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RESEARCH ARTICLE

BIOCHEMCIAL, ANTIDIABETIC AND CHARACTERIZATION OF MEDICINAL PLANT *Achyranthes aspera* L. PLANTS

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Abstract

The antimicrobial properties of diethyl ether, benzene, alcohol, acetone, ethyl acetate, distilled water extracts of medicinal plant sample were investigated against seven infectious bacterial strains and six fungal strains. The medicinal plant sample employed for the present tests, which include, *Achyranthes aspera.* The medicinal plant sap was extracted using separating funnel apparatus; concentrated and then it was investigated for antibacterial and antifungal activity by disc diffusion technique. The inhibitory effect widely varied with the solvents diethyl ether, benzene, alcohol, acetone, ethyl acetate, distilled water plant extracts to the test organisms employed. Results showed that diethyl ether extract of *Achyranthes aspera* possess better antibacterial and antifungal activity. The benzene, ethyl acetate, and distilled water extracts of *A. aspera* showed moderate activity of both antifungal and antibacterial activity. The liver and kidney exhibit numerous morphological and functional alterations during diabetes. Since both diabetes and hyperlipidemia are considered to be major risk factors for the premature atherosclerosis and essentially all the cholesterol in atherosclerotic plaques is derived from that of circulatory cholesterol. The antihyperlipidemic and antiperoxidative effect of extract of *A.aspera* in particular could be considered as of possible therapeutic value.The bio-chemical analysis of compound was done by radial paper chromatography technique. In this, amino acid like Tryptophan, Tyrosine and Arginine were identified. Also lipids and vitamin were separated by paper chromatography.

Key words: *Achyranthes aspera,* antibacterial and antifungal activity, diabetes and hyperlipidemia, antihyperlipidemic and antiperoxidative effect, radial paper chromatography,

Introduction

The ethnic and rural people of India have preserved a large bulk of traditional knowledge of medicinal uses of plants growing around them. This knowledge is handed down to generations through word of mouth and is extensively used for the treatment of common diseases and conditions. Rural women of India commonly experience gynecological problems due to unhygienic living conditions, malnutrition and hard physical work, often even during pregnancy. In every village some women, locally known as 'Daiya, specialize in phytotherapy of these diseases and conditions using commonly available plants. However, the number of these lady-healers is fast decreasing as younger generation is showing little interest in learning this valuable science of healing. Native medicines were used to induce abortion, to induce labor pains, to expel dead fetus, to

IJCRCPS
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expel the remains of placenta after abortion, excessive hemorrhage during pregnancy, excessive hemorrhage during early pregnancy, post-partal hemorrhage, post-partal body aches, post-partal fever, post-abortion abdominal pain, post-partal loss of appetite (Anorexia), prolonged menstrual flow, amenorrhoea, dysmenorrhoea, menaxenia (abnormal menses), leucorrhoea, habitual abortion, abnormal secretion of lochia, costodynia (pain in ribs), post delivery / abortion jaundice, infertility in women .

Apamarga known to the research world as *Achyranthes aspera* traces its existence in manuscripts of Ayurveda and Chinese medicines. It is described in 'Nighantas', ancient Indian treatise as purgative, pungent, digestive, a remedy for inflammation of the internal organs, piles, itch, abdominal enlargements and enlarged cervical glands. The diuretic properties of this plant are well known to the Indian and European Physicians.

Preclinical studies reveal that the saponins present in Achyranthes aspera shows stimulant action on the myocardium of rat and also increased the phosphorylase activity of the heart, the effect being comparable to that of adrenaline. These saponins also caused significant increase, in force of contraction of the isolated hearts of frog, guineapig and rabbit. Achyrahthine, a water-soluble alkaloid, present in whole plant of Achyranthes aspera is reported to dilate the blood vessels, lower the blood-pressure, depress the heart, and increase the rate and amplitude of respiration.
This plant is erect or procumbent, annual or perennial herb, often with a woody base. The whole plant contains the alkaloids achyranthine and betaine. The plant is much valued in the indigenous medicine. It is reported to be pungent, astringent, pectoral and diuretic. It is used as an emmenagogue and in piles and skin eruptions.

Traditional medicine is an important part of the health-care system of Tanzania. In spite of an extensive programme to create health centers and to train rural medical aids and medical assistants, the traditional healer is still the only medical practitioner available, within reasonable distance to many Tanzanians living in the rural parts of the country. The number of traditional healers has been estimated to be about 30,000 to 40,000 in comparison with about 600 Western – trained doctors, most of whom are working in hospitals in big cities. Most of the healers use various parts of plants from the flora as remedies. Only a small number of these plants have hitherto been identified. Haerdi (1964) identified 625 plants used by healers in villages around the town of Ifakara in central Tanzania. The Government Chemical Laboratory in Dar es Salaam has compiled a list of about 500 plants used in traditional medicine in various parts of Tanzania. Kokwaro (1976) has published a book listing plants used in traditional medicine in East Africa (Kenya, Tanzania and Uganda), but unfortunately the occurrence of the plants in the various countries not has been mentioned.

The researches have developed a test for antidiabetic activity based on inhibition of a digestive enzyme (pancreatic alpha amylase). This enzyme is involved in the breakdown of dietary starch to glucose. A diabetic patient him does not produce enough insulin to cope with rapid rises in blood glucose levels. Slowing the rate of starch breakdown, by blocking alpha-amylases, can lead to more even trickle of glucose into the blood stream from the intestine.

A. aspera L. is a common plant found in wastelands. The plant is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dropsy, dog bite, dysentery, ear complications, headache, leucoderma,

IJCRCPS
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pneumonia, renal complication, scorpion bite, snake bite, skin diseases etc,

Antibiotics have been used to treat infections since 1940's but bacteria are now becoming resistant to them. Multi drug resistance has been documented in many pathogenic microorganisms (Wicher *et al*., 1999). It is also becoming widely recognized that designing even more deadly drugs to kill bacteria is no longer a viable option. Resistance to new drug is increasing rapidly, often with in a year of their introduction. Furthermore, drugs toxic to bacteria are often toxic for human beings as well and the 'side-effects' may become much worse than the cure. As the awareness regarding the problems associated with overprescription and misuse of drugs has increased, people are becoming increasingly respective to the use of antimicrobials derived from natural resources. Many plants have been studied for their medicinal and antimicrobial properties (Babu *et al*., 2002)

Nature posses large number of chemical compounds. The properties of these compounds are to be described. It has turned out that it is on easy task to present the resulting vast stock of information as apart of curriculum with in a prescribed time frame. There are, besides other factors, which add to the complexity of the situation resulting from the explosive growth of the subject, the range and diversity encountered in the structures and properties of organic compounds of natural origin and imperative to acquire an understanding of their biological function in terms of chemical structure. It is estimated that about 2 % of all carbon photosynthesis by plants is converted into flavonoids or closely related compounds.

In plants, flavonoids aglycones occur in a variety of structural forms. All contain carbon atoms in their basic nucleus and these are arranged in a $C_6 – C_3 – C_6$ configuration. For convenience the rings are labeled A, B and C and the individual carbon atoms are referred to by a number system which utilizes original numerical for the A and C rings and primed numericals for the B-ring. Naturally occurring polyphenols have two alternative modes of sugar linkage, namely O-glycosylation [II] or C-glycosylation [III].
The flavonoids are responsible for the yellow color of certain flowers although more usually this may as well be due to the presence of anthocyanidins (W), which constitute one of the important groups of flavonoids. Many of these compounds have structure base on "flavone" skeleton and are named as flavonoids. *Achyranthese aspera* families contained one general and two species it is widely distributed, in N-W India.

Flavonoids

The flavonoids, one of the most numerous and widespread groups of natural secondary constituents are important to man not only because these contribute to plant color but also because many members are physiologically active. Certain flavones are among the earliest known dyestuffs. The pigments responsible for flora colors are located in the vacuoles. The term flavonoids (Lat, flavus = Yellow) were originally coined in 1952 by Geissmen and Heinreiner to embrace all those compounds having the flavone (2-phynyl chromone II) skeleton.

Flavonoids form the largest single-family and oxygen containing heterocycilcs. The flavone moiety consists of two benzene rings (A and B) linked together by a three-carbon unit, which forms the (central) pyrone ring. The various classes of flavonoids differ from one another only in the state of oxidation of this central heterocyclic viz. the three-carbon unit. There is a limitation to the number of structures commonly found in nature, which vary in their state of oxidation from flavon –3 –ol III a (catechins) to flavanols (3 hydroxy

IJCRCPS

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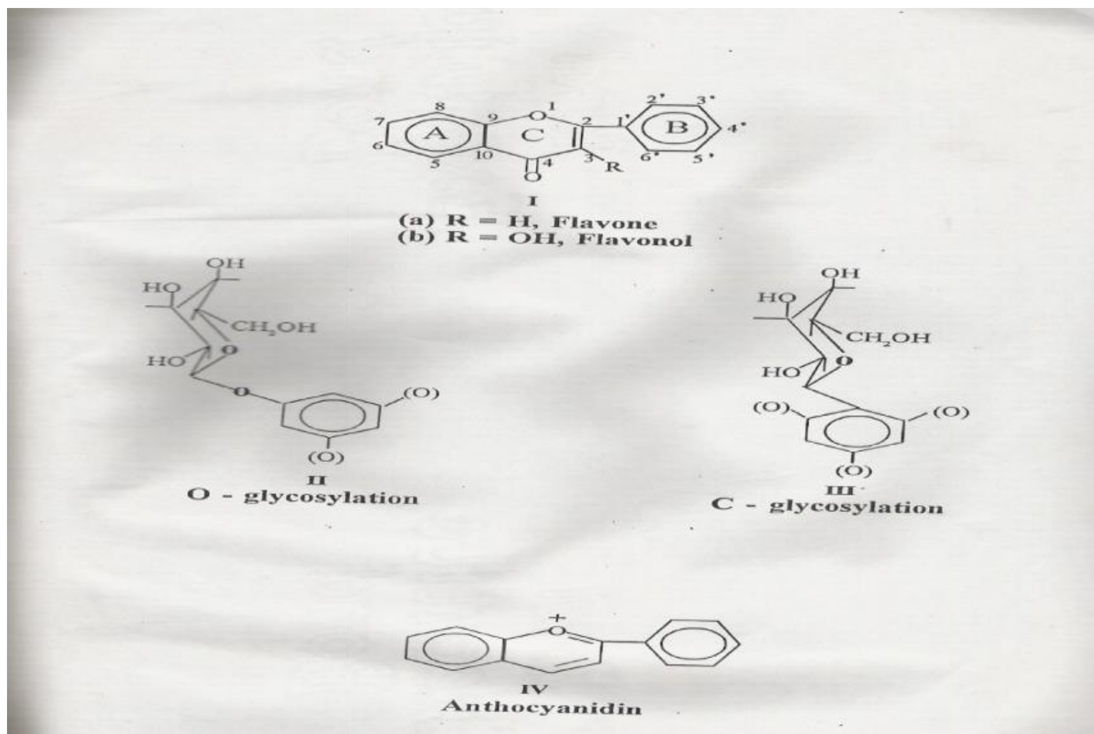
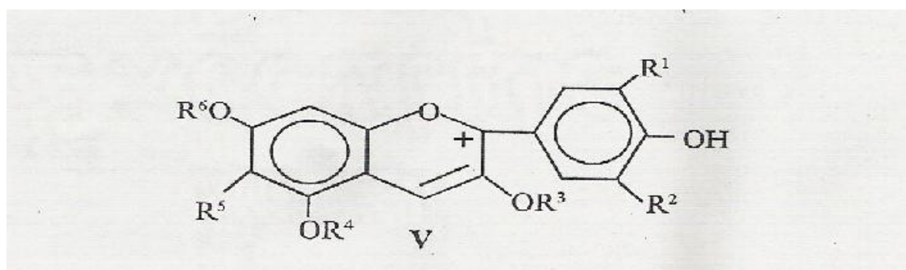
Figure.1 structure of Anthocyanidin





1. R^1 = OH; R^2, R^3, R^4, R^5, R^6, = H; Cyanidin
2. R^1 = OH; R^3 = Glc; R^2, R^4, R^5, R^6 = H; Cyanidin-3-Glucoside
3. R^1 = OH; R^3, R^4 = Glc; R^2, R^5, R^6 = H; Cyanidin-3, 5-diglucoside
4. R^1, R^2 = OH; R^3, R^4, R^5, R^6 = H; Delphinidin
5. R^1, R^2 = OH; R^3 = Glc; R^4, R^5, R^6 = H; Delphinidin-3-glucoside
6. R^1, R^2 = OH; R^3, R^4 = Glc; R^5, R^6 = H; Dephinidin-3, 5-diglucoside
7. R^1 = OMe; R^2 = OH; R^3, R^4, R^5, R^6 = H; Petunidin
8. R^1 = OMe; R^2 = OH; R^3 = Glc; R^4, R^5, R^6 = H; Pentunidin-3-glucoside
9. R^1= OMe; R^2 = OH; R^3, R^4 = Glc; R^5, R^6 = H; Pentunidin-3-5 diglucoside
10. R^1, R^2, R^3, R^4, R^5, R^6 = H; Pelargonidin
11. R^1, R^2,R^5,R^6 = H; R^3,R^4 = Glc; Pelargonidin-3-5-digulcoside
12. R^1, R^2 = OMe; R^3, R^4, R^5, R^6 = H; Malvidin
13. R^1, R^2 = OMe; R^3 = Glc, R^4, R^5, R^6 = H; Malvidin-3-glucoside
14. R^1,R^2 = OMe; R^3, R^4, R^5, R^6 = H; Malvidin-3,5-glucoside

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flavones III) and anthocyanins III. Also include in this category are the flavones.

Aim and scope

The present investigation was initiated to study the effect of *A. aspera* extract with the following objectives.

Collection of *A. aspera*
Processing of *A. aspera*
Preparation of *A. aspera*
To analyze the compounds by Chromatographic techniques.
To test the antimicrobial activity of *A. aspera* extract against human pathogenic bacteria and Fungi
To study the effect of *A. aspera* extract on diabetic in rats.

Materials and methods

Description of *Achyranthes aspera*

Vernacular names

Family : Amarantaceae
Sanskrit : Apamarga
Hindi : Chirchita, Onga, Latkana, Latjira, Apamarg
Tamil : Nayrivi
Tibetan : Abamarga
English : Prockly chaff flower
Teluge : Uttarene, Antisha
Malyalam : Katalati
Marathi : Agadha
Gujarati : Aghedo
Bengal : Apang
Parts used : Roots, Leaves, Seeds.

General description of the plant

This is a common roadside weed with spike. Inflorescence flower in throughout year. It is of common occurrence in throughout India.

A perennial, twinning or creeping herb generally cultivated as an annual. Most of the varieties have a twinning habit; a few are bushy, prostrate or semi – erect, leaves trifoliate.

The plant is considered to be Asian in origin. It is very variable. The plant is cultivated mostly as an annual, distributed throughout the tropical and temperate regions of Asia, Africa and America. It is grown in India as a garden crop and never as a field crop. Several types, size, shape and texture of pods and size of seeds are recognized.

Properties and therapeutic utility

Apamarga is pungent in taste an vipaka (taste that emerges after digestion), hot in potency and laxative. It reduces kapha, medas(fat) and vayu. It is depleting.

There are about fifty species under this genus and four species are native to India. *Achyranthes aspera* “Rough chaff flower”.

It is an erect herp (or) under shrub, which attains a maximum height up to 1 metre. Stem is stiff and not much branched. Branches are pubescent, tereate and striate. Leaves are few, thick, and elliptic or operate, pubescent, usually rounded at the apex. This species is distributed in different parts of India, tropical Asia, Africa, Australia, Ceylon and Pakistan. It is a common weed in Garhural regions up to 1000-meter elevation. The plants are also occurring commonly in Damtee of ulter khashi between 1000-1800 m and in chnindwara district of M.P at rater.

Medicinal properties

The plant is reported to possess anti - diabetic and anti - rheumatic properties and used beneficially in abdominal tumors.

Seeds

The seeds power is used beneficially in treatment of bleeding piles. The decoction

of plant is diuretic and used in renal dropsy and generalized anasarca. The seeds are used to treat snakebites, hydrophobia and itching. Seeds are emetic and used as a brain tonic. Painful delivery. The juice of the plant is used to stop bleeding of wounds.

Roots:

The root has been used as stomachic and digestant is said to be useful for the treatment of pneumonia.

Roots are also useful in toothache. The extracts of the roots are also used to treat menstrual disorders and dysentery. The root paste is said to be an ant fertility drug.

Uses

The plants are used medicinally for several diseaces such as piles, coilc, boils, etc., It is pungent, purgative, diuretic and astringent. Roots are used for pyorrhea. Also used in couch and fevers.

Collection of the plant

The whole plant *Achyranthes aspera* were collected from Thellar, during the month of May. The plants were dried in shade. Dried plant samples were used to prepare the extract.

Seperation of amino acid by radial paper chromatography

Procedure:

To begin with the Whattmann No:1 disc paper was cut into a suitable diameter.
The paper was saturated with aqueous phase for 30 minutes.
A circle was drawn at the center of the disc (3cm in dm) and with the help of the capillary tube standard and unknown samples are spotted.
Care was taken to use separate capillary tube for individual standard sample. After spotting the paper disc was placed on a petridish containing the mobile phase.
A small wick was inserted in the center of the paper to provide the movement of the solvent by capillary action.
The chromatography was allowed to run for 2 hours and then the paper was taken with the help of a pencil.
The paper was dried before spraying with the locating agent.
The paper was activated at 110°C in a hot air over for 2 minutes.
The amino acids in the mixture were identified by comparing their R_f values with the standard.

Calculation

R_f : (Resolution front)

R_f is the ratio of the distance traveled by the solute to that of the solvent. Both measured from the point of application.

$$R_f = \frac{\text{Distance Traveled by the solute from the point of origin}}{\text{Distance traveled by the solvent form the point of origin.}}$$

SEPERATION OF VITAMINS BY RADIAL PAPER CHROMATOGRAPHY

Procedure

To begin with the Whattmann No :1 disc paper was cut into a suitable diameter.
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A small wick was inserted in the center of the paper to provide the movement of the solvent by capillary action.

The chromatography was allowed to run for 2 hours and then the paper was taken with the help of a pencil.
The paper was dried before spraying with the locating agent.
The paper was activated at 110°C in a hot air over for 2 minutes.
The R_f value was calculated.

Calculation

R_f : (Resolution front)

R_f is the ratio of the distance traveled by the solute to that of the solvent. Both measured from the point of application.

$$R_f = \frac{\text{Distance Traveled by the solute from the point of origin}}{\text{Distance traveled by the solvent form the point of origin.}}$$

Seperation of lipids by radial paper chromatography

Procedure

To begin with the Whattmann No :1 disc paper was cut into a suitable diameter.
The paper was saturated with aqueous phase for 30 minutes.
A circle was drawn at the center of the disc (3cm in dm) and with the help of the capillary tube standard and unknown samples are spotted.
Care was taken to use separate capillary tube for individual standard sample. After spotting the paper disc was placed on a petridish containing the mobile phase.
A small wick was inserted in the center of the paper to provide the movement of the solvent by capillary action.
The chromatography was allowed to run for 2 hours and then the paper was taken with the help of a pencil.
The paper was dried before spraying with the locating agent.
The paper was activated at 110°C in a hot air over for 2 minutes.
The R_f values were calculated.

Calculation

R_f : (Resolution front)

R_f is the ratio of the distance traveled by the solute to that of the solvent. Both measured from the point of application.

$$R_f = \frac{\text{Distance Traveled by the solute from the point of origin}}{\text{Distance traveled by the solvent form the point of origin.}}$$

Effect of *a.aspera* extract on diabetics in rats

Experimental animals

Male rats of body wt. 180–200 g were used for the study.

Drug and chemicals

Ethanolic extract of medicinal plant of *Achyranthres aspera* was used in this study.
The *Achyranthres aspera* samples was extracted individually and soaked overnight in 1.5 liters of 95% ethanol.
This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 h and filtered again.
The two filtrates were pooled and the solvents were evaporated in a rotavapor at 40° – 50°C under reduced pressure

Drug administration

Residue of ethanol extract of *A. aspera* was suspended in distilled water and administered orally through intragastric tube at the following doses of 50, 100 and 200-mg/kg body weight.

Experimental induction of diabetes in rats

The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile

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normal saline at a dose of 150 mg/kg body wt Katsumata et al.,1999).

After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycemia (i.e. with a blood glucose of 200–300 mg/dl) were used for the experiment.

Experimental design

In the experiment, a total of 6 rats were used. The rats were divided into six treatments of one rat each after the induction of alloxan diabetes.

Treatment1:

Normal treated rat.

Treatment 2:

Normal rat given aqueous solution of ethanol extract of *A.aspera* (200 mg/kg body weight) daily using an intragastric tube for 30 days.

Treatment 3:

Diabetic control rat.

Treatment 4:

Diabetic rat given aqueous solution of ethanol extract of *A.aspera* (50 mg /kg body weight) daily using an intragastric tube for 30 days.

Treatment 5:

Diabetic rat given aqueous solution of ethanol extract of *A.aspera* (100 mg /kg body weight) daily using an intragastric tube for 30 days.

Treatment 6:

Diabetic rat given aqueous solution of ethanol extract of *A.aspera* (200 mg /kg body weight) daily using an intragastric tube for 30 days.

At the end of 30 days, all the rats were killed by decapitation under pentobarbitone sodium (60 mg/kg) anaesthesia. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose and plasma was separated for the assay of insulin. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris – Hcl buffer, pH 7.5. After centrifugation at 200 rpm for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS) and hydroperoxides.

For the determinations of lipids the liver and kidney tissues were weighed and lipids were extracted from tissues by the method of Folch *et al*., (1957) using chloroform – methanol mixture ($CHCl_3$: MeOH)(2:1 v/v).

Biochemical analysis

Estimation of blood glucose and plasma insulin

Blood glucose was determined by the O-toluidine method Sasaki *et al.*(1972).

Estimation of lipid peroxidation

Lipid peroxidation in liver and kidney were estimated colorimetrically by thiobarbituric acid reactive substances hydroperoxides by the method of Niehius and Samuelsson(1968) and Jiang *et al.*,(1992) respectively.

In brief, 0.1 ml of tissue homogenate (Tris-Hcl buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and

15% TCA) and placed in water bath for 15 min, cooled.

The absorbance of clear supernatant was measured against reference blank at 535 nm.

0.1ml of tissue homogenate was treated with 0.9 ml of Fox reagent (88 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250 mM sulphuric acid) and incubated at 37°C for 30 min.

The color developed was read at 560 nm colorimetrically. Hydroperoxides was expressed as mM/100 g tissue.

Estimation of lipids

Lipids were extracted from tissues by the method of Folch *et a., l*(1957) using chloroform – methanol mixture ($CHCl_3$: MeOH) (2:1 v/v).

The total cholesterol was estimated by the method of Zlatkis *et al.*,(1953).

To 0.1 ml of the lipid extract, 9.9 ml of ferric chloride-acetic acid reagent was added and allowed to stand for 15 min and then centrifuged.
To 5 ml of the supernatant, add 3 ml of Conc. H_2So_4.
The colour developed was read after 20 min at 560 nm against a reagent blank. Values were expressed as mg/100 g tissue.

Triglycerides were estimated by the method of Foster and Dunn(1973).

To an aliquot of lipid extract, evaporated to dryness. 0.1 ml of methanol was added followed by 4 ml of isopropanol.

0.4 g of alumina was added to all the tubes and shaken well for 15 min. Centrifuged and then 2 ml of the supernatant was transferred to labeled tubes. The tubes were placed in a water bath at 65°C for 15 min for saponification after adding 0.6 ml of the saponification reagent followed by 0.5 ml of acetyl acetone reagent.
After mixing, the tubes were kept in a water bath at 65°C for 1 h, the contents were cooled and absorbance was read at 420 nm.
The triglyceride content was expressed as mg/100 g tissue.

Phospholipid content was determined by the method of Zilversmit et al., (1950).

To 0.1 ml of lipid extract, added 1 ml of 5 N H_2SO_4 and 1 ml of concentrated nitric acid and digested to a colourless solution.
The phosphorus content in the extract was determined by the method of Fiske and Subba Row (1920).
The values were expressed as g/100 g tissue.

Free fatty acids were estimated by the method of Falholt *et al* .,(1973).

0.1ml of lipid extract was evaporated to dryness. 1 ml of phosphate buffer, 6 ml of extraction solvent and 2.5 ml of copper reagent were added.
All the tubes were shaken vigorously.
200 mg of activated silicic acid was added and left aside for 30 min.
The tubes were centrifuged and 3 ml of the copper layer was transferred to another tube containing 0.5 ml of diphenyl carbazide and mixed carefully. The absorbance was read at 550 nm immediately. The amount of free fatty acids was expressed as mg/100 g tissue.

Results and discussion

Effect of *A.aspera* on Diabetics

Table 3 shows the level of blood glucose and plasma insulin in control and experimental animals. There was a significant elevation in blood glucose level with significant decrease in plasma insulin

IJCRCPS

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levels in alloxan diabetic rat, compared with normal rat. Administration of extract of *A.aspera* tended to bring blood glucose and plasma insulin towards near normal levels.

The effect of extract of *A.aspera* at 200 mg /kg was significantly better than 50 and 100 mg/kg, therefore the higher dose was used for further biochemical studies. The administration of extract of *A.aspera* normal rat showed a significant effect in lowering blood glucose and increasing plasma insulin.

The concentration of hydroperoxides in tissues of control and experimental animals. There was a significant elevation in tissue hydroperoxides during diabetes, when compared to the corresponding control group. Administration of extract of *A.aspera* tends to bring the values to near normal.

The levels of cholesterol, triglycerides, free fatty acids and phospholipids in liver and kidney of control and experimental rat respectively. Liver and kidney of diabetic rat showed significantly increased levels of cholesterol, triglycerides, free fatty acids and phospholipids, when compared with normal rats.

In rat treated with extract of *A.aspera* there was a significant decrease in the content of cholesterol, triglycerides, free fatty acids and phospholipids in both the tissues, when compared with diabetic control rat.

Diabetes mellitus is one of the most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complications of both clinical and experimental diabetes (Bierman *et al.*,1975). Alloxan, a beta cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Omamoto *et al.*, 1981).

In our study, we have observed that extract of *A.aspera* decreases blood glucose in alloxan diabetic rat. The possible mechanism of action of extract could be correlated with the reminiscent effect of the hypoglycemic sulphonylureas that promote insulin secretion by closure of K^+-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion. In this context, number of other plants have also been reported to have antihyperglycemic and insulin stimulatory effects (Venkateswaran and Pari,2002 ; Latha and Pari,2003).

Like other plant extract, Extract of *A.aspera* produced significant reduction in blood glucose levels of alloxan diabetic rats. Since alloxan is known to destroy pancreatic -cells, the present findings appear to be in consonance with the earlier suggestion of Jackson and Bressler(1981) that sulphonylureas have extra- pancreatic antihyperglycemic mechanism of action secondary to their insulin secreting effect and the attendant glucose uptake into, and utilization by, the tissues.

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids (Loci et al., 1994). During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids in to the circulation (Agardh *et al.*, 1999). Increased fatty acids concentration also increases the -oxidation of fatty acids, producing more acetyl CoA and cholesterol during diabetes.

In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes

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hypercholestrolemia. Hypercholestrolemia and hypertriglycridemia have been reported to occur in diabetic rats (Bopanna *et al.,*1977).

The increased concentration of cholesterol could result in a relative molecular ordering of the residual phospholipids resulting in a decrease in membrane fluidity (Dario *et al*.,1996). The increased concentration of free fatty acids in liver and kidney may be due to lipid breakdown and this may cause increased generation of NADPH, which results in the activation of NADPH dependent microsomal lipid peroxidation. Liver and kidney phospholipids were increased in diabetic control rats. Phospholipids is present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasma environment and non-polar lipoprotein of lipoprotein core (Cohn and Roth,1996).

Phospholipids are vital part of biomembrane rich in PUFA, which are susceptible substrate for free radicals such as $O_2^{\bullet-}$ and $OH^{\bullet}$ radicals (Ahmed *et al.,* 2001). Increased phospholipids levels in tissues were reported by (Venkatateswaran *et al*., 2002 and Pari and Amarnath Satheesh,2004) in streptozotocin diabetic rats. Administration of extract of *A.aspera* decreased the levels of tissue free fatty acids and phospholipids.

Accumulation of triglycerides is one of the risk factors in Coronary Heart Disease (CHD). The significant increase in the level of triglycerides in liver and kidney of diabetic control rats may be due to the lack of insulin. Since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis triglycerides (Frayn,1993). extract of *A.aspera* reduces triglycerides in tissues of alloxan-induced diabetic rats and may prevent the progression of CHD.

The results show increased lipid peroxidation in the tissues (liver and kidney) of diabetic control group. Previous studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha and Pari,2003; Ananthan *et al.,*2004). This may be because the tissues contain relatively high concentration of easily peroxidizable fatty acids. Liver during diabetes, showed a relatively severe impairment in antioxidant capacity than kidney. The kidney exhibits a characteristic pattern of changes during diabetes (Aragno *et al*., 1999). The increase in oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon autoxidation generate free radicals and secondarily due to the effects of diabetogenic agent alloxan (Szkudelski ,2001).

In diabetes, hypoinsulinaemia increases the activity of the enzyme, fatty acyl coenzyme, coenzyme A oxidase, which initiates - oxidation of fatty acids resulting in lipid peroxidation(Oberley ,1998 and Baynes,1995). Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane-bound enzymes (Baynes,1995). Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with atherosclerosis and brain damage (Baynes,1995) .

Administration of extract of *A.aspera* reduced the lipid peroxidative markers in liver and kidney tissues of diabetic rats. This indicates that extract of *A.aspera* inhibit oxidative damage due to the antiperoxidative effect of ingredients present in extract of *A.aspera*. This could be correlated with previous study that reported that *Cassia auriculata* (Pari and Latha, 2002), *Syzigium cumini* (Prince and Menon,1998; Prince *et al*., 2004)*Tinospora cardifolia* (Prince *et al*., 1999), and *Scoparia dulcis* (Latha and Pari,2003) (ingredients of extract of *A.aspera*) have antiperoxidative

and antihyperlipidemic effect of diabetic animals.

Antidiabetic and antihyperlipidemic effect of extract of *A.aspera* may be due to the effect of active constituents of different parts, viz, alkaloid and pectins from *Coccinia indica* (Hossain *et al.,*1992) alkaloids from *Tinospora cordifolia*(Prince *et al.*,2002), emlicanin A and B from *Emblica officinalis* Aruna Bhattacharya *et al.*,1999) trigonelline and scopoltin from *Trigonella foenum graecum* (Jachak and Sanja,2002)loid-6-methoxybenzoxazolinone and terpenoids such as scoparic acids A,B,C and scopadulcic acid A and B from 'scoparia dulcis' (Pari and Latha,2004), which may be responsible for scavenging free radicals liberated by alloxan in diabetic rats.

On the basis of above results, it could be concluded that extract of *A.aspera* medicinal plants exert a significant antihyperlipidemic and antiperoxidative effect. This could be due to different types of active principles, each with a single or a diverse range of biological activities, which serves as a good adjuvant in the present armamentarium of antidiabetic drug.

Chemical analysis of a.aspera

Separation of amino acid:

R_f : (Resolution front)

R_f is the ratio of the distance traveled by the solute to that of the solvent. Both measured from the point of application.

$$R_f = \frac{\text{Distance Traveled by the solute from the point of origin}}{\text{Distance traveled by the solvent form the point of origin.}}$$

2.0/ 4 = 0.55 (Tryptophan)

1.8/ 4 = 0.46 (Tyrosin and Arginine)

Separation of vitamin:

R_f : (Resolution front)

R_f is the ratio of the distance traveled by the solute to that of the solvent. Both measured from the point of application.

$$R_f = \frac{\text{Distance Traveled by the solute from the point of origin}}{\text{Distance traveled by the solvent form the point of origin.}}$$

1.5/ 5 = 0.3

2.6/ 5 = 0.5

Separation of lipids

R_f : (Resolution front)

R_f is the ratio of the distance traveled by the solute to that of the solvent. Both measured from the point of application.

$$R_f = \frac{\text{Distance Traveled by the solute from the point of origin}}{\text{Distance traveled by the solvent form the point of origin.}}$$

0.8/6 = 0.13

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