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Research

Pharmacological Evaluation Of Anti-Atherosclerotic Activity Of Tinospora Cordifolia In Animal Model

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Abstract

Objective: To investigate the antiatherosclerotic activity of ethanol extract of *Tinosporacordifolia* in male Wistarrats.
Material & method: In this model of atherosclerosis, 30 adult male Wistar rats were evenly divided into 5groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4and5 were the treatments groups which were simultaneously treated with standard, 200and400mg/kg extract respectively along with High Fat Diet. On last day, blood samples for biochemical parameters, were obtained under inhaled diether anaesthesia.
Results: HFD caused atherosclerosis as evidenced by marked elevation in Cholesterol, Triglycerides, LDL, VLDL and decrease in HDL levels. Co-administration of extract with HFD decreased rise Cholesterol, Triglycerides, LDL, VLDL and incr
ease in HDL levels.
Conclusion: It was observed that the ethanol extract of *Tinosporacordifolia* conferred Anti- atherosclerotic activity by biochemical observation against HFD induced atherosclerosis in rats. In the near future could constitute lead to discovery of an ovel drug for treatment of drug induced atherosclerosis.

Keywords: Antiatherosclerotic activity,Tinosporacordifolia,Ethanol extract

INTRODUCTION

HERBAL PLANTS

Herbal plant is a valuable plant that you can use some or almost every part of it for many treatments. Some people use its part such as dried leaves, roots, flowers, etc for curing diseases. Some use its chemical substance such as its extract oil for therapy. Furthermore, you can also use herbs as cooking recipes. Consequently, herbs have many benefits but the main purpose of using them is to maintain good health.

There are many kinds of herbal plants and each part of herbal plant used is different. Herbal plants can be used for various purposes that depend on your demand. You can use them for relief healing such as Aloe and some kinds of herbs and can be grown for a garden full of their sweet smelling. Crafting with herbs can make a satisfactory and beautiful home. Artemisia is a beautiful herb that is easily grown in the garden or Giver King is a lovely herb that has a fresh herbal scent and dried floral such as sunflower, roses or anything else are suitable for your crafting.

Herbs are natural in the kitchen such as lemon, pepper, chili or anything else. Many excellent cosmetics come from herbs. Almost all herbs are used for improvement human health. Herbs have long been known for therapy. You can make excellent skin tonic and fragrance in the soap and hair conditioner from herbs. Some kinds of herbs have been used in the control of acne and eczema. You can use some kinds of herbs for relieving headache and as a stimulant and tonic.

There are several reasons why herbal plants become popular plants. One of the main reasons, of course, is that each herbal plant has various properties. For example, Lavender has long been known for its classic fragrance. Although Lavender is a fragrant flower, it is also an important medicinal herb. The oil from flowers can be used to protect cloths and store linens from moths. It can be used as a scent in air fresheners. The oil can be applied as a stimulant, tonic, and headache relief and for relief of intestinal gas. Disinfecting wounds can be cured by using the oil from Lavender. In addition, Lavender oil can relieve neuralgic pains, sprains and sore joints.

Furthermore, Rosemary has been named the herb of the year by the International Herb Association. In ancient Greece and Rome rosemary was believed to strengthen memory. Rosemary has long been known for its therapeutic power. If you suffer from nightmares, you should try placing a sprig under the pillow of a sleeper. It will treat you. A product of Rosemary, for example, Rosemary tea helps digestion but it takes time as a soothing drink to calm the nerves and induce sleep. Rosemary oil is an excellent conditioning effect on the hair, helping to control dandruff and even, be alleged and curing baldness.

Moreover, Aloe Vera is known as a medicinal plant because it has many useful parts to treat many diseases. For example, the clear gel has a dramatic ability to heal wounds, ulcers and burns by putting a protective coating on the affected areas and speeding up the healing rate. As a food supplement, Aloe is said to facilitate digestion, aid in blood and lymphatic circulation. It helps cleanse the digestive tract by exerting a soothing, balancing effect because it has three anti inflammatory fatty acids. And for another thing, Aloe has a moisturizing effect on the skin. It is a common remedy for sun burn and skin irritation. It can relieve itching due to insect bites.

Another useful plant is Ginkgo, which is the oldest living tree species and its dried leaf can be used as a sacred herb. Ginkgo is often used to treat elderly persons with Alzheimer's and other symptoms of cerebral insufficiency because it has benefits of enhancing circulation in the brain including improvement of short and long-term memory which increases reaction time and improves mental clarity. Moreover, Ginkgo may help to counteract the effects of aging, including mental fatigue and lack of energy. It has been used to relieve tension and anxiety, improve mental alertness and elevate mood and restore energy.

This herb is also used as a treatment for vertigo, ringing in the ears and a variety of neurological disorders and circulation problems; for example, the Ginkgolides have been shown to control allergic inflammation, anaphylactic shock and asthma. For one thing, Ginkgo may also help control the transformation of cholesterol to plague associated with the hardening of arteries, and can relax constricted blood vessels. [1,2,3]

Cardiovascular disease (CVD) is the leading cause of death in the world and accounts for well over one million deaths each year in the United States. Of the more than two million deaths in the United States in 1998, CVD was listed as the primary or contributing cause in 70% of cases.1 According to the Centres of Disease Control and Prevention (CDC) and the National Health and Nutrition Examination Survey III, the probability at birth of dying from CVD is 47%, compared to 22% from cancer, 2% from diabetes, and less than 1% from human immunodeficiency virus (HIV) disease. The largest proportion of this high mortality is attributed to coronary artery disease (CAD) or coronary heart disease (CHD), which was the primary contributing cause of death in 459,841 Americans in 1998.[4]

CVD includes hypertension, coronary artery disease (CAD), congestive heart failure (CHF), congenital cardio-vascular defects, and stroke. The prevalence of these entities in the United States surpasses 60 million cases. Although these diseases are associated with a high mortality, the associated morbidity affects all walks of life and has a great impact on the quality of life of affected individuals. This chapter presents a brief overview of common cardio-vascular conditions and their implications for the practice of dental medicine.

ATHEROSCLEROSIS

Atherosclerosis can affect arteries in the heart, brain, arms, legs pelvis and intestines leading to disease of those organs. There are 4 types of atherosclerosis which include as follows:.

Coronary artery disease (CAD)

When plaque build-up in the coronary arteries, supply of oxygen rich blood to heart is reduced leading to chest pain and ultimately heart attack. [2]

Carotid artery disease or cerebrovascular disease

When plaque builds up in carotid arteries, the supply of oxygen rich blood to the brain is reduced leading to a stroke. [5]

Peripheral Arterial Disease (PAD)

When plaque builds up in arteries supplying blood to leg, arms and pelvis, the oxygen rich blood supply to these parts is restricted leading to numbness, pain and dangerous infections. [2]

Abdominal Angina and a Bowel Infraction

Atherosclerosis leads to narrowing of arteries supplying blood to the intestines causing abdominal pain and is called abdominal angina. Complete or sudden blockage of blood supply to intestines leads to bowel infection.

In service cause, atherosclerosis could also lead to narrowing of arteries of kidney leading to renal artery stenosis. Millions of Americans are diagnosed to be suffering from atherosclerosis and millions more have the diseases but are unaware it. Atherosclerosis accounts for about 75 percent of all deaths from cardiovascular diseases. Men, African –Americans and all individuals over 65 years of age have the highest risk of developing advanced atherosclerosis. [5,6]

SYMPTOMS OF ATHEROSCLEROSIS:

Unfortunately, atherosclerosis produces no symptoms until the damage to arteries is severe enough to restrict blood flow. * Restriction of blood flow to the heart muscle due to atherosclerosis can cause angina pectoris or a myocardial infarction (heart attack). * Narrowing of the arteries supplying blood to the brain may cause transient ischemia attacks (symptoms and signs of stroke listing less than 24 hrs) and episodes of dizziness, or ultimately, to a stroke itself.[7,8,9]

MATERIALS AND METHODS

MATERIALS

Plant material

The plant material used for the study is

The ethanolic extract of *Tinosporacordifolia* plant.

Collection of plant material

The aerial part of *Tinosporacordifolia* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

METHOD

Preparation of the plant extract

Preparation of *Tinosporacordifolia* extract

- → The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh.
- → About 100g of powdered materials were extracted with Ethanol (90%) using Soxhlet apparatus.
- → The extraction was carried out until the extractive becomes colourless.
- → The extracts is then concentrated and dried under reduced pressure.
- → The solvent free semisolid mass thus obtained is dissolved in Tween 80 and used for the experiment.

Preliminary phytochemical analysis

The crude and successive extracts were tested for the following phytoconstituents, carbohydrate, alkaloids, glycosides, tannins, flavonoids, phytosterols, fats and oil. The extracts were subjected to the following chemical test for the identification of various active constituents.

1. Test for alkaloids

Dragondroff's test: To 1ml of the extract, add 1ml of Dragondroff's reagent, an orange red precipitate indicates the presence of alkaloids.

Mayer's test: To 1ml of the extract, add 2ml of Mayer's reagent, a cream coloured precipitate reveal the presence of alkaloids.

Wagner's test: To 1ml of the extract, add2ml of Wagner's reagent, the formation of reddish brown precipitate indicates the presence of alkaloids.
Hager's test: To 1ml of the extract, add 3ml of Hager's reagent the formation of yellow precipitate confirms the presence of alkaloids.

2. Test for carbohydrates
Molisch test: To 2ml of the extract, add 1ml of α-naphthol solution and then add concentrated sulphuric acid through the sides of the test tube, purple or reddish violet ring at the junction of the two reveals the presence of carbohydrates.
Fehling's test: To 1ml of the extract, add an equal quantity of Fehling's solution A and B and heat. The formation of the brick red precipitate indicates the presence of carbohydrates.
Benedict's test: To 5ml of Benedict's reagent add 1ml of extract solution and boil for 2minutes and cool. Formation of a red precipitate shows the presence of carbohydrates.
Barfoed's test: To 5ml of Barfoed's reagent, add 1ml of the extract solution and heat to boil, a red precipitate of copper oxide was formed and confirms the presence of carbohydrates in the test extract.

3. Test for steroids and sterols
LibermannBurchard test: Dissolve the extract in 2ml of chloroform in a dry test tube. Add ten drops of acetic anhydride and two drops of concentrated sulphuric acid. The solution becomes red, then blue and finally bluish green, indicating the presence of steroids.
Salkowaski test: Dissolve the extract in chloroform and add volume of concentrate sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and whereas the acid layer assumes marked green florescence, represents the steroid and sterol components in the tested extract.

4. Test for glycosides
Legal test: Dissolve the extract in pyridine and add freshly prepared sodium nitroprusside solution to make it alkaline. The formation of pink to red colour shows the presence of glycoside.
Baljet test: To 1ml of the test extract add 1ml sodium picrate solution and the yellow to orange colour reveals the presence of glycoside.
Borntrager"s test: Add a few ml of diluted sulphuric acid to 1ml of the extract solution. Boil, filter and the filtrate extract with chloroform. Separate the chloroform layer and treat with 1ml ammonia. The formation of red colour shows the presence of anthraquinone glycoside.
Keller Mililani test: Dissolve the extract in acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually becomes blue, confirms the presence of deoxy sugar attached to the aglycon part of glycoside.

5. Test for saponins
Foam test: About 1ml of alcoholic extract, dilute separately with 20ml of distilled water and shake in a graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponins. To 1ml of the extract, add alcoholic vanillin solution and a few drops of concentrated sulphuric acid. A deep violet colour confirms the presence of saponins.

6. Test for flavonoids
Shinoda test: To 1ml of the extract, add magnesium turnings and 1-2 drops of concentrated hydrochloric acid. Formation of pink or red colour shows the presence of flavonoids. To 1ml of extract, add 1ml of ferric chloride, the formation of brown colour confirms the presence of flavonoids.

7. Test for triterpenoids
Dissolve two or three granules of tin metal in 2ml of thionyl chloride solution. Then add 1ml of the extract into test tube. The formation of a pink colour indicates the presence of triterpenoids.

8. Detection of phenolics and tannins
Ferric chloride test: The extract was treated with few drops of neutral ferric chloride solution. The formation of bluish black colour indicates the presence of phenolic nucleus.
Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicates the presence of tannins.
Lead acetate test: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

RESULTS

PLANT EXTRACT

The fresh aerial parts of *Tinosporacordifolia*were dried under shade and powdered in grinder. 300gm of powder was extracted with methanol (MEDB) by soxhletion in soxhlet apparatus. The yield of extract was 30.27 gm and the percentage yield was 15.13%. The extract was stored in air tight container and further used for pharmacological screening.

Priliminary Phytochemical Screening

Phytochemical screening of *Tinosporacordifolia*was done using Methanol, the extract showed the presence of Alkaloids, carbohydrates, glycosides, saponins, flavonoids and tannins. Results are shown in the following table.

Acute Toxicity Studies

The acute toxicity studies of *Tinosporacordifolia*was carried out as per OECD guidelines 423. There was no gross evidence of any abnormalities observed up to a period of 4-6hrs and no mortality was observed at the maximum tolerated dose(MTD) level of 2000mg/kg bw. per oral. The maximum tested dose was 2000mg/kg body weight. Further pharmacological screenings were carried out with two dose ranges i.e. 1/10 of MTD (200 mg/kg bwp.o.), 1/5 of MTD (400mg/kg bwp.o)..They were taken as Test doses T_1 and T_2 respectively.

Effect Of Ethanolic Extract Of *Tinospora Cordifolia*on Hfd, Atherosclerosis Profile In Rats
Biological Parameters

a. Body Weights

HFD and MSG fed rats significantly gained weight compared to the normal rats. Oral administration of EETC had reduced the weight gained. EETC at a dose of 200 mg/kg b.w. p.o. significantly decreased ($p<0.05$) weights, while EETC at 400 mg/kg b.w. p.o.and orlistat had decreased the body weight significantly ($p<0.01$) at the end of week 4. Results are presented in the following table-4, depicted in graph-1.

b. Liver Weights

The livers in negative control group were enlarged and produced a yellow color, indicating liver steatosis and increased weights. The livers were appeared yellow and bulky due to presence of fat. On the other hand group given with orlistat reversed the conditions of liver to remain normal and healthy. Significant reduction ($p<0.05$) was seen with EEPG at 200mg/kg whereas significant reduction ($p<0.01$) was observed with EEPG at 400mg/kg and orlistat.

Table 1: Priliminary Phytochemical Analysis

S.No.	Test	Result
1.	**ALKALOIDAL TEST**	
	a.Dragondroffs test	Positive
	b.Mayer's test	Positive
	c.Wagner's test	Positive
	d. Hager's test	Positive
2.	**CARBOHYDRATES TEST**	
	a.Molish's test	Positive
	b.Fehling's test	Positive
	c.Benedict's test	Positive
	d. Baeford's test	Positive
3.	**STEROIDS TEST**	
	a.LibermannBuchard test	Negative
	b. Salwoski test	Negative
4.	**GLYCOSIDES TEST**	
	a.Legal test	Positive
	b.Baljet test	Positive
	c.Killerkilaini test	Positive
	d. Borntagers test	Positive
5.	**SAPONINS TEST**	
	a.Foam test	Positive
6.	**FLAVONOIDS TEST**	
	a.Shinoda test	Positive

7.	**TRITERPINOIDAL TEST**	Negative
8.	**TANNINS TEST** a.Ferric chloride test b.Gelatin test c.Lead acetate test	 Positive Positive Positive
9.	**PROTIEN& AMINOACIDS TEST** a.Buret's test b.Ninhydrin test c.Xanthoprotic test	 Negative Negative Negative

Table 2: Acute toxicity study results

Alertness	Behavioral Responds	↓
Stereotypy		-
Irritability		↓
Fearfulness		↑
Touch responds		↑
Analgesia		N
Spontaneous activity		↓
Grooming		↑
Restfulness		↑
Inclined plane test		↑
Body Temperature		↑
Righting responds	Neurological Responds	-
Limb tone		N
Grip strength		+
twitching		-
Abdominal tone		+
Pinnal reflex		N
Corneal reflex		N
Straub tail		+
Tremors		-
Convulsions		-
Catalepsy		-
Writhing	Autonomic Responds	+
Defecation		↑
Urination		↑
Piloerection		+
SMA		N
Respiration		↑
Pupil size		N
Cyanosis		N
Heart rate		N
Ataxia		+
Ptosis		-
Salivation		-
Lachrymation		-

(↑) Increased (↓) Decreased (+) Presence (-) Absence (N) None

Table 3: Effect of PG on body weights of rats (HFD Model)

	Differences in body weights (gm) (Mean ± SEM)			
Group (n=5)	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Group I Normal control group	30.5 ± 3.52	37.5 ± 1.5	40.6 ± 3.6	44.12 ± 3.1
Group II Negative control group HFD	34.6 ± 6.48	79.9 ± 0.1	104.8 ± 2.1	115.8 ± 1.0
Group III Positive control group Orlistat 50mg/kg b.w. p.o	31.9 ± 2.24	71.6 ± 3.8	90.8 ± 6.1	82.1 ± 8.1
Group IV T_1 TC 200mg/kg b.w. p.o	33.6 ± 2.5	78.5 ± 2.9	96.2 ± 1.5	91.2 ± 6.8
Group V T_2–TC 400mg/kg b.w. p.o	35.2 ± 6.1	78.8 ± 2.0	99.0 ± 8.2	85.51 ± 2.6

Table-3 Values are expressed as Mean ± SEM (n=5) *p<0.05, **p<0.01 was considered significant compared to normal and untreated groups.

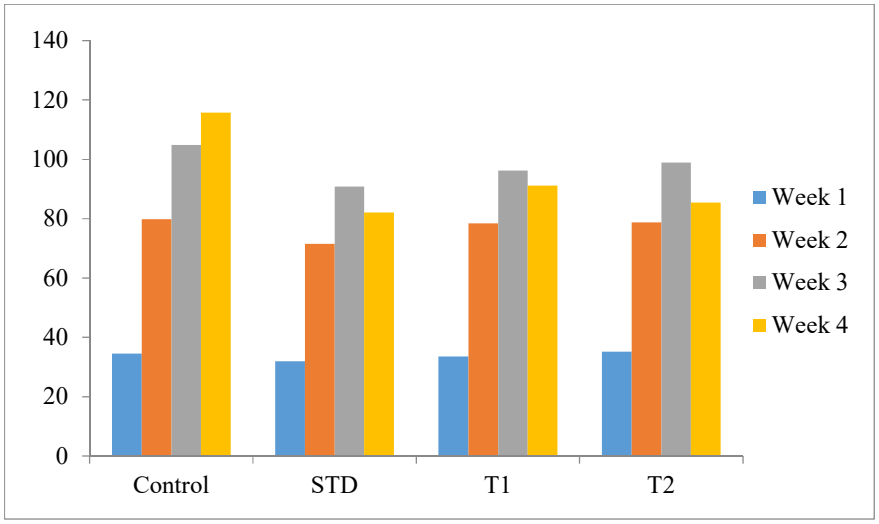

Fig 1: Effect of PG on body weights of rats

ANOVA followed by Dunnet's t-testValues are expressed as Mean ± SEM (n=5)*p<0.05,**p<0.01 was considered significant compared to normal and untreated groups.

CONCLUSION

Phytochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardio tonic aglycones, flavonoids, saponins, carbohydrates, proteins, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg and 400mg/kg. Anti atherosclerotic activity was performed by using the high fat diet induced method. In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from other lipid was observed. It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and MDA which are actually raised in atherogenic diet, can be lowered significantly with *Tinosporacordifolia*. And total proteins and antioxidant parameters SOD, GSH which are actually lowered in atherogenic diet can be raised significantly with *Tinosporacordifolia.* From this we can conclude that the extract (***Tinosporacordifolia***) Showed the anti atherosclerotic activity.

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