. The aqueous extract was filtered and concentrated.

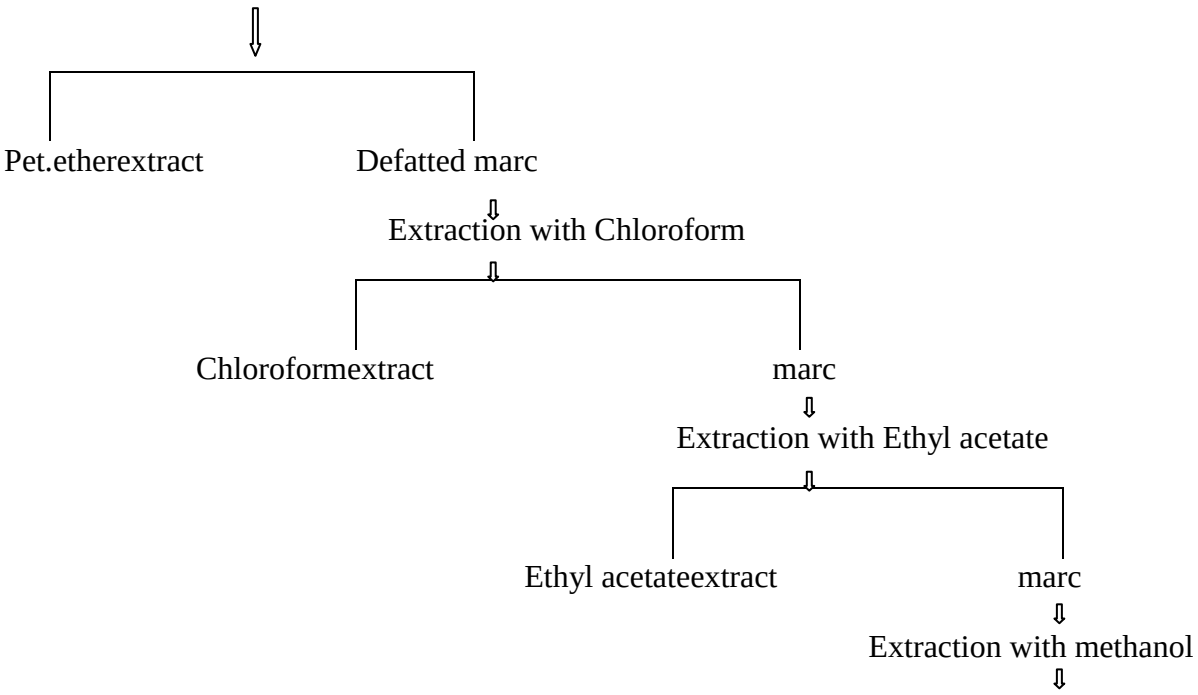
2.2 Direct methanolic extraction:

Two times extraction of the plant is done in the gap of 15 days. It is done by the Soxhlet Apparatus. Methanol is used as a solvent.

Plant material was collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant material was then sun dried and pulverized in a hand mill followed by sieving (sieve no. 40) to obtain coarse powder. The powdered leaves were extracted with petroleum ether (40-60^{0}C) and methanol for 48 h in soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.
Leaves of *Gymnemasylvestre*.extract were shade dried at room temperature. The dried plant material was coarsely powdered and subjected to extraction with petroleum ether in soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.The marc obtained after petroleum ether was subjected to methanol in Soxhlet apparatus. The extraction was continued for the period of 48 hours. The extract was then concentrated and finally dried to a constant weight.

Fig. No.1 Extraction





2.3. Procedure: (Continuous Hot Percolation Process or Soxhlet Extraction)

- The dried plant was powdered (without fruits and seeds) with the help of hand mill in the lab.
- This powdered plant material was passed through a specific grade sieve (for coarse powder sieve No. 10 and sieve No. 44 were used) to get the required size particles.
- Dried powdered material was weighed by using analytical balance.
- Weighed quantity of drug was filled in a wider part of the extractor.
- Coarsely powdered plant material was subjected to extraction with petroleum ether in soxhlet apparatus.
- The extraction was continued till the defatting of the material had taken place.
- Marc obtained after petroleum ether, was subjected to methanol.
- Methanol was placed with boiling chips into the flask.
- Temperature was mentained at 65^0C.
- Extraction was done for 48 hrs.

2.4. Phytochemical screening of extract

1. Test for Alkaloids
2. Test for Glycosides
3. Test for Sterols
4. Test for Triterpinoids
5. Test for Protein and amino acid
6. Test for Tannins

7. Test for Soponin
8. Test for carbohydrates
9. Test for Flavanoids

Table No.01: Table of Phytochemical Screening [37]

	TEST	OBSERVATION	INTERPRITATION
ALKALOIDS	Powered drug + Mayer's reagent (Potassium mercuri-iodide solution)	Yellow precipitate	Present
	Powered drug + Hager's reagent (Saturated solution of Picric Acid)	Yellow precipitate	Present
	Powered drug + Wagners reagent (Iodine in Potassium iodide)	Brown precipitate	Present
	Powered drug + Dragendroff's reagent (Potassium bismuth Iodide)	Orange precipitate	Present
GLYCOSIDES	Powered drug + Dilute KOH (Borntrager's Test)	Red color	Present
	Powered drug + Pyredine + 2% Sodium nitroprusside + 20% NaOH (Legal's Test)	Absence of red color	Cardiac glycoside Absent
STEROLS	Powered drug + Acetic anhydride (containing Sulphuric acid) (Lieberman's Test)	Blue-green color	Present
TRITERPINOIDS	Powered drug + $CHCl_3$ + H_2SO_4 (Salkowski's Test)	Yellow color which changes to red	Present
	Powered drug + $CHCl_3$ + acetic anhydride + conc. H_2SO_4	Reddish-violet color	Present
	TEST	OBSERVATION	INTERPRITATION
PROTEIN	Powdered drug+ 4% NaOH + 1% $CuSO_4$ (Biuret Test)	Pink color	present

TENNIN	Powdered drug + 1% gelatin solution containing 10% sodium chloride (Gelatin Test)	White precipitate	Present
SAPONIN	Drug solution + water and shake (Froth Test)	stable froth be noted	Present
CARBOHYDRATE	Drug solution + Molisch's reagent (Molisch's test)	purple to violet colour ring appeared at the junction	Present
FLAVANOIDS	Drug solution + Mg-ribbon + Conc. HCl(Shinoda Test /Magnesium Hydrochloride reduction test)	Blue color	Present

Storage: Powdered drug Stored at room temperature.

Table No.02: Physical examination of extract

Name of extract	**Consistency**	**colour**	**odour**	**taste**	**Yield %**
Petrolium ether extract	Semi-solid	Dark green	Characteristic	Bitter	6.3 %
Chloroform extract	Semi-solid	Dark green	Characteristic	Bitter	1.2 %
Ethyl acetate extract	Semi-solid	Dark green	Characteristic	Bitter	2.6 %
Methanolic extract	Semi-solid	Dark brown	Pungent	Bitter	5.4 %
Methanolic extract (Direct)	Semi-solid	Dark brown	Pungent	Bitter	6.2 %
Aqueous extract	solid	Brown	Characteristic	Bitter	3.8 %

Table No.3: Phytochemical screening of different extracts of*Gymnemasylvestre*

Chemical test		**Pet. ether**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Aqueous**
ALKALOID	Mayer's test	-ve	-ve	+ve	+ve	-ve
	Dragendorff's test	-ve	-ve	-ve	+ve	-ve
	Hager's test	-ve	-ve	-ve	+ve	-ve
	Wagner's test	-ve	-ve	-ve	-ve	-ve
GLYCOSIDES	Brontrager's	-ve	-ve	-ve	+ve	+ve
	Legal's Test	-ve	-ve	-ve	-ve	+ve

STEROLS	Lieberman's Test	-ve	+ve	+ve	-ve	-ve
TRITERPINOIDS	Salkowski's test	+ve	+ve	+ve	+ve	-ve
	Molescott's Test	+ve	+ve	+ve	+ve	-ve
PROTEIN	Biuret test	+ve	+ve	+ve	+ve	+ve
TANNINS	Gelatin Test	+ve	+ve	+ve	+ve	+ve
SAPONINS	Froth test	+ve	+ve	+ve	+ve	+ve
CARBOHYDRATES	Molisch's Test	+ve	+ve	+ve	+ve	+ve
FLAVANOIDS	Magnesium ribbon Test	-ve	-ve	-ve	+ve	+ve

2.5. Oral Acute Toxicity Study

2.5.1. Purpose: To provide information on health hazards likely to arise from a short-term exposure to Gymnemasylvestre extract by the oral route.

2.5.2. Summary: An Acute oral toxicity test was conducted with rats to determine the potential for *Gymnemasylvestre* extract to produce toxicity from a single dose via the oral route. Based on the results of this study, the single oral dose of the test substance is greater than 2000mg/kg of body weight.

2.5.6. Animals:

- Number of Animals: 06
- Sex : 3 male and 3 female
- Species/strain: Rat/ Wistar, albino
- Age: Young adult
- Body weight: 150-186 g at experimental start.

2.5.7. Method:

A) Husbandry:
   1. Housing: Each group was housed in plastic caging.
   2. Animal Room: Temperature range: 20-25^{0}C
   3. Photo-period: 12 hrs. Dark/light cycle.
   4. Acclimation Period: 15 days
   5. Water: Filtered tap water was supplied *ad libitum* by an automatic water dispensing bottle.
   6. Food:

B) Identification:
   1. Cage: Each cage was identified with a cage-card indicating the study number and identification and sex of animals.

2. Animal: A mark of different colors (For male: Black, Green, Blue; For female: Pink, orange, Red) were given to each rat. These colors constituted unique identification.

2.5.8 Procedures:

The acute oral toxicity study was carried out according to OECD 423 guidelines. Referring to old research papers and to avoid unnecessary harm and loss of animals on repetitive work, this was opted that only one group of animals should be subjected to the drug dose. Six albino rats were grouped and an oral dose of 2000mg/kg of body weight of plant extract was administered.

A. **Selection of animals:** Prior to dosing, a group of animals was fasted for approximately 17 hrs. by removing feed from their cages. During the fasting period, the rats were examined for health and weight (initial). Six (3 male and 3 female) healthy rats were selected for test.
B. **Dose calculation:** Doses were calculated based on the initial body weight.
C. **Dosing:** Each animal received 2,000 mg/kg of the test-substance, by stomach intubation. After administration, each animal was returned it its designated cage. Feed was replaced approximately 3.0 hrs. After dosing. The day of administration was considered day-zero of the study.
D. **Body weight:** Individual body weight of the animals were recorded prior to test substance administration and again on day 7 and 14.
E. **Cage Side Observations:** The animals were observed for mortality, signs of gross toxicity and behavioral changes at 1 and 3 hrs. Post-dosing and at least once daily there after 14 days. Observations included gross evaluation of skin and fur, eyes, respiration, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convultions, salivation, diarrhea and comma.

   Dose was selected on the basis of maximum tolerable dose, as there was no lethality observed up to 2000 mg/kg. Thus dose was selected as 1/10 and 1/5 of 2000mg/kg (i.e.200 mg/kg and 400 mg/kg) for further investigation.

2.5.9. Grouping of animals for main experiment

ANIMALS

Albino rats of either sex , weighing between 150-200g, procured from the Animal House and acclimatized under standard laboratory conditions at 25±2ºC , 50 ±15% RH and normal photoperiod 12:12 hour light: dark cycle for 7 days, were used.

The room temperature was maintained 25±2ºC with food and water *ad libitum*. The animals transferred to the laboratory at least one hour before the start of the experiment. The experiments were performed during day.

ANIMAL DETAILS:

STRAIN:	Albino Wistar Rats
AGE:	4-5 weeks

GENDER: Male/ Female
BODY WEIGHT: 180-210 gm

5 groups with six animals each of 180-210 gm. Albino Rats

Group-I: Control (Normal Group)

Group-II: STZ induced DM (50mg/kg)

Group-III: Diabetic Rats received Methanolic Extract of *A. Gymnemasylvestre* (200mg/kg)

Group-IV: Diabetic Rats received Methanolic Extract of *Gymnemaslyvestre*(400mg/kg)

Group-V: Standard Drug (**Amitriptyline**) (05 mg/kg)

3. INDUCTION OF DIABETES [49]

a. Preparation of citrate buffer[50]
b. Preparation of STZ solution
c. Administration of STZ

a. Preparation of citrate buffer: 10 g of Sodium citrate and 5.90 g of Sodium chloride were dissolved in 900 ml. of distilled water. pH of the solution was adjusted by addition of Hydrochloric acid and at last sufficient water was added to produce 1000ml.

b. Preparation of drug solution:
Calculation and weighing for the amount of STZ for all animals to be injected were done. Calculation was done for the buffer I need to get the concentration of 50mg/ml. Weighed quantity of STZ was dissolved in 0.1M cold citrate buffer (pH 4.5 and) immediately before use.

c. Administration of STZ: All the rats were fasted overnight before the administration of Streptozotocin. A freshly prepared solution of STZ (50mg/kg body weight) in 0.1M citrate buffer, pH 4.5 was injected (1.0-mL syringe) intra-peritoneally in a volume of 1ml/kg body weight to overnight fasted rats. For intra-peritoneal injection of STZ, the rat was held in one hand in dorsal position, the injection site was swabbed using 70% alcohol and designated amount of STZ was injected in the caudal abdominal cavity using sterile 25g needle. After the injection they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycaemic shock. The development of diabetes was confirmed after 48hrs of Streptozotocin injection. The animals having fasting blood glucose level more than 225mg/dl were considered as diabetic rats and used for the experimentation. Diabetic animals were grouped five days after induction of diabetes Effect of Methanolic Extract of *Gymnemasylvestre* in streptozotocin induced diabetes in rats.

4. PREPRATION OF INTERVENTIONS:

Dried extract was suspended in distilled water using 0.3% CMC as suspending agent. The standard drug Amitriptyline (05mg/kg body weight) was also prepared in a similar manner. The test and standard drugs were administered by oral route.

5. EVALUATION OF DIFFERENT PLANT EXTRACT

5.1Biochemical examination:

At the end of the treatment period, all rats were fasted for 8 hours. The blood was collected into tubes and serum was separated by centrifugation and used for biochemical analysis. The biochemical investigations were performed by using a Biochemical semi auto analyzer (ERBA-Chem-5 Plus. V2., West-Germany). The biochemical parameters considered were : Serum AST (SGOT) i.e. Asparate transaminase, ALT (SGPT) i.e. Alanine aminotransferase and total protein.

Fig.No.2: Blood Sampling

- **Biochemical studies:** In non heparinised tubes the blood was collected and centrifuged for 10 min at 3000 rpm. To analyze the enzymes, the serum was separated.

  Biochemical data of Albino Rats as per CPCSEA guidelines:

  1. Glucose: 50-135 mg/dL
  2. Total protein: 5.6-7.6 g/dL
  3. Cholesterol: 40-130 mg/dL
  4. Triglycerides: 25-165 mg/dL
  5. SGPT: 0-40 IU/L
  6. SGOT:5-34 IU/L

Study design for blood glucose:
Apparatus: ACCU-CHECK
Principle: Amperometry: The glucose dehydrogenase enzyme, in the presence of coenzyme (PQQ), on the test strip converts the glucose in the blood sample to gluconolactone. This reaction creates a harmless electrical current that glucometer interprets for blood glucose.

Reaction:

$$Glucose \xrightarrow{\text{Glucose dehydrogenase}} gluconolactone + e^-$$

Procedure:

1. Insert the test strip into the meter. The meter turns on.
2. Make sure the code number on the display matches the code number on the test strip container.
3. Obtain a drop of blood using the lancing device.
4. Touch and hold the drop of blood to the edge of the test strip. Do not put blood on the top of the test strip.

TRIGLYCERIDES TEST

Principle

Triglyceridesare hydrolysed by lipoprotein lipase (LPL) to produce glycerol and free fatty acid (FFA). In presence of glycerol Kinase (GK), Adenosine Triphosphate (ATP) phosphorylates Glycerol to produce Glycerol 3- phosphate and Adenosine Diphosphate(ADP). Glycerol 3-phosphate is further oxidized by glycerol 3-phosphate oxidase(GPO) to produce Dihydroxy acetone phosphate(DAP) and H_2O_2. In presence of peroxidase (POD), Hydrogenperoxide couples with 4-aminoantipyrine (4-AAP) and 4-Chlorophenol to produce red Quinonemine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to Triglycerides concenteration in the sample.

REACTION:

(i) Triglycerides ----------› Glycerol + FFA

(ii) Glycerol + ATP ----------› Glycerol 3-Phosphate + ADP

(iii) Glycerol 3-Phosphate + O_2 ----------› DAP + H_2O_2

(iv) $2H_2O_2$ + 4-AAP + 4-Chlorophenol ----------›Quinonemine dye + $4H_2O$

PROCEDURE:

Pipette into tube marked	Blank	Standard	Test
Serum/Plasma	-	-	10 µL
Reagent 2	-	10 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL

Mix well. Incubate at 37ºC for 10 minutes.

Programme the analyser as per above assay parameters.

1. Blank the analyser with Reagent Blank.

2. Measure absorbance of standard followed by the Test.
3. Calculate results as per given calculation formula.

CALCULATION:

$$\text{Triglycerides (mg/dL)} = \frac{Absorbance\ \ of\ Test}{Absorbance\ \ of\ Standard} \times 200$$

For Glycerol free Triglyceride

Glycerol free Triglyceride = Calculated Triglyceride – 10 mg/dL

TOTAL PROTEIN TEST

PRINCIPLE:

The Peptide bonds of Proteins react with cupric ions in alkaline solution to form a coloured chelate, the absorbance of which is measured at 578nm. The Biuret reagent contains Sodium-Potassium Tartrate, which helps in maintaining solubility of this complex at alkaline pH. The absorbance of final colour is proportional to the concentration of Total protein in the sample.

REACTION:

Protein + Cu^{++} ----------› Cu-Protein Complex

PROCEDURE:

Pipette into tube marked	Blank	Standard	Test
Serum/Plasma	-	-	10 µL
Reagent 2	-	10 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL

Mix well. Incubate at 37ºC for 10 minutes.

Programme the analyser as per above assay parameters.

1. Blank the analyser with Reagent Blank.

2. Measure absorbance of standard followed by the Test.

3. Calculate results as per given calculation formula.

CALCULATION:

$$\text{Total Protein Concentration (g/dL)} = \frac{Absorbance\ \ of\ Test}{Absorbance\ \ of\ Standard} \times 6.5$$

Globulins = Total Protein – Albumin

Conversion factor

Total Protein Concentration (g/L)= Total Protein Concentration in g/dL × 10

S.G.P.T.

Principle:

L-Alanine + 2-Oxoglutarate ---------> Pyruvate + L-Glutamate

Pyruvate + NADH ---------> L-Lactate + NAD

Procedure:

Allow the working Reagent to attain 37ºC before performing the test.

Pipette	Volume
Working Reagent	1000 µl
Test	100 µl

Mix well and aspirate.

CALCULATION:

The general formula for converting absorbance change into International Units(IU) of activity is:

$$\text{IU/L} = \frac{\left(\frac{\Delta A}{min}\right) \times T.V. \times 103}{S.V. \times Absorptivity \times P}$$

Where:

T.V. = Total reaction Volume (µl)

S.V. = Sample Volume (µl)

Absorptivity = millimolar absorptivity of NADH at340nm = 6.22

P = cuvette lightpath (cm)

= 1 cm

Activity of ALT = Δ Abs/min × 1768

S.G.O.T.

Principle:

L-Aspartate + 2-Oxoglutarate ---------> Oxaloacetate + L-Glutamate

Oxaloacetate + NADH ---------> Malate + NAD

Sample Pyruvate + NADH ---------> L-Lactate + NAD

Procedure:

Allow the working Reagent to attain 37ºC before performing the test.

Pipette	Volume
Working Reagent	1000 µl
Test	100 µl

Mix well and aspirate.

CALCULATION

The general formula for converting absorbance change intointernationalUnits(IU) of activity is:

$$IU/L = \frac{\left(\frac{\Delta A}{min}.\right)\times T.V.\times 103}{S.V.\times Absorptivity\ \times P}$$

Where:

T.V. = Total reaction Volume (µl)

S.V. = Sample Volume (µl)

Absorptivity = millimolar absorptivity of NADH at340nm = 6.22

P = cuvette lightpath (cm)

= 1 cm

Activity of AST = Δ Abs/min × 1768

6. PREPARATION OF DOSE (FOR EXTRACTED DRUG)

Solvent used: Distilled water

Suspending Agent: CMC (1%)

Calculation for doses: As per weight of the animal

- 200mg/kg body weight

  Solution for the dose of 200mg/kg body weight: Stock solution was prepared by dissolving the 5g of extracted powdered drug in 100 ml.

- 400/kg body Weight

  Solution for the dose of 400mg/kg body weight:Stock solution was prepared by dissolving the 10g of extracted powdered drug in 100 ml.

Table No. 4: Calculations for dose required (of extract) as per body weight

Groups	Group-III		Group-IV	
	Weight of Rats	dose as per 200mg/kg	Weight of Rats	Dose as per 400mg/kg
R1	197	$\frac{200}{1000}\ X\ 197 = 39.4$mg	201	$\frac{400}{1000}\ X\ 201 = 80.4$mg
R2	199	$\frac{200}{1000}\ X\ 199 = 39.8$mg	184	$\frac{400}{1000}\ X\ 184 = 73.6$mg
R3	206	$\frac{200}{1000}\ X\ 206 = 41.2$mg	201	$\frac{400}{1000}\ X\ 201 = 80.4$mg
R4	205	$\frac{200}{1000}\ X\ 205 = 41$mg	206	$\frac{400}{1000}\ X\ 206 = 82.4$mg
R5	193	$\frac{200}{1000}\ X\ 193 = 38.6$mg	187	$\frac{400}{1000}\ X\ 187 = 74.8$mg
R6	187	$\frac{200}{1000}\ X\ 187 = 37.4$mg	200	$\frac{400}{1000}\ X\ 200 = 80$mg

Table 5: Quantity of plant- extract- solution required for the administration in the rats

	Group-III (200mg/kg body weight)	Group-IV (400mg/kg body weight)
R_1	$\frac{100}{5000} X\ 39.4 = 0.78ml$	$\frac{100}{10000} X\ 80.4 = 0.80ml$
R_2	$\frac{100}{5000} X\ 39.8 = 0.79ml$	$\frac{100}{10000} X\ 73.6 = 0.73ml$
R_3	$\frac{100}{5000} X\ 41.2 = 0.82ml$	$\frac{100}{10000} X\ 80.4 = 0.80ml$
R_4	$\frac{100}{5000} X\ 41 = 0.82ml$	$\frac{100}{10000} X\ 82.4 = 0.82ml$
R_5	$\frac{100}{5000} X\ 38.6 = 0.77ml$	$\frac{100}{10000} X\ 74.8 = .74ml$
R_6	$\frac{100}{5000} X\ 37.4 = 0.74ml$	$\frac{100}{10000} X\ 80 = 0.80ml$

Solution of standard drug (Amitriptyline):

Marketed brand of Amitriptyline was purchased.The tablets were crushed, suspended in distilled water and given to diabetic rats at the dose level 05 mg/kg body weight, daily by gastric intubation.

Each Uncoated tablet contains:

Amitriptyline I.P……5mg

Company Name: Sanofi India Limited

Storage: Below $+25^0$C

Dose of Amitriptyline: 05mg/kg body weight

Solvent Used: Distilled Water

7. RESULTS:

Effect on fasting blood glucose level:

Effect of multi dose extract of *Gymnemasylvestre*.on **blood glucose level** in different groups of Rats

DAY / GROUPS	0^{th} day	7^{th} day	14^{th} day	21^{st} day	28^{th} day
NORMAL	72.166	73.333	71.666	72.5	73.333
DIABETIC(UNTREATED)	278.5	272.0	281.166	290.0	287.333
DIABETIC(Am-200mg/kg)	264	231	194.666	159.833	135.833
DIABETIC(Am-400mg/kg)	272	209.166	160.5	111.333	90.333
DIABETIC (St.DRUG)	278.5	103.333	100.166	93.833	81.666

The results of the study are depicted in above table. During the experiment the diabetic rats had an improvement in the normalization of blood glucose levels.

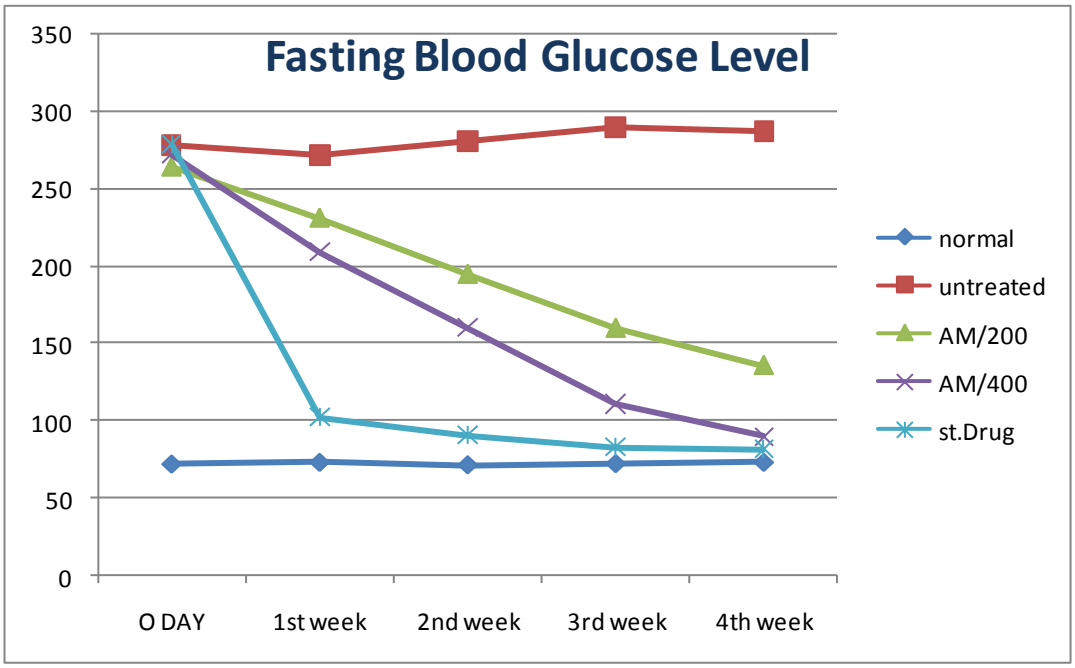


Fig.3. Effect of different plants extracts and Amitriptyline on glucose level at the 1st, 2nd,3rd, and 4th week of the treatment.

Effect on oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) of normal non-diabetic and diabetic rats were shown in Fig.2 , the blood glucose level of normal non-diabetic rats had fasting blood glucose level 72.5±1mg/dl that was much lower than that of the diabetic rats; reached its peak value at 60 minutes following glucose intake (3 gm/kg B.W.) and began to decrease during the next 60 minutes to reach 97.33 after two hours of glucose administrationIn the diabetic non-treated albino rats, blood glucose level also attained its maximal level after 60 minutes of glucose administration recording 343.6 , 357,354, 366and 372 after the first, second, third and fourth weeks. Subsequently, these values begin to decline during the next 60 minutes but in slower rate but still elevated than that of the normal rats.When the diabetes was treated with *Gymnemasylvestre* extract and amitryptiline there was a noticeable hypoglycemic effect in diabetic treated animalscompaired with the diabetic non-treated groups.

Continuous treatment with the tested material for two weeks had beneficial effects on OGTT values alleviating hyperglycemia. Gymnemasylvestre with 200mg/kg body weight exhibited, a mild hypoglycemic effect, while amitryptiline treatment showed remarkable effect as compairedwith the other diabetic groups.Prolonged treatment of the Diabetic rats with each of the tested extracts as well as with amitryptiline for four weeks showed a more beneficial effect on OGTT. All changes were statistically highly significant. Fasting glucose was lower than that of the first and second weeks treated groups.

During latency period observations of OGTT at different weeks and their graphs are as follows:

0- Day Observations

Table:6

Groups Time (min)	Normal	Diabetic (untreated)	Diabetic (A.m.200)	Diabetic (A.m.400)	Diabetic (Amitriptyline)
0	72.166	278.5	264	272.5	256.5
30	132.46	295	297	303	284
60	197.72	343.6	355	364	312
90	157.77	324	336	326	293
120	97.33	296	288	291	271

7th Day Observations

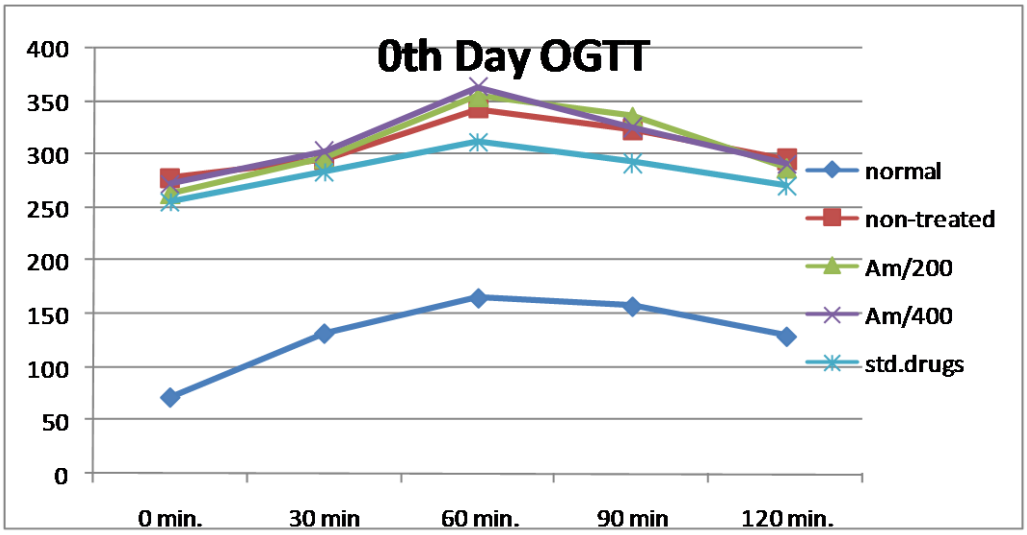
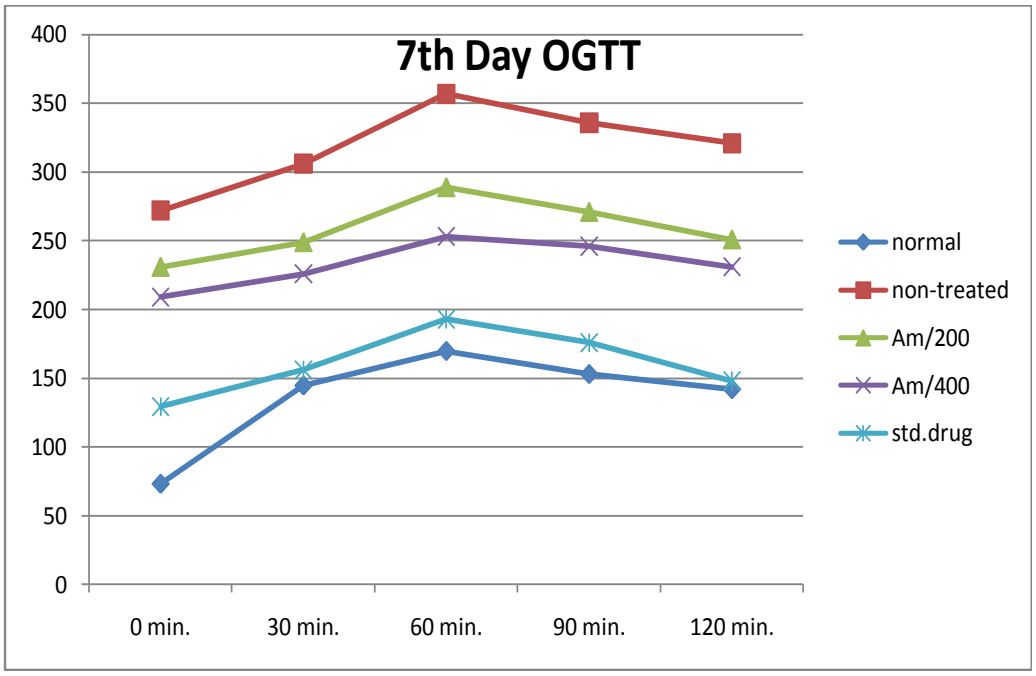


Table:7

Groups Time (min)	Normal	Diabetic (untreated)	Diabetic (A.m.200)	Diabetic (A.m.400)	Diabetic (Amitrityline)
0	73.333	272.0	231	209.166	129.333
30	144.66	306	249	226	156
60	169.71	357	289	253	193
90	153	336	271	246	176
120	142	321	248	231	148

Graph:

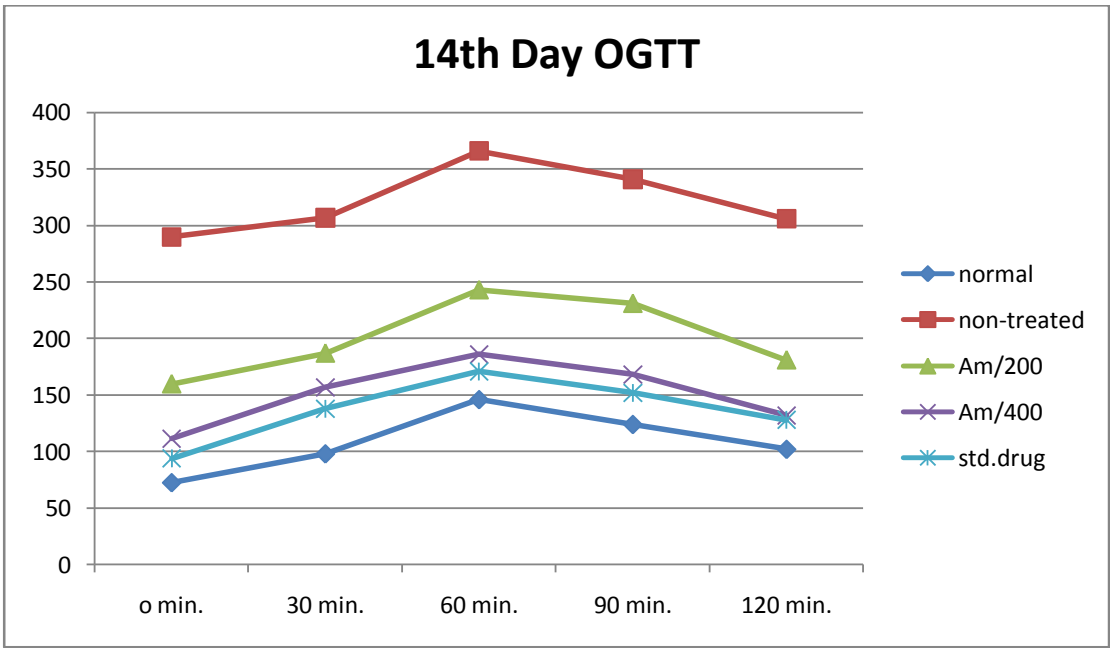


14th Day Observation

Table:8

Groups / Time (min)	Normal	Diabetic (untreated)	Diabetic (A.m.200)	Diabetic (A.m.400)	Diabetic (Amitrityline)
0	71.666	281.166	194.666	160.5	100.166
30	146.75	309.2	208	195	133
60	171.68	354	268	256	183
90	135	321	241	231	169
120	124	292	203	197	128

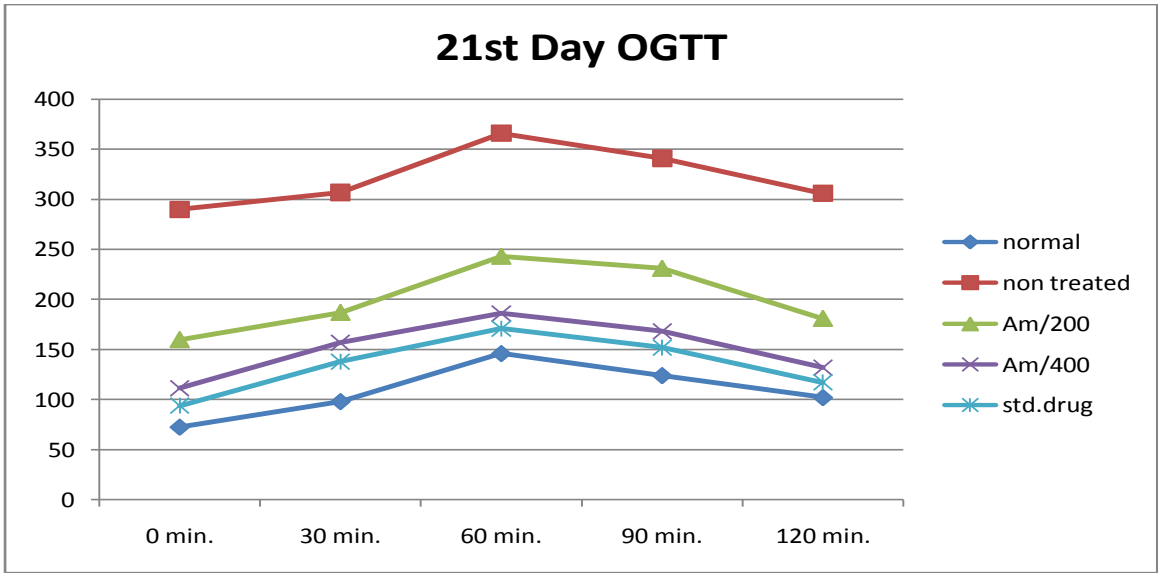
Graph:



21st Day Observation:

Table:9

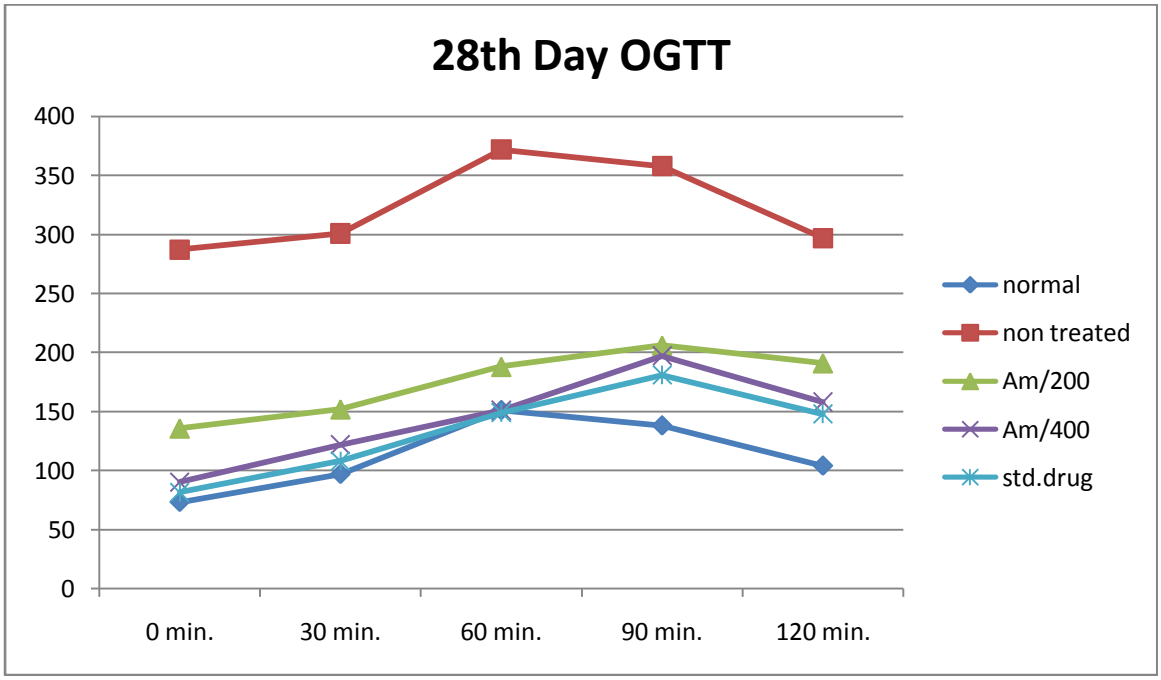
Groups / Time (min)	Normal	Diabetic (untreated)	Diabetic (A.m.200)	Diabetic (A.m.400)	Diabetic (Amitriptyline)
0	72.5	290	159.833	111.333	93.833
30	98	307	187	157	138
60	146	366	243	186	171
90	124	341	231	168	152
120	102	306	181	132	117



28th Day Observation

Table:10

Groups / Time (min)	Normal	Diabetic (untreated)	Diabetic (A.m.200)	Diabetic (A.m.400)	Diabetic (Amitriptyline)
0	73.333	287.333	135.833	90.333	81.666
30	97	301	152	122	108
60	151	372	188	151	149
90	138	358	206	197	181
120	104	297	191	158	148



Discussion:

1. It was found that Gymnemasylvestre was claimed to show Diabetic Neuropathy activity.
2. Extract of leaf of *Gymnemasylvestre*.was collected, dried and powdered.
3. Selection of solvent, extraction of plant *Gymnemasylvestre.*
4. Extraction of plant *Gymnemasylvestre*
5. Phytochemical screening of the extract.
6. Acute toxicity studies will be done based on OECD guidelines.

- Literature review show that *Gymnemasylvestre*. showed various activities.
- Shade dried areal parts (leaves and stem) of *Gymnemasylvestre* subjected to extraction yielded crude extract.
- The ethanolic extract showed presence of phytochemical constituents which may be responsible for its diabetic neuropathy activity.

So we can say that the extract possess diabetic neuropathy activity showing by decreasing blood glucose levels to normal. Hence this plant looks promising in the treatment of diabetes. It is very difficult to mention which of the ingredients were responsible for this favorable response.

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