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Evaluation Of Anti-Hypertensive Activity of Methanolic Extract of *Operculina Turpethum* Linn. Silva Manso Stem Bark: An *In-Vivo* Approach

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Abstract:

Hypertension, a prevalent cardiovascular disorder, continues to be a significant public health concern globally. This study aimed to evaluate the potential anti-hypertensive activity of the methanolic extract derived from *Operculina turpethum* Linn Silva Manso stem bark through an in-vivo approach. Male Wistar rats were divided into different groups and subjected to various doses of the methanolic extract. Blood pressure was measured using non-invasive tail-cuff method at regular intervals following extract administration. Additionally, biochemical parameters including lipid profile and renal function tests were assessed to evaluate the safety and efficacy of the extract. The results revealed a dose-dependent reduction in blood pressure levels in rats treated with the extract, with a significant decrease observed in systolic and diastolic blood pressure compared to the control group. Furthermore, the extract exhibited no significant adverse effects on lipid profile or renal function, indicating its potential safety for therapeutic use. These findings suggest that the methanolic extract of *Operculina turpethum* Linn Silva Manso stem bark possesses promising anti-hypertensive properties, warranting further exploration of its therapeutic potential for the management of hypertension.

Key Words: *Operculina turpethum*, hypertension, methanolic extract, in-vivo study, anti-hypertensive activity, Wistar rats, blood pressure, lipid profile, renal function tests

INTRODUCTION:

Hypertension (HTN) is the most prevalent clinical symptom arising from various cardiovascular disorders. Likewise, it is considered a precursor or squeal to the development of acute coronary artery disease and congestive heath failure. Hypertension has been considered a cardinal criterion to determine cardiovascular function. It has been identified by WHO as one of the most significant risk factors for morbidity and mortality worldwide and is responsible for the deaths of approximately nine million people annually[1].

The treatment of hypertension mainly relies on synthetic medicines. Several drug classes have been used in the treatment of hypertension in the past forty years. These include diuretics, beta blockers (β-blockers), calcium channel blockers (CCB's) and more recently, angiotensin

converting enzyme (ACE) inhibitors and angiotensin II receptor blockers. The data from more than 20 randomized controlled trials demonstrate reductions in both mortality and morbidity with these drug classes[2].

The use of these synthetic medicines, however, has some negative effects. Most drugs used to treat hypertension have been evaluated for several specific patient populations; these include ACE inhibitors, β-blockers, CCB's and diuretics in patients with concomitant diabetes, nephropathy, coronary and cerebrovascular disease, heart failure, and left ventricular hypertrophy[3]. Side effects of these synthetic medicines have also been reported. For example, dry cough is a common side-effect of ACE inhibitors and is a major limiting factor of their use[8]. Secondly, despite the availability of useful non-drug therapy and potent medications, treatment is too often ineffective, mainly because of the patient's lack of compliance with therapeutic regimens[3]. Moreover, because of limited resources, synthetic drug treatment may not be affordable to the majority of hypertensive patients[4].

There are many herbal medicines traditionally used to treat hypertension in many countries. These herbal medicines are much easier and cheaper to obtain than synthetic medicines, and fewer side effects are reported.

A global perspective

Hypertension is becoming one of the most prevalent diseases all over the world. Elevation of both systolic and diastolic blood pressures is associated with increased risk of cardiovascular diseases, like left ventricle hypertrophy (LVH), myocardial infarction, heart failure, strokes, and so on. Blood pressure depends on the amount of blood that the heart pumps out with each contraction, as well as the ease with which this blood flows through even the smallest blood vessels. The narrower the blood vessel, the more difficult it is for the blood to flow through and the higher the blood pressure gets. A definition of hypertension should therefore include both diastolic and systolic blood pressure criteria. The World Health Organization (WHO) defines hypertension as the situation when arterial pressure is greater than 140/90 mmHg for an extended period[5].

Epidemiology:

Hypertension is a significant contributor to mortality in India. Hypertension exerts a substantial public health burden on cardiovascular health status and healthcare systems in India. HTN is directly responsible for 57% of all stroke deaths and 24% of all coronary heart disease deaths in India. In an analysis of worldwide data for the global burden of HTN, 20.6% of Indian men and 20.9% of Indian women were suffering from HTN in 2005. The rates for HTN in percentage are projected to go up to 22.9 and 23.6 for Indian men and women, respectively by 2025[6]. Recent studies from India have shown the prevalence of HTN to be 25% in urban and 10% in rural people in India. According to the WHO 2008 estimates, the prevalence of raised BP in Indians was 32.5% (33.2% in men and 31.7% in women). However, only about 25.6% of treated patients had their BP under control, in a multicenter study from India on awareness, treatment, and adequacy of control of Hypertension[6].

Etiology:

There are two main types of high blood pressure:

1) Primary hypertension (Essential hypertension)
2) Secondary hypertension (non-essential hypertension)

1) Primary hypertension: Also called Essential hypertension. This is the most common type of high blood pressure. For most adults, there's no identifiable cause of high blood pressure. It tends to develop gradually over many years. Plaque buildup in the arteries, called atherosclerosis, increases the risk of high blood pressure.

It is further divided into two types namely: Benign hypertension and malignant

hypertension.

Benign hypertension: Here, there is a moderate increase in blood pressure with systolic pressure of 200 mm Hg and the diastolic pressure of above 100 mm Hg.

Malignant hypertension: Here, the blood pressure elevated largely of about 250mmHg of systolic pressure and 150 mmHg of diastolic pressure. It produces severe symptoms like renal disease, retinal disease, and being a fatal disease, it causes death within few years.

Some of the characteristics of primary or essential hypertension are:

- The mean arterial pressure is increased 40-60%.
- The renal blood flow in the later stages decreased by about one half of normal.
- The resistance to blood flow through the kidney is increased 2-4-fold.
- The kidneys will not excrete adequate amounts of salt and water unless the arterial pressure is high.

2) Secondary hypertension:

This high blood pressure is caused by another medical condition or use of certain medicines. It tends to appear suddenly and cause higher blood pressure than does primary hypertension.

The different forms of secondary hypertension are:

- Cardiovascular hypertension
- Renal hypertension
- Endocrine hypertension
- Neurogenic hypertension

Figure: 1 Etiology of hypertension

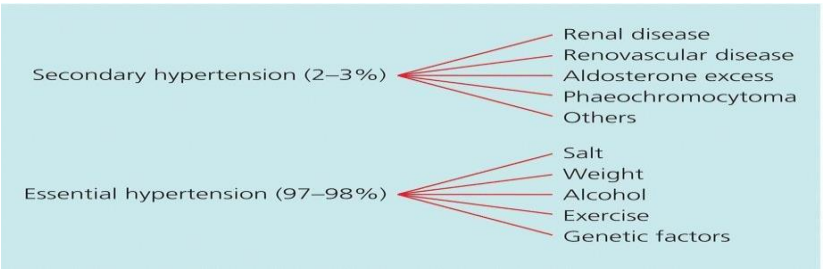


Table 1: Classification of blood pressure for adults aged 18 years and older:[7]

Category	Systolic Blood Pressure (mm Hg)		Diastolic Blood Pressure (mm Hg)
Normal	<120	and	<80
Prehypertension	120-139	or	80-89
Hypertension			
Stage 1	140-159	or	90-99
Stage 2	≥ 160	or	≥ 100

Table 2: Factors influencing prognosis of patients with hypertension[8].

Risk factors for cardiovascular disease	Target-organ damage (TOD)	Associated clinical conditions (ACC)
Levels of systolic and diastolic blood pressure (grades 1-3) Males > 55 years Females > 65 years Smoking	Left ventricular hypertrophy (electrocardiogram or echocardiogram)	Diabetes Cerebrovascular disease Ischemic stroke Cerebral

Total cholesterol >6.1 mmol/l (240 mg/dl) or LDL-cholesterol >4.0 mmol/l (160 mg/dl) HDL-cholesterol M <10 F <1.2 mmol/l (<40,45 mg/dl) History of cardiovascular disease in first-degree relatives before age 50 Obesity, physical inactivity	Micro albuminuria (20-30 mg/day) Radiological or ultrasound evidence of extensive atherosclerotic plaque (aorta, carotid, coronary, iliac and femoral arteries) Hypertensive retinopathy grade III or IV	hemorrhage Transient ischemic attack heart disease Myocardial infarction Angina Coronary revascularization Congestive heart failure Renal disease **Plasma creatinine concentration:** Females 1.5 mg/dl (120, 133 µ mol/l) Albuminuria >300mg/day Peripheral vascular disease

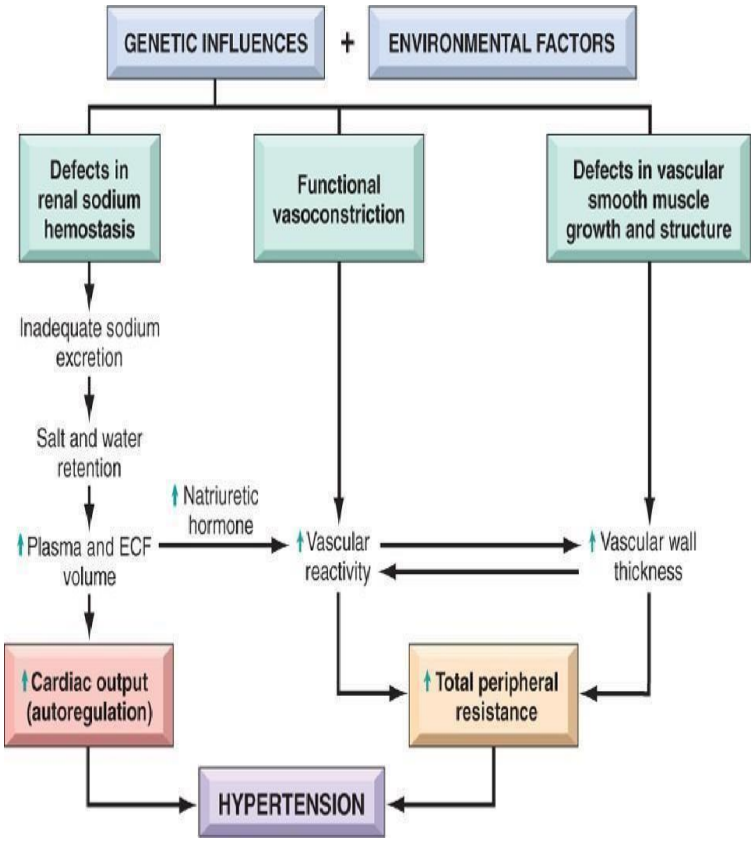


Fig 2: Pathophysiology of hypertension

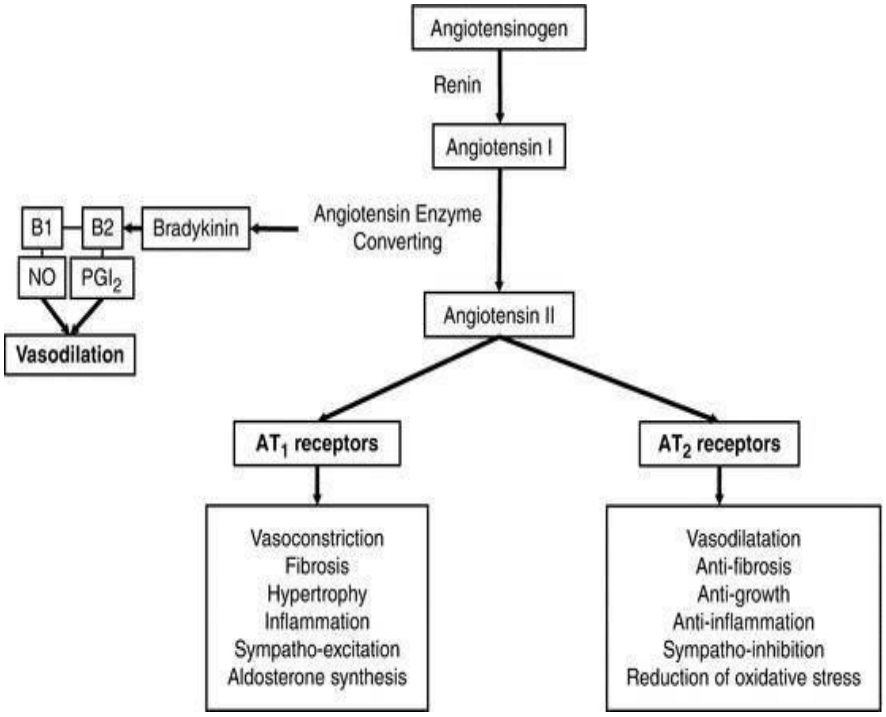


Fig 3: Physiology of hypertension

Complications of hypertension: [9]

- **Heart attack or stroke:** Hardening and thickening of the arteries due to high blood pressure or other factors can lead to a heart attack, stroke, or other complications.
- **Aneurysm:** Increased blood pressure can cause blood vessels to weaken and bulge, forming an aneurysm. If an aneurysm ruptures, it can be life-threatening.
- **Heart failure:** When you have high blood pressure, the heart has to work harder to pump blood. The strain causes the walls of the heart's pumping chamber to thicken. This condition is called left ventricular hypertrophy. Eventually, the heart can't pump enough blood to meet the body's needs, causing heart failure.
- **Kidney problems:** High blood pressure can cause the blood vessels in the kidneys to become narrow or weak. This can lead to kidney damage.
- **Eye problems:** Increased blood pressure can cause thickened, narrowed, or torn blood vessels in the eyes. This can result in vision loss.
- **Metabolic syndrome:** This syndrome is a group of disorders of the body's metabolism. It involves the irregular breakdown of sugar, also called glucose. The syndrome includes increased waist size, high triglycerides, decreased high-density lipoprotein (HDL or "good") cholesterol, high blood pressure and high blood sugar levels. These conditions make you more likely to develop diabetes, heart disease and stroke.

- **Changes with memory or understanding:** Uncontrolled high blood pressure may affect the ability to think, remember and learn.
- **Dementia:** Narrowed or blocked arteries can limit blood flow to the brain. This can cause a certain type of dementia called vascular dementia. A stroke that interrupts blood flow to the brain also can cause vascular dementia.
- **Renal complications:** Renal disease initially revealed by micro albuminemia may progress slowly and becomes evident in later years.
- **Diagnosis:**
  High blood pressure (hypertension) is diagnosed if the blood pressure reading is equal to or greater than 130/80 mm Hg. A diagnosis of high blood pressure is usually based on the average of two or more readings taken on separate occasions. Blood pressure is grouped according to how high it is. This is called staging. Staging helps guide treatment.[10]
  Stage 1 hypertension:
  The top number is between 130- and 139-mm Hg, or the bottom number is between 80- and 89-mm Hg.
  Stage 2 hypertension:
  The top number is 140 mm Hg or higher or the bottom number is 90 mm Hg or higher.
  Tests[11]
  - Ambulatory monitoring
  - Lab tests
  - Electrocardiogram (ECG or EKG)
  - Echocardiogram

Medical Plants with antihypertensive effects[12]

Traditional medical plants

A medicinal plant is any plant which provides health-promoting characteristics, temporary relief or symptomatic problems or has curative properties.

Medicinal plants have played a very important role since the existence of human beings. As early as 2800 B.C., the first independent system about Traditional Chinese Medicine (TCM) appeared on the earth, which is called "The Medical Classic of the Yellow Emperor"[9]. This book is written in the form of a dialogue in which the Yellow Emperor (the legendary first ancestor of the Chinese nation) discusses medicine with his ministers and some well-known doctors. No researchers of TCM in China could escape knowing about this text. Till now over 40% of medicines prescribed in the United States contain chemicals derived from plants (http://www.nps.gov). Furthermore, most developing countries are endowed with vast resources of medicinal and aromatic plants, much of which are still under-explored, especially in the rural areas.The scientific literature relating medicinal plants to blood pressure is extensive and can be datedback to more than 100 years ago. With the development of technology, people do not settle for taking infusions or decoctions orally anymore. They start to locate the effective components and look for the right doses. More and more traditionally used medicinal plants have been studied to investigate the principles or mechanisms of their blood pressure lowering effect.

Medicinal plants, including Olive, Camellia, Claviceps purpurea, Coffea arabica, Hawthorn, Linden blossom, Yarrow, Cramp bark, and Valerian (http://www.healthy.net), are a broad range of remedies that have the observed effect of lowering elevated blood pressure. They appear to work in a variety of ways, such as cardiac tonics, diuretics, nervines and anti-spasmodic.

In South Africa, traditional medicine is an integral part of cultural life. It is estimated that between 12 and 15 million South Africans still depend on traditional herbal medicine from as many as 700 indigenous plant species.

The traditional use of wild olive in South Africa has lasted for hundreds of years[12]. The dried

leaves are most often used, followed by the roots or the stem bark. The scientific study seems to start from 1960's, which determined the main use of this plant as a hypotensive to lower blood pressure and to enhance renal function. Researchers screened 20 Zulu medicinal plants and indicated that 6 of them have high level of ACE inhibition activities, including Adenopodia spicata, Diets iridoids, Mesembryanthemum, etc.

Advantages and disadvantages of medicinal plant medicine[13]

Traditional medicinal plants are potential sources of new drugs, sources of cheap starting materials for synthesis of known drugs. It is claimed that the human body better accepts drugs derived from natural sources than synthetic substances invented in the laboratory. Furthermore, traditional plant medicines have fewer side effects when compared with synthetic products.

Traditional plant medicines also have some disadvantages. Plants from different geographic areas may have biologic diversity. Secondly, for most traditional medicines, the specific constituents that cause a therapeutic effect are often not known. There exist many different constituents in the plant, and it is likely that they work together to produce the desired therapeutic effect. The exact combination of active ingredients is often the crucial problem for traditional plant medicine development. Olives have traditionally been used for hundreds of years and more and more research proved its lowering blood pressure effect, it should get our more attention on the mechanism and comparative effects with classic synthetic medicines.

1.6 Animal models of hypertension:[14]

Recent research during this period has also added considerably to our knowledge of the mechanisms involved in the pathogenesis of hypertension due to use of animal models of hypertension shares many features which are common to human hypertension. Excessive salt intake, hyperactivity of renin angiotensin-aldosterone system (RAAS) and genetic factors are some of the etiological factors that are presumed to be responsible for human hypertension.

An ideal animal model of hypertension should fulfill the following criteria:

- It should be feasible in small animals.
- It should be simple to perform and uniformly reproducible.
- It should be able to predict the potential antihypertensive properties of an agent.
- It should consume minimal quantities of compounds.
- It should be comparable to some form of human hypertension.

Rat models of hypertensions[15]

Type of hypertension/ Syndrome	**Model**
Systemic	Spontaneously Hypertensive Rats (SHRs)
	Stroke-prone SHR (SHR-SP)
	Mineralocorticoids (DOCA–salt)
	NO synthase inhibition (L-NAME administration)
	Transgenics (TGR (mREN2)27rats)
	Diabetic hypertensive rats (STZ-SHR, Zucker)
Renal	Renal artery occlusion (1K1C, 2K1C)
Pulmonary	Hypoxia (normobaric, hypobaric)
	Monocrotaline

Rat models of hypertensions Measurement of BP in animal models:

Repeated measurement of BP is needed in experiments on animal models of hypertension. The BP in the following animal models is measure either directly (intravascular) or indirectly (bloodless).

Direct methods:

a) A week before the experiment, each rat is anaesthetized with 40 mg/kg pentobarbital. Left or right carotid artery or femoral artery (for recording BP) is cannulated under aseptic conditions with polyethylene cannula filled with 1% heparin in normal saline[18]. Free end of the cannula is passed under the skin and allowed to protrude 3-4 cm from the skin behind the ears of the rat. The skin incisions are sutured, and a plastic skin dressing is applied. After recovery from anesthesia (2-2.5h) each rat is placed in an individual cage for 24h habituation period[19].

On the day of the experiment, a pressure tube filled with 200 U/ml heparin in saline is tied to the implanted catheter and connected to a pressure transducer and then to the pre-amplifier and recorded on the polygraph or physiography[16, 18].Alternatively, Condon ‘s mercury manometer can also be used for recording BP in rats[17].Popovic *et al* (1960) used animals for 40 days before cannula was blocked[18].

Indirect methods

a) Tail cuff
b) Tail swelling
c) Foot swelling

Bhansali, et al (2014) determined the prevalence of hypertension (HTN) and its risk factors in urban and rural India. In Phase I of the Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study, individuals aged ⩾20 years were surveyed using a stratified multistage sampling design, in three states (Tamil Nadu, Maharashtra and Jharkhand) and one union territory (Chandigarh) of India. The overall age-standardized prevalence of HTN was 26.3% (self-reported: 5.5%; newly detected: 20.8%). Urban residents of Tamil Nadu, Jharkhand, Chandigarh, and Maharashtra (31.5, 28.9, 30.7 and 28.1%) had significantly higher prevalence of HTN compared with rural residents (26.2, 21.7, 19.8 and 24.0%, respectively). They concluded that the prevalence of undiagnosed HTN is high in India and this calls for regular screening[20].

Gupta *et al*., (2015) studied urban-rural convergence of hypertension. Result showed that prevalence of hypertension in urban locations has stabilized to about 25-30% but it has increased in rural populations from 15 to 25%. This urban-rural convergence of hypertension in India is due to rapid urbanization of rural populations with consequent changes in lifestyles (sedentariness, high dietary salt, sugar and fat intake) and increase in overweight and obesity[21].

MATERIALS AND METHODS

Drugs and chemicals:

Serotonin hydrochloride (5-HT), meta-chlorophenyl piperazine (m-CPP; 5-HT2A/2B/2C agonist), Phenyl biguanide (PBG; 5-HT3 agonist), Ketanserin (5-HT2A antagonist), Noradrenaline (NA), Adrenaline (Adr), Phenylephrine (PhE), Acetylcholine (ACh), Urethane and were purchased from Sigma (Sigma Chemicals, Hyderabad). Fructose was purchased from SD Fine Chemicals. DOCA was dispersed in cottonseed oil. Diazepam (GABA agonist) (Calmpose, Ranbaxy Laboratories, India), Ondansetron (5-HT3 antagonist) (Emset, Cipla, India), Haloperidol (D2 antagonist) (Searle, India), Lithium Sulphate (Glenmark, India) and Pentobarbital (GABA agonist) were used in the study. Pet ether (60°- 80° C), Ethyl acetate Methanol, n-butanol, and toluene were purchased from Modem Scientific, Nashik. The EAF of

fenugreek seeds was suspended in PEG-400 (just sufficient to dissolve- not exceeding 0.005% of the total volume).

Collection of plant material

The fresh stem bark of Operculina turpethum (L.) was collected from, Talakona hills, Thirupathi, Andhra Pradesh, India in the month of February 2023. The plant material was identified and authenticated by Dr.K. Madhav Cheety, Plant taxonomist (IAAT:337), Assistant professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P, India, and voucher number (0887) were deposited at S.V. University, Thirupathi, Andhra Pradesh, India for future reference.

Fig 4: Powdered materials of *Operculina turpethum* stem bark before extraction.

Preparation of extract

Take one kg stem bark of *Operculina turpethum* (L.) was crushed into a coarse powder using for extraction. The extraction was carried out by using a cold maceration method. Methanol is used as a solvent. The plant material (150 g) was first macerated with hexane and then the residue was macerated with chloroform and 70% methanol successively for 48 hrs. with intermittent shaking every 3 hrs. The respective extracts were filtered with Whatmann No. 1 filter paper and were dried in a hot air oven at 40°C. The *O. turpethum* extract (OTE) was stored in a refrigerator at 4°C until time of use[22].

Fig 5: Extraction of *Operculina turpethum*

Preliminary phytochemical screening

Qualitative phytochemical analysis Preliminary chemical tests were carried out for crude powder and methanolic extract to identify different phyto-constituents[23, 24].

Alkaloids

The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was dissolved

in 2 N HCl. The mixture was filtered, and the filtrate was divided into 3 equal portions. One portion was treated with few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent, and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicated the presence of respective alkaloids. A (+) score was recorded if the reagent produced only a slight opaqueness; a (++) score was recorded if a definite turbidity, but no flocculation was observed, and a (+++) score was recorded if heavy precipitate or flocculation was observed[25].

Flavonoids Shinoda test

The presence of flavonoids was estimated by Shinoda test. The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was treated with a few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red color within few minutes indicated the presence of flavonoids.

Alkaline reagent test

The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was treated with a few drops of diluted sodium hydroxide (NaOH) separately. The formation of intense yellow color which turned colorless on addition of few drops of diluted HCl indicated presence of flavonoids.

Cardiac glycosides

The Keller-kiliani test was performed for the presence of cardiac glycosides. The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was treated with 1 ml mixture of 5% FeCl3 and glacial acetic acid (1:99 v/v). To this solution, a few drops of concentrated H2SO4 were added. Appearance of greenish blue color within few minutes indicated the presence of cardiac glycosides[26].

Phlobatannins

The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was boiled with 1% aqueous HCl. Deposition of red precipitate was taken as evidence for the presence of phlobatannins[27].

Saponins

The presence of saponins was determined by the Frothing test. The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: no froth indicates absence of saponins and stable froth for more than 1.5 cm indicated the presence of saponins[28].

Steroids

Liebermann-Burchard reaction was performed for the presence of steroids. A chloroform solution of the crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was treated with acetic anhydride and a few drops of concentrated H_2SO_4 were added down the sides of test tube. A blue green ring indicated the presence of steroids.

Tannins

The crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was treated with alcoholic ferric chloride (FeCl3) reagent. Blue color indicated the presence of tannins[29.]

Triterpenes

Chloroform extract of the crude powder and methanol extract of *Operculina turpethum* (L.) stem bark was treated with concentrated sulphuric acid (H_2SO_4). The appearance of reddish-brown ring indicated the presence of triterpenes[30].

Experimental design

Experimental Protocol *(In vivo* method) Fructose Induced hypertension.

Animals

Male albino rats (Wistar strain) weighing between 150 and 200 g were obtained from local

breeders. Animals were housed into groups of five under standard laboratory conditions of temperature 25°C±1°C with free access to food (Hindustan Lever, India) and water. The experiments were performed during the light portion (9–14 h). The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India, and approved by the Institutional Animal Ethical Committee with CPCSEA Reg No:1636/PO/Re/S/12/CPCSEA/2023/020. All animal management and procedures were performed in accordance with recommended guidelines.

Fig 6: Male Wistar albino rats

Acute oral toxicity (AOT) studies

Toxicity test studies conducted as per internationally accepted protocol drawn under OECD guidelines. Healthy male Wister rats (200-250g) were subjected to AOT studies as per Organization for Economic Co-operation and Development (OECD) guidelines 2001 (AOT-423). Then the extracts (stem and roots) were administered orally at the dose of 2000 mg/kg by intragastric tube. Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, behavior pattern and mortality were noted[30].

Grouping of animals:

Male Wistar rats (200-250 g) were randomized and divided into following groups of 5-6 animals each.

Group 1: Control: Animals received no medication but were given distilled water for drinking.

Group 2: F-10: Animals received 10% fructose solution instead of drinking water, *ad libitum* for 21 days.

Group 3: F-10 +CAP-20: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with Captopril (20 mg/kg/day, p.o.) for 21 days.

Group 4: F-10+ME-100: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with Methanolic extraction of *O. turpethum* (ME) (100 mg/kg/day, p.o.) for 21 days.

Group 5: F-10+ME300: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with Methanolic extraction of *O. turpethum* (ME) (300 mg/kg/day, p.o.) for 21 days Hypertension was induced experimentally in male Wistar rats (200-250 gm) by giving 10% fructose solution to drink *ad libitum* for five to six weeks. Fructose solution was prepared every two days by dissolving the fructose in distilled water. Ordinary tap water was given to control animals to drink throughout the whole experimental period. Consumption of fructose leads to development of hypertension through activation of the sympathetic nervous system, increased salt r**e**tention and enhanced renin-angiotensin.

Principle of recording B.P:

The animal was placed in the NIBP restrainer and an appropriate cuff with sensor was then mounted on its tail and warmed to about 33-35 °C. The tail cuff was inflated to a pressure well above the expected systolic blood pressure i.e. 250 mm Hg and slowly released during which

the pulse was recorded by using Power Lab data acquisition system and computer. Systolic blood pressures (SBP), diastolic and mean arterial pressure (MAP) were measured for each rat. Mean arterial pressure is the average pressure in arteries during one cardiac cycle and it is considered as a better indicator to perfusion of coronary arteries, brain, and kidneys[31].

Mean arterial pressure can be calculated by MAP = SBP+2(DBP)

The animals SBP, DBP and MAP were measured using the tail cuff method by using noninvasive BP (NIBP) system for rodents on day 0 d 7th, 14th, 21st days.

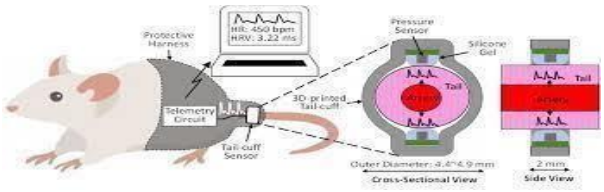


Fig 7: A Miniature of non-invasive tail cuff method for measurement of B.P

RESULTS:

Extraction of plant material:

The powdered material of *Operculina turpethum* was subjected to cold maceration method using methanol as solvent.

Table 3: Color, consistence and yield of the plant extract:

Plant	Color	Consistency	Yield
Operculina turpethum	**Brown**	**Oily**	**7.33%**

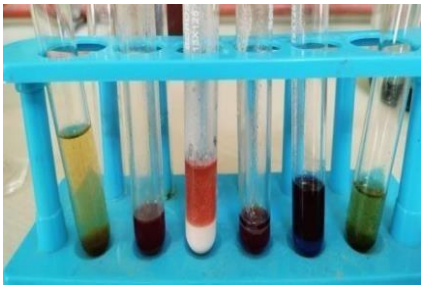
Preliminary phytochemical studies for *Operculina turpethum Linn Silva Manso*

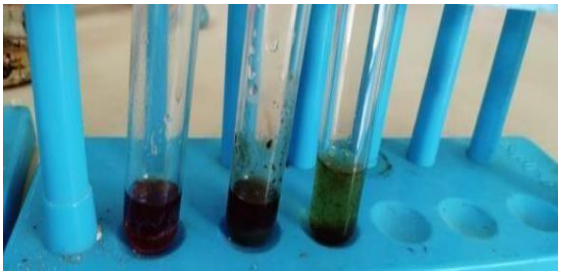
Preliminary phytochemical evaluation revealed that *Operculina turpethum Linn Silva Manso* extracts contain carbohydrates, proteins and amino acids, alkaloids, phenolic compounds and tannins, flavonoids, and glycosides

Table 4: Phytochemical screening for *Operculina turpethum Linn Silva Manso*

Note: The symbol (+) denotes presence and (-) denotes absence of phytoconstituents

S. No	Test	Result
1	Carbohydrates	+
2	Proteins and amino acids	+
3	Glycosides	+
4	Saponins	+
5	Flavonoids	+
6	Alkaloids	+
7	Phenols	-
8	Fixed oils and sterols	-
9	Terpenoids	-





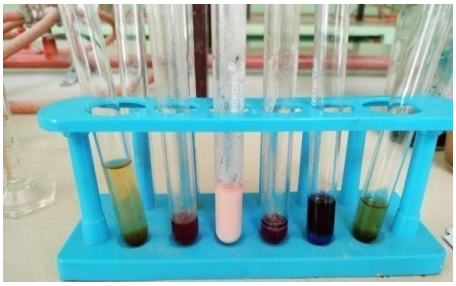


Fig 8: Tests for Carbohydrates

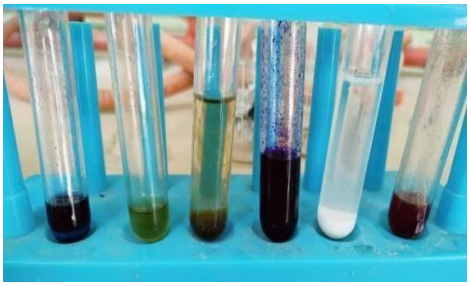


Fig 9: Tests for alkaloids

Fig 10: Tests for Saponins

Fig 11: Tests for Glycosides

Fig 12: Tests for Flavonoids

Fig 13: Tests for Phenols

Acute oral toxicity studies

Extract of *Operculina turpethum Linn Silva Manso* were non-toxic when subjected to acute toxicity study at a dose of 2000 mg/kg. No characteristic behavioral, autonomic, and neurological effects were observed; except moderate diarrhea in *Operculina turpethum Linn Silva Manso*100mg/kg and 300mg/kg were selected as dose of extract for entire study for plant.

Table 5: Acute oral toxicity *Operculina turpethum Linn Silva Manso*

Treatment	Dose(mg/kg)	Number of animals	Mortality			Toxicity Profile
			After 24hrs	After 7days	After 14 days	
O. turpethum extract	2000	5	0	0	0	Safe

Table 6: Acute toxicity parameters observed

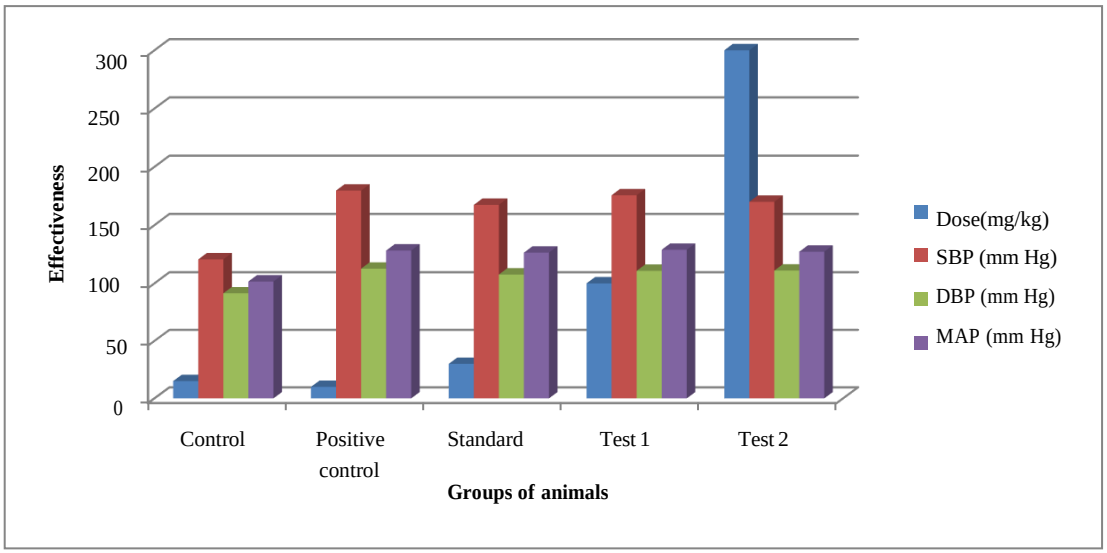
Parameter observed	Treatment	Response of O. turpethum
Behavioural Response	Alertness	N
	Stereo type	N
	Irritability	-
	Fearfulness	-
	Touch Response	N
	Pain Response	N
	Spontaneous action	N
	Grooming	-
	Restlessness	-
Neurological Response	Righting reflex	N
	Limb Tone	N
	Twitching	-
	Abdominal tone	N
	Pinna reflex	N
	Corneal reflex	N
	Straub tail	-
	Tremors	-
	Convulsions	-
Autonomic Response	Writhing	N
	Defecation	+
	Urination	+
	Piloerection	-
	Heart rate	N
	Respiration	N
	Pupil size	N
	Skin color	N

Fructose induced hypertension:

The methanolic extract of *Operculina turpethum* exhibited a significant ($p<0.05$) decrease in SBP, DBP and MAP antihypertensive effect in 0 d, 7th day at the dose of 100 and 300 mg/kg when compared with fructose induced hypertensive control groups (table 1 and table 2). On 14thday the test extracts at the doses of 300 mg/kg significantly reduces only DBP and MAP ($p<0.05$) and on 21st day treatment only decrease in DBP was recorded however the treatment is continued but no significant activity observed for SBP and MAP in the hypertensive rats. However antihypertensive activity is continued for 14th and 21st days and found that test extracts decreased all the parameters in dose dependent manner in the hypertensive control group.

Table 7: Effect of *Operculina turpethum* on blood pressure in fructose induced hypertensive rats on 0 day.

Groups	Treatment	Dose (mg/kg)	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)
1	Control	15	120.6±1.53	91.4±1.50	101.6±1.02
2	Positive control (10% fructose)	10	179.6±1.20	112.6±1.02	128.2±0.66
3	(10% fructose + Captopril)	10+20	167.4±1.20	107.6±1.12	126.4±0.74
4	10% Fructose 10 + Methanolic extract of *O. turpethum*	100	175.6±0.97*	110.8±1.15*	128.8±0.96*
5	10% Fructose + Methanolic extract of *O. turpethum*	300	170±0.83*	111±1.22*	127.2±0.86*



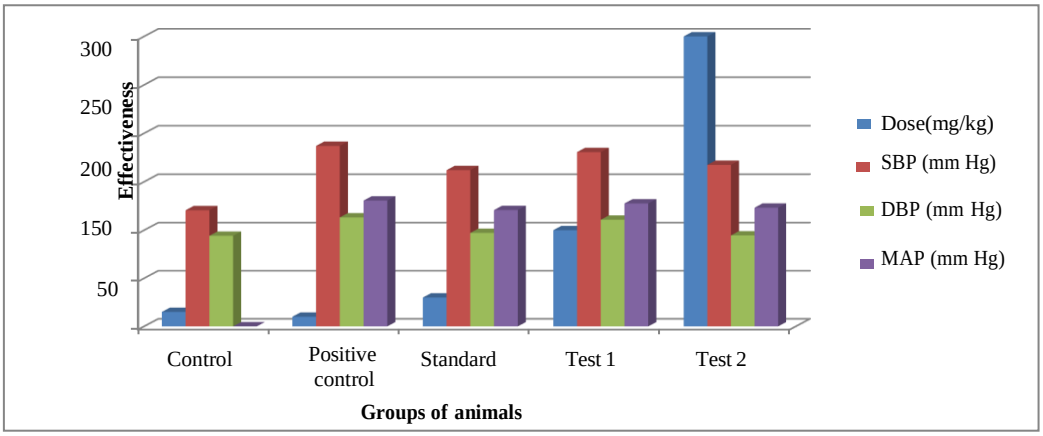
Graph 1: SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean Arterial blood pressure.

Data are expressed as Mean±SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control, *P<0.05 significant.

Table 8: Effect of *Operculina turpethum* on blood pressure in fructose induced hypertensive rats on 7th day.

Groups	Treatment	Dose(mg/kg)	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)
1	Control	15	120.6±0.81	94.2±1.35	105.2±1.71
2	Positive control (10% fructose)	10%	187±0.89	113.2±1.01	130.6±0.97
3	(10 % fructose + Captopril)	10+20	162±1.14	97.2±1.01	120.8±0.96

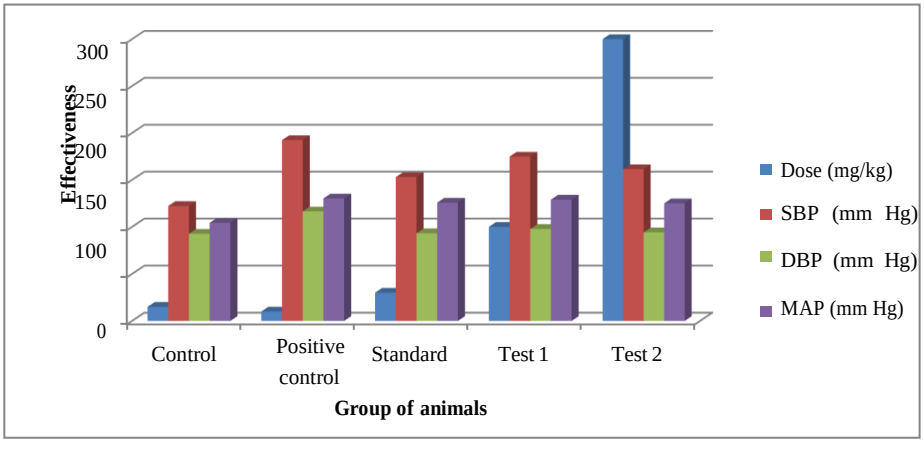
4	10% Fructose + Methanolic extract of *O. turpethum*	100	180.6±0.81*	110.8±0.96*	127.6±0.81*
5	10% Fructose + Methanolic extract of *O. turpethum*	300	167.4±0.87*	94.6±1.02*	123.2±1.01*



Graph 2: Effect of *Operculina turpethum* on fructose induced hypertensive rats taking groups of animals against effectiveness of the extract. Data are expressed as mean ± SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control, *P<0.05 significant.

Table 9: Effect of *Operculina turpethum* on blood pressure in fructose induced hypertensive rats on 14th day.

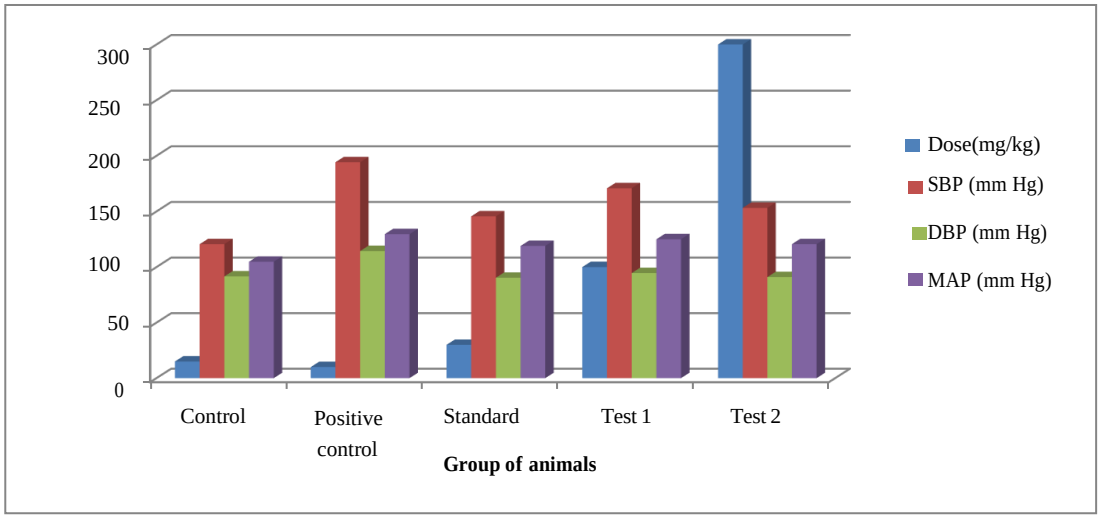
Groups	Treatment	Dose(mg/kg)	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)
1	Control	15	122.2±1.49	92.6±1.16	104.2±1.28
2	Positive control (10 % fructose)	10	192.4±0.97	116.4±1.12	130.2±1.11
3	(10 % fructose+ captopril)	10+20	153±1.14	93.2±0.73	125.8±0.66
4	10 %Fructose 10 + Methanolic extract of *O. turpethum*	100	174.8±1.06	97.8±0.66*	129±0.99*
5	10 % Fructose + Methanolic extract of *O. turpethum*	300	161.4±0.97	94.2±0.66*	125±0.70*



Graph 3: Effect of *Operculina turpethum* on fructose induced hypertensive rats taking groups of animals against effectiveness of the extract. Data are expressed as mean ± SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal, control, *P<0.05 significant.

Table 10: Effect of *Operculina turpethum* on blood pressure in fructose induced hypertensive rats on 21st day.

Groups	Treatment	Dose(mg/kg)	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)
1	Control	15	120.6±1.88	91.6±1.20	104.8±0.96
2	Positive control (10% fructose)	10	194.2±0.66	114.4±1.16	129.6±0.50
3	(10% fructose+ captopril)	10+20	145.6±0.81	90.4±1.20	119±0.83
4	10% Fructose10 + Methanolic extract of *O. turpethum*	100	170.8±1.15	94.6±0.74*	125±0.89
5	10% Fructose + Methanolic extract of *O. turpethum*	300	153.2±1.01	91±0.99*	120.6±1.16



Graph 4: Effect of *Operculina turpethum* on fructose induced hypertensive rats taking groups of animals against effectiveness of the extract. Data are expressed as mean ± SEM; n=6. One way ANOVA followed by Tukey's multiple comparison test when compared with normal control. *P<0.05 significant.

DISCUSSION

Hypertension is the most prevalent clinical symptom arising from various cardiovascular disorders. Likewise, it is considered a precursor or sequelae to the development of acute coronary artery disease and congestive heath failure. Hypertension has been considered a cardinal criterion to determine cardiovascular function. It has been identified by WHO as one of the most significant risk factors for morbidity and mortality worldwide and is responsible for the deaths of approximately nine million people annually. The treatment of hypertension mainly relies on synthetic medicines. Several drug classes have been used in the treatment of hypertension in the past forty years. These include diuretics, beta blockers (β-blockers), calcium channel blockers (CCB's) and more recently, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers.

The present study evaluated the anti-hypertensive activity of *Operculina turpethum* by using methanolic extract in fructose induced hypertension rats. The hypertension was induced by using fructose induced hypertension model in male Wister rats of weight 200-250g.

The plant was powdered and subjected to cold maceration process with hexane and methanol. The derived plant extract was stored at 4^0c for further use. Then plant extract was subjected to conduct phytochemical screening for the screening of phytochemical constituents for the presence of Carbohydrates, proteins, alkaloids, Saponins, terpenoids and glycosides. All the tests show possible outcomes and results are recorded.

Acute oral toxicity studies are conducted according to OECD guidelines 2001 (AOT-423). Extract of *Operculina turpethum Linn Silva Manso* were non-toxic when subjected to acute toxicity study at a dose of 2000 mg/kg. No characteristic behavioral, autonomic, and neurological effects were observed; except moderate diarrhea in *Operculina turpethum Linn Silva Manso* 100mg/kg and 300mg/kg were selected as dose of extract for entire study for plant.

The methanolic extract of *Operculina turpethum* showed a significantly ($P<0.05$) decrease in the SBP, DBP and MAP in fructose induced hypertensive rats at the doses of 200 mg/kg and 400 mg/kg in 0 and 7 th day of treatment. However antihypertensive activity is continued for 14^{th} and 21^{st} days and found that test extracts decreased all the parameters in dose dependent manner in the hypertensive control group.

The decrease in blood pressure by an ethanolic and aqueous extract of *Operculina turpethum* in hypertensive rats could be linked to several mechanisms. Previous experimental studies demonstrated that glucose and fructose contribute to the increase in blood pressure. It has been clearly stated that one of the reasons for glucose-induced hypertension is an increase in sympathetic activity. Increase in sympathetic activity by any mean usually contributes to increase in heart rate and blood pressure. In the present investigation, the extracts tested were found to significantly show hypotensive effect could be a strong reason of its antihypertensive effect in hypertensive rats. Endothelial dysfunction and oxidative stress are the important factors which favors hypertension. It is also well-known fact that high quantity of sugar consumption is associated to increased tissue production of reactive forms of oxygen. Moreover, in hypertensive patients, lower concentration of antioxidants have been documented. Furthermore, an increased blood glucose level has also been involved in a reduction in nitric oxide levels ultimately resulting in an increased hypertension.

CONCLUSION

The outcome of this study provides evidence that the methanolic extract of *Operculina turpethum* most likely contains certain active principles which exert an antihypertensive effect in fructose induced rats. Moreover, the present investigation shows that *Operculina turpethum* is safe for use. The anti-hypertensive activity of methanolic extract Operculina turpethum may be due to presence of Turpethosides A, B, C. which may alter the RAAS system. The exact

mechanism of action is unknown. However, more research is needed to isolate the phytoconstituents, identify the lead molecule and validate its exact mechanism of antihypertensive effect.

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