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Original Research Article

Evaluation of Hypoglycemic Effect of Crude Extracts and Different Fractions of Stem Bark of Acacia nilotica

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Abstract: Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances. Diabetes is a major degenerative disease in the world today, affecting at least 150 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders. The crude methanolic extract of Acacia nilotica bark with different soluble partitionates were subjected to investigate for the evaluation of analgesic, hypoglycemic, CNS depressant and antidiarrheal activity on mice and thrombolytic, anthelmintic, antimicrobial, antioxidant along with cytotoxicity different in vivo experiment. Hypoglycemic activity of the plant Acacia nilotica was evaluated and the result depicted that the Methanolic crude extract, Dichloromethane fraction at a dose of 400mg/kg body weight showed significant hypoglycemic activity compared to the standard.
Keywords: Hypoglycemic effect, Acacia nilotica, Crude extracts.

INTRODUCTION

Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia [1], lipoprotein abnormalities [1], raised basal metabolic rate [2], defect in reactive oxygen species scavenging enzymes [3] and altered intermediary metabolism of major food substances. Diabetes is a major degenerative disease in the world today [4], affecting at least 150 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders.

Yet with the tremendous scientific advances witnessed in this century medical science cannot claim it knows all that needs to be known about this disease, including its management. At present, the oral anti-diabetic agents belong to sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones and meglitinide derivatives. The major limitations of these drugs are their side effects. The high cost of modern treatment of diabetes indicates a great need for the development of alternative strategies for prevention and treatment of diabetes and since plants are the basic source of modern medicine, recently the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine and so far a number of hypoglycemic agents have been derived from different plant origin [5].

Plants with a traditional indication for diabetes are more likely than randomly selected plants to show activity in standard hypoglycemic assays. Due to the phylogenetic relationship of Phlogacanthus thyrsiflorus (Acanthaceae) with Adhatoda vasica (Acanthaceae) which has antidiabetic activity reported in reputed journal, the present study was conducted to screen the hypoglycemic effect of methanolic crude extract of bark of Acacia nilotica and its different fractions both in normal and glucose overloaded Swiss albino mice.

The plant family: fabaceae [6](a,b)

The plant under investigation is Acacia nilotica belongs to the family Fabaceae. The Fabaceae, also called Leguminosae or bean and pea family, is the third largest family in terms of agricultural and economic importance. Legumes includes a large number of domesticated species harvested as crops for human and animal consumption as well as for oils, fiber, fuel, fertilizers, timber, medicinals, chemicals, and horticultural varieties[7]. In addition, the family includes several species studied as genetic and genomic model systems.

Growth pattern

Legumes vary in habit from annual and perennial herbs to shrubs, trees, vines/lianas, and even a few aquatics. Ranging in size from some of the smallest plants of deserts and arctic/alpine regions to the tallest of rain forest trees, legumes are a conspicuous, and often dominant, component of most of the vegetation types distributed throughout temperate and tropical regions of the world [8]. Legumes are particularly diverse in tropical forests and temperate shrub lands with a seasonally dry or arid climate. This preference for semi-arid to arid habitats is related to a nitrogen demanding metabolism. While many species have the ability to colonize barren and marginal lands because of their capacity to "fix" atmospheric nitrogen via a symbiotic association with root-modulating bacteria, this is just one of several ways in which legumes obtain high levels of nitrogen to meet the demands of their metabolism [9]. Over the past 30 years, the study of legume classification and biology has benefitted from major advances in understanding of the morphology, evolution and systematics, and ecology of the family [10].

Characteristics

Morphologically, Fabaceae is characterized by leaves simple to compound (pinnate, rarely palmate, or bipinnate), unifoliate, trifoliate (Medicago, Trifolium), sometimes phyllodic (many species of Acacia), or reduced to a tendril (as in Lathyrus), spirally arranged, with stipules present that are sometimes large and leaf-like (Pisum) or developed into spines (Prosopis, Robinia).

Flowers are usually regular or irregular (i.e., actinomorphic to zygomorphic in symmetry, respectively), bisexual, with a single superior carpel (hypogynous to perigynous), pentamerous, arranged singly or in racemes, spikes, or heads. The principal unifying feature of the family is the fruit, the legume [10]. With a few exceptions, legumes are typically one-chambered pods (one locule), with parietal placentation along the adaxial suture, ovules 2 to many, in two alternating rows on a single placenta, typically dry and dehiscent along one or both sutures (legume).

TAXONOMY [11]

Taxonomically, Fabaceae has been traditionally divided into three subfamilies-

- Caesalpinioideae
- Mimosoideae
- Papilionoideae

The recognition of three subfamilies is based on characteristics particularly of the flower, aestivation of petals, sepals (united or free), stamen number and heteromorphy, pollen (single or polyads), leaf complexity, and presence of root nodules. Differences in these characteristics led to the view that the Mimosoideae and Papilionoideae are unique and distinct lineages in the family which arose independently within a paraphyletic "basal" caesalpinioid assemblage.

Agricultural & Economic Importance of Legumes

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago, their use as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and their use by the Roman Empire as a food source and for soil improvement [12]. Today legumes are an increasingly invaluable food source not just for humans, accounting for 27% of the world's primary crop production, but also for farm animals [12]. Legumes were grown on more than 13% of the total arable land under cultivation in the world in 2004 [14]. Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans, while soybeans (*Glycine max*) and peanut (*Arachis hypogeae*) provide more than 35% of the world's processed vegetable oil and a rich source of dietary protein for the poultry and pork industries [12].

While they produce nitrogen-containing protein in abundance, legumes are deficient in sulfur containing amino acids and other nutrients needed by people and animals. For this reason, legumes and cereal crops are often raised together to account for the amino acids and other elements they are each deficient in [13]. The primary dietary legumes grown, such as bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), and lentils, include representatives of each of the four clades within papilionoids, the genistoids, dalbergioids, Hologalegina, and phaseoloid/millettioids.

Industrially, legumes have many uses in making biodegradable plastics, oils, dyes, and biodiesel fuel. Legumes are used traditionally in folk medicines, but also demonstrate importance in modern medicine. Isoflavones commonly found in legumes are thought to reduce the risk of cancer and lower cholesterol and soybean phytoestrogens are being studied for use in postmenopausal hormone replacement therapy. Legumes also produce a hypoglycemic effect when eaten, making them a recommended food for diabetics [13].

The plant: Acacia nilotica [14-17]

Acacia nilotica is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50^{o} C) and air dryness. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India.

Synonyms

ACAR11	*Acaciaarabica* (Lam.) Willd.
MINI2	*Mimosanilotica* L.

Taxonomical classification

Kingdom Plantae – Plants
Subkingdom *Tracheobionta*– Vascular plants
Superdivision *Spermatophyta*– Seed plants
Division *Magnoliophyta*– Flowering plants
Class *Magnoliopsida*– Dicotyledons
Subclass *Rosidae*
Order *Fabales*
Family *Fabaceae*– Pea family
Genus *Acacia Mill.* – acacia
Species *Acacianilotica* (L.) Willd. ex Delile – gum arabic tree

Plant Description

Acacia nilotica is a single stemmed plant with a well-developed deep root system.

Height

The average height of the plant has been 15-18 m in height and 2-3 m in diameter.

Pods and Seeds

Pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance. Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa [18].

Leaves

The leaves are bipinnate, pinnate 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long [19]

Flowers

Flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow color, develop either in axillary or whorl pattern on peduncles 2-3 cm long located at the end of branches [20].

Stem

Stems are usually dark to black colored, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum [20].

Bark

The bark a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns [21].

Thorns

Thorns are thin, straight, light grey exist in axillary pairs (usually 3-12), 5-7.5 cm long in young trees.

Root

Root is generally of brown color in older and whitish in younger regions.

Gum

The gum varies in color from very pale yellowish brown to dark reddish brown depending on the quantity of tannins in the sample. The lighter, more highly valued gums are soluble in water and very viscous; the tannins in the darker gum reduce the solubility. The gum has a moisture content of about 13% and is slightly dextrorotary [22].

Growth pattern and germination

Acacia nilotica is a tropical species found throughout India and occurs from sea-level to over 2000 m altitude. Prickly Acacia germinates in rainfall in the wet season. But some seeds may still germinate up to 15 years after seed drop. Seedlings grow rapidly near water but more slowly in open grasslands. It grows in average annual temperatures range from 15–28°C, being frost sensitive when young and withstanding daily maximum temperatures of 50°C [23]. The mean maximum temperature of the hottest month is 25–42ºC and the mean minimum temperature of the coldest month 6–23°C. Babul plant prefers dry conditions, with an annual rainfall of (100–) 250–1500(–2300) mm. This subspecies is commonly found on soils with high clay content, but may grow on deep sandy loam in areas of higher rainfall. It commonly grows close to waterways on seasonally flooded river flats and tolerates salinity well [24]. Trees can flower and fruit two to three years after germination, but after high rainfall it is more quickly, usually between March and June [25]. Pods are formed between July and December. Most leaf fall between June and November and seed pods drop during October to January [26]. Seeds are very simple. Inner integument degenerates completely and the testa is formed by the outer integument [27, 28]. Meharia has observed that A. nilotica is more productive than A. tortilis after slat treatment. It grows well in two types of soils i.e. riverian alluvial soil and black cotton soil [29].

DISTRIBUTION

The native distribution of Acacia nilotica includes much of Africa and the Indian subcontinent [16]. From the GRIN database, the native distribution includes [30] - Africa: Algeria, Angola, Botswana, Egypt, Ethiopia, Gambia, Ghana, Guinea-Bissau, Kenya, Libya, Malawi, Mali, Mozambique, Niger, Nigeria, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe Asia: Iran, Iraq, Israel, Oman, Saudi Arabia, Syria, Yemen, India, Nepal, Pakistan. In Bangladesh it is found in Bogra, Faridpur, Jessore, Kushtia, Pabna, Rajshahi also planted by the road sides and embankments throughout the country.

Table−1: Some common medicinal uses of different parts of Acacianilotica

Part used	Uses
Root	The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen[31].
Leaf	Chemoprventive, anitmutagenic, anti bacterial, anticancer, astringent, anti microbial activity Tender leaves are used to treat diarrhea, Aphrodisiac, dressing of ulcers,anti-inflammatory and Alzheimer's diseases[32].
Gum	Astringent, emollient, liver tonic, antipyretic and antiasthmatic[33].
Stem bark	Anti bacterial, antioxidant, anti-mutagenic, cytotoxic bark is used as astringent, acrid cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, biliousness, burning sensation, toothache, leucoderma, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leukoderma [34].
Seeds	Spasmogenic activity and antiplasmodial activity [35].
Pods	Anti hypertensive and antispasmodic, anti-diarrhoerial, astringent,anti-fertility and against HIV-1 PR, Inhibited HIV-1 induced cythopathogenicity, antiplatelet aggregatory activity and anti oxidant [36].

Fig−1: Flower of Acacia nilotica

Fig−2: Bark of Acacia nilotica

Fig–3: Whole tree of Acacia nilotica

MATERIALS FOR PARTITIONING AND EXTRACT PREPARATION

Glass wares

Table–2: List of glass wares

Materials	Source
Distilled machine	BDH Laboratory Equipments
Conical flasks (250 ml)	BDH Laboratory Equipments
Beakers (100 ml, 500 ml)	BDH Laboratory Equipments
Test tubes	BDH Laboratory Equipments
Funnels	BDH Laboratory Equipments
Measuring cylinders	BDH Laboratory Equipments
Pipettes	BDH Laboratory Equipments
Automatic pipette puller	Bel-Art Products, USA

Solvents

Table–3: List of solvents

Materials	Source
n-Hexane	Merck
Carbon tetrachloride (CCl_4)	Merck
Dichloromethane (CH_2Cl_2)	Merck
Ethyl acetate ($CH_2CH_3OOCCH_3$)	Merck
Methanol	Scharlau
Acetic acid	Merck
Ethanol	Merck
Distilled Water	-

Filter aid

Table–4: List of filter aid

Filter aids
Filter Paper (Whatman no. 1)
Normal Cotton

Equipments

Table−5: List of equipments

Equipments	Source
Rotary vacuum evaporator	-
Electronic balance	Denver Instruments M-
Table-top UV detector (252 & 366	*CAMAG*
Grinding machine	-
Oven (0^0C-210^0C)	Gallen Kamp Hotbox
Solvent distillation plant	University Instruments
Distilled water plant	University Instruments

Collection and preparation of plant material

Plant sample (bark) of Acacia nilotica was collected from Pabna, Bangladesh in April 2012. Then proper identification of plant sample was done by an expert taxonomist. The bark was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried bark was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy; University of Dhaka.

Extraction of the Plant Material

About 950 gm of the powdered material was taken in separate clean, round bottomed flask (4.5 liters) and soaked in 5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 21 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 39ºC with a Heidolph rotary evaporation. The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 22 gm.

Solvent-solvent partition of crude extract

Solvent-solvent partitioning of crude methanolic extract was done following Modified Kupchan Partition [37].

Preparation of mother solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This was the mother solution which was partitioned off successively by four solvents of different polarity. In subsequent stages each of the fractions was analyzed separately for the detection and identification of compounds having antibacterial, cytotoxic, antioxidant and other pharmacological properties.

Partition with n-hexane

The mother solution was taken in a separating funnel. 100 ml of the n-hexane was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice (100 ml × 3). The n-hexane fraction was then air dried for solid residue.

Partition with carbon tetrachloride

To the mother solution left after partitioning with n-hexane; 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with carbon tetrachloride (CCl_4). The process was repeated thrice (100 ml × 3). The carbon tetrachloride fraction was then air dried for solid residue. The aqueous fraction was preserved for the next step.

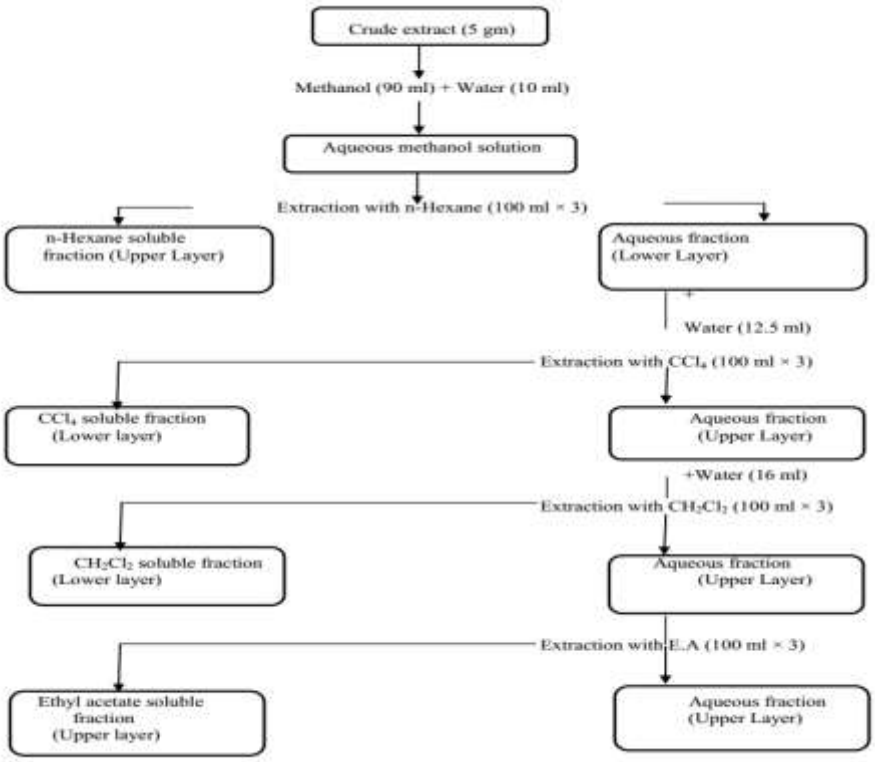
Partition with dichloromethane

To the mother solution that was left after partitioning with petroleum ether and carbon tetrachloride; 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with dichloromethane (CH_2Cl_2) (100 ml × 3). The dichloromethane soluble fractions were collected together and air dried. The aqueous fraction was preserved for the next step.

Partition with ethyl acetate

To the mother solution that was left after washing with petroleum ether, carbon tetrachloride and dichloromethane; was then taken in a separating funnel and extracted with ethyl acetate (100 ml × 3). The ethyl acetate soluble fractions were collected together and air dried.

Flow Diagram



Fig−4: Schematic representation of the modified Kupchan Partitioning of methanolic crude extract of Acacia nilotica.

After evaporation the weight of the different fractions obtained are as follows:

Table−6: Amount of fractions after fractionation of crude methanolic extract

Fraction	Weight
n-Hexane soluble fraction	0.50 g
Carbon tetrachloride soluble fraction	0.90 g
Dichloromethane soluble fraction	1.25 g
Ethyl acetate soluble fraction	1.80 g

PRINCIPLE OF ORAL GLUCOSE TOLERANCE TEST IN SWISS ALBINO MICE

A **glucose tolerance test (GTT)** is one of the most acceptable methods to evaluate the hypoglycemic activity. It is a medical test in which glucose is given and blood samples taken afterward to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia or rarer disorders of carbohydrate metabolism. Many variations of the GTT have been devised over the years for various purposes, with different standard doses of glucose, different routes of administration, different intervals and durations of sampling, and various substances measured in addition to blood glucose [38].

In this method, different fractions of the bark of Acacia nilotica (i.e. ethyl acetate, dichloromethane and carbon tetrachloride) and the methanolic crude extract of bark were administered orally to the experimental animals at the fasting condition. Then the animals were given glucose load at a certain dose 30 minutes after administration of crude extracts, their fractions, standard and control. The blood glucose levels of the experimental animals were measured by using a glucometer (Bioland G-423 S from Hong Kong) and Glucose oxidase-peroxidase reactive strips at 30 minutes, 90 minutes and 150 minutes interval. The hypoglycemic effect of the test samples were then compared with relative to that of control (vehicle containing 1% Tween 80 and DMSO in saline) and standard (vehicle containing Glibenclamide) group.

EXPERIMENTAL ANIMALS

Swiss-albino mice of either sex, aged 4-5 weeks, weighing 20-25 gm each obtained from the Animal Resource Branch of Jahangirnagar University were used for the experiment. They were kept in clean and only dry polypropylene cages with 12 hours light dark cycle at 25±2°C and 45-55% relative humidity.

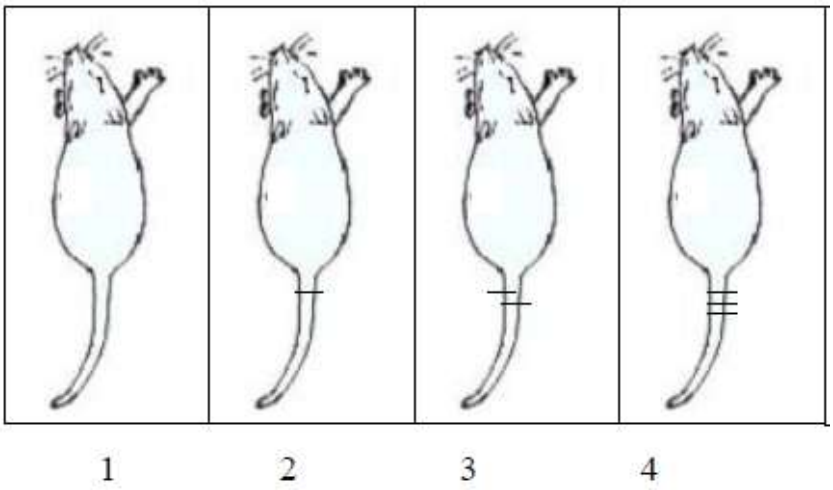
The animals were fed with pelletized mice feed supplied from ICDDR,B and tap water.

EXPERIMENTAL DESIGN

Twenty four experimental animals were randomly selected and divided into six groups denoted as group-I, group-II, group-III, group- lV, group-V and group-VI consisting of 4 mice in each group. Each group received a particular treatment i.e. control, standard and dose of the extract of different fractions of bark of the plant respectively. Prior to any treatment, each mouse was weighed properly and the doses of the test samples, standard and control materials were adjusted accordingly.

METHOD OF IDENTIFICATION OF ANIMALS

Each group consisted of five mice. As it was difficult to observe the biologic response of five mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way (Figure 7.1) and marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3, M-4=Mice 4 and M-5=Mice 5.



Fig–5: Identification of each test animals per group

PREPARATION OF TEST MATERIALS

In order to administer the extracts and different fractions at doses of 400 mg/kg body weight of rats, 150 mg samples were measured and were triturated unidirectional way by the addition of small amount of Tween-80 (suspending agent). After proper mixing of sample and suspending agent, normal saline was slowly added. The final volume of the suspension was made 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture.

PREPARATION OF STANDARD SAMPLE

For the preparation of Glibenclamide at the dose of 10-mg/kg-body weight, 5 mg of Glibenclamide was taken and dissolved in 5 ml of normal saline.

PREPARATION OF CONTROL SAMPLE

Tween-80 (1%) and DMSO are mixed properly in the normal saline and the volume was made up to 3 ml.

PREPARATION OF GLUCOSE SOLUTION

For the preparation of Glucose solution at the dose of 2g/kg-body weight, 10 g glucose was dissolved in 100 ml of distilled water.

Table–7: Particulars of test materials used in the evaluation of hypoglycemic activity of Methanol extract and its different fractions of Acacia nilotica bark

Test samples	Group	Identification	Dose (mg/kg)	Route of administration
1%Tween 80 and DMSO in saline	I	Control Group	200	Oral
Glibenclamide	II	Standard Group	5	Oral
MEAN	III	Test sample	400	Oral
EAAN	IV	Test sample	400	Oral
DMAN	V	Test sample	400	Oral
CTAN	VI	Test sample	400	Oral
5% glucose solution	-	-	2g/kg body weight	Oral

PROCEDURE

- The animals were weighed and randomly divided into six groups consisting of 4 mice in each group.
- To perform the glucose tolerance test, mice were kept fasting overnight.
- Blood glucose level of each mouse under fasting condition was measured using glucometer and glucose oxidase-peroxidase reactive strips and it was denoted as 0 min record.
- Blood sample were collected by cutting the tail tips with a sharp blade.
- Blood samples were exposed to glucose test strips and blood glucose level was visualized in the glucometer.
- After collection of blood the tail tips were exposed to Povidone Iodine ointment to counteract the possibility of infection and inflammation.
- Among the mice groups, group I was considered as control receiving 0.3 ml of Tween 80 and DMSO in normal saline each, group II was considered as standard receiving 0.2ml Glibenclamide each, group III-VI was kept as test groups receiving bark crude extract 0.3 ml each, pet ether fraction 0.3 ml each, chloroform fraction 0.3 ml each, leaf extract 0.3 ml each respectively.
- The extracts, standard and control samples were given orally with the help of feeding needle.
- All the groups were administered 0.4 ml (2g/kg body weight) glucose solution orally 30 minutes after receiving the treatment.
- Blood glucose levels were again measured using glucometer 30 min, 90 min and 150 min after glucose administration.

Then the result of hypoglycemic effects of the test sample is compared in relative to control and standard groups.

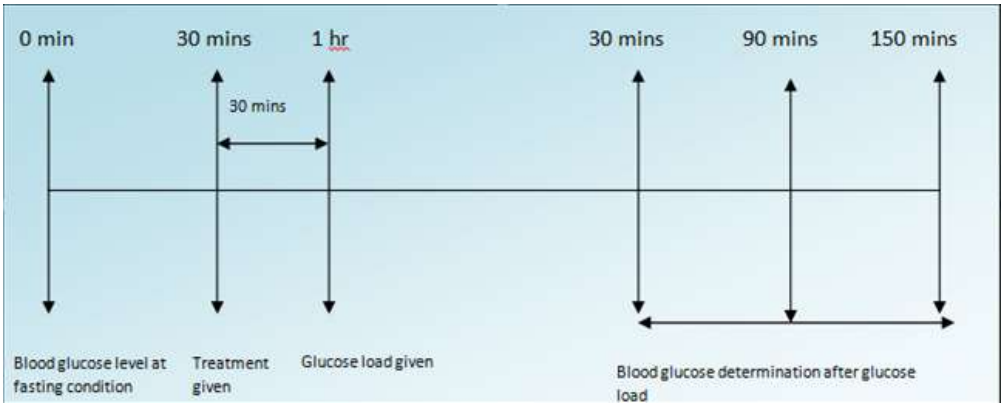
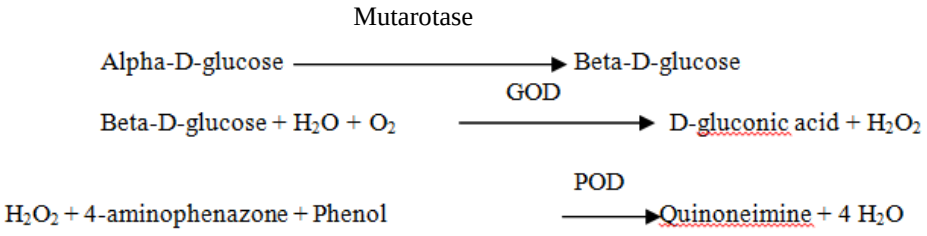


Fig-6: Pictorial diagram of the procedure of evaluation of hypoglycemic effect of different fractions and crude extracts of Acacia nilotica.

Determination of Plasma Glucose Level

The glucose level is determined after the enzymatic oxidation in the presence of glucose oxidase. The resultant Hydrogen peroxide (H_2O_2) is oxidatively coupled with 4-aminophenazone and phenol in the presence of peroxidase (POD) to yield a red-violet quinoneimine dye as an indicator, the concentration of which is proportional to the concentration of glucose. (Trinder. P 1969)



RESULTS

The methanolic crude extract of *Acacia nilotica* bark and three solvents (Ethyl acetate, dichloromethane and carbon tetrachloride) soluble fractions of crude extract at a dose of 400 mg/kg body weight were subjected to screening for hypoglycemic activity by oral glucose tolerance test.

Table−9: Data representing concentration of glucose in plasma (mmol/l) of mice at different time intervals after the administration of normal saline at the dose of 400mg/kg body weight

0 minutes		30 minutes		90 minutes		150 minutes	
Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean
8.1	7.2	11.3	10.4	9.5	8.6	8.0	7.1
7.3		10.5		8.7		7.2	
6.3		9.5		7.7		6.2	
7.1		10.3		8.5		7.0	

Table−10: Data representing concentration of glucose in plasma (mmol/l) of mice at different time intervals after the administration of Standard drug (Glibenclamide) at the dose of 10mg/kg body weight

0 minutes		30 minutes		90 minutes		150 minutes	
Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean
8.2	7.5	9.02	8.3	5.7	5.0	5.2	4.5
7.4		8.22		4.9		4.4	
7.8		8.62		5.3		4.8	
6.5		7.32		4		3.5	

Table−11: Data representing concentration of glucose in plasma (mmol/l) of mice at different time intervals after the administration of methanolic crude extract at the dose of 400mg/kg body weight (MEAN)

0 minutes		30 minutes		90 minutes		150 minutes	
Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean
6.5	7.5	7	8	6.7	7.7	5.9	6.9
7.8		8.3		8.0		7.2	
8.2		8.7		8.4		7.6	
7.5		8		7.7		6.9	

Table−12: Data representing concentration of glucose in plasma (mmol/l) of mice at different time intervals after the administration of ethyl acetate fraction at the dose of 400mg/kg body weight (EAAN)

0 minutes		30 minutes		90 minutes		150 minutes	
Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean
7.8	6.6	8.3	7	8.4	7.1	8.1	6.8
6.3		6.8		6.9		6.6	
5.6		6.1		6.2		5.9	
6.5		7.0		7.1		6.8	

Table−13: Data representing concentration of glucose in plasma (mmol/l) of mice at different time intervals after the administration of dichloromethane fraction at the dose of 400mg/kg body weight (DMAN)

0 minutes		30 minutes		90 minutes		150 minutes	
Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean
8.1	6.9	8.7	7.45	8.3	7	7.9	6.69
6.6		7.2		6.8		6.4	
5.9		6.5		6.1		5.7	
6.8		7.4		7.0		6.6	

Table-14: Data representing concentration of glucose in plasma (mmol/l) of mice at different time intervals after the administration of carbon tetrachloride fraction at the dose of 400mg/kg body weight (CTAN)

0 minutes		30 minutes		90 minutes		150 minutes	
Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean	Indv. Data	Mean
7.3	7.6	7.9	8.1	7.7	7.9	7.0	7.2
7.8		8.4		8.2		7.5	
8.7		9.3		9.1		8.4	
6.5		7.1		6.9		6.2	

Table-15: Hypoglycemic activity of methanolic extract and its different fractions of Acacia nilotica

Group	Plasma level of glucose (Mean ± SEM)			
	0 min	30 min	90 min	150 min
Control	7.2 ± 0.369	10.4 ± 0.371	8.6 ± 0.357	7.1 ± 0.361
Standard	7.5 ± 0.363	8.3 ± 0.364	5.0 ± 0.365	4.5 ± 0. 365
Crude	7.5 ± 0.362	8 ± 0.363	7.7 ± 0.362	6.9 ± 0.362
EAAN	6.6 ±0.459	7 ± 0.459	7.1 ± 0.458	6.8 ± 0.458
DMAN	7.6 ± 0.461	8.1 ± 0.460	7.9 ± 0. 461	7.2 ± 0.461
CTAN	6.9 ± 0.459	7.5 ± 0.46	7.0 ± 0. 459	6.7 ± 0.459

Blood glucose curves of different groups of mice receiving different samples

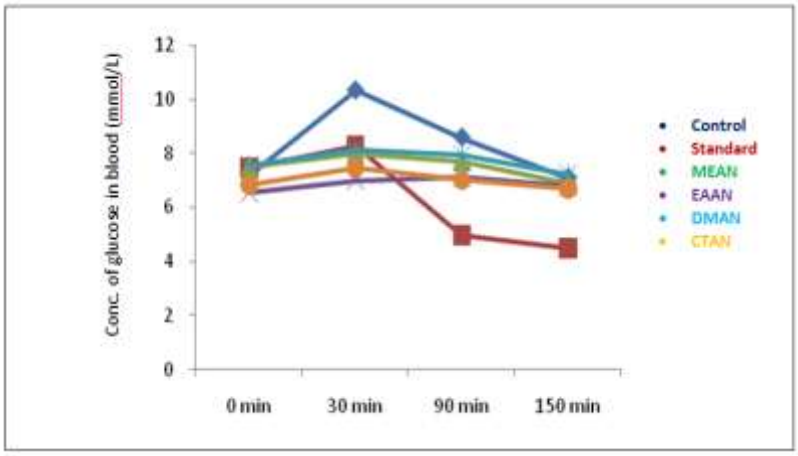


Fig-7: Curves of glucose concentration in blood of different groups of mice receiving different samples. EAAN=Ethyl acetate fraction of Acacia nilotica. CTF= carbon tetrachloride fraction of Acacia nilotica DMAN=Dichloromethane fraction of Acacia nilotica

Comparative study of conc. of glucose in blood in diff groups of mice receiving diff samples

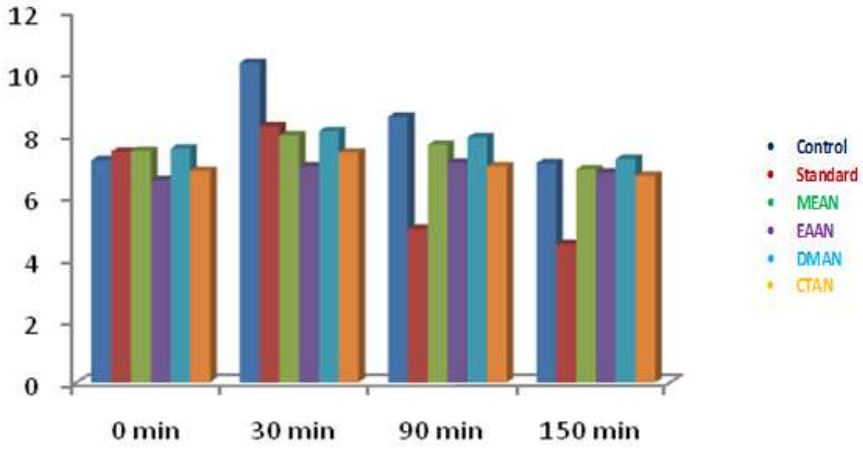


Fig-8: Concentration of glucose in blood in different groups in different times

DISCUSSION

The result depicted that the Methanolic crude extract, Dichloromethane and Carbon tetrachloride fraction at a dose of 400mg/kg body weight showed significant hypoglycemic activity compared to the standard. However, Ethyl acetate fractions didn't have shown any significant decrease of blood glucose level. The study is only preliminary and based on the present findings; antidiabetic potential of the extract can further be studied in diabetic rats. In addition the extract could further be subjected to bioactivity guided drug discovery to isolate compounds that are responsible for such activity.

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