Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Evaluation of Antihypertensive Activity of Methanolic Leaf Extract of *Adina cordifolia*using a Fructose-Induced Hypertensive Rat Model

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Conflict of interest
The authors declare that they have no conflict of interest.

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Abstract
A common cardiovascular condition, hypertension remains a major global public health concern. Using an in-vivo method, this study sought to assess the methanolic extract made from Adina cordifolia leaves' possible anti-hypertensive properties. Male Wistar rats were split up into groups and given varied amounts of the methanolic extract. The non-invasive tail-cuff method was used to test blood pressure at regular intervals after extract administration. Biochemical measures such as lipid profiles and kidney function tests were also evaluated to determine the extract's safety and effectiveness. Rats given the extract showed a dose-dependent drop in blood pressure, with the systolic and diastolic blood pressures much lower than those of the control group. Additionally, the extract showed no discernible negative effects on renal function or lipid profile, suggesting it may be safe for therapeutic use. These results imply that Adina cordifolia Linn's methanolic extract has encouraging anti-hypertensive qualities, indicating the need for additional research into its potential as a treatment for hypertension.

Key Words:*Adina cordifolia***,** hypertension, methanolic extract, in-vivo study, anti-hypertensive activity, Wistar rats, blood pressure.

1. INTRODUCTION

Hypertension, a menace to the cardiovascular system, is depicted by prolonged blood pressure higher than the generally approved normal maximum level for a particular age group (Solomon and Emelike, 2014). It is globally seen as a major cause of death among adults due to associated complications including coronary heart disease, stroke, sudden cardiac arrest, congestive heart disease, renal insufficiency, and aneurysm, among others (Etuk, 2006).

In recent times, hypertension and other related cardiovascular diseases have become a major public health concern in both developed and developing countries; because it is a silent killer and it has no early significant symptoms. The prevalence of the disease and its related conditions has been identified to be emerging in developing countries as recorded for developed countries (Grosskurt, 2019). This is due to an increase in contributing factors of lifestyle and genetic traits such as obesity, unhealthy diet intake, lack of physical activity, and increased alcohol intake (Yoshihiro *et al*., 2019).

The World Health Organization (WHO) reported that hypertension is a condition that causes 7.1 million early deaths and 4.5% of the infection load yearly around the globe (Peprah *et al*., 2016).

Studies on hypertension in globally have indicated a raw prevalence between 25% and 48%, using the threshold of 140/90 mmHg with the prevalence higher in urban areas than in rural areas; with the rural areas accounting for a prevalence of 35% (Bosu, 2010). In the Greater Accra Region, it was noticed that hypertension has moved from fourth to second as the leading cause of outpatient morbidity in 2007 (Bosu, 2010). Thus, the need to control hypertension becomes very imperative, as its complications are associated with high mortality and morbidity rates (WHO, 2002). Providentially, hypertension can be detected easily for treatment to be undertaken.

The treatment of hypertension primarily depends on synthetic drugs and lifestyle modifications. Few medication classes have been exploited in the treatment of hypertension in the previous forty years. These involve diuretics, beta-blockers, calcium channel blockers (CCBs), and more recently, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers. There have been results from more than 20 assigned controlled ordeal proving reductions in both mortality and morbidity with these medication classes (Neal *et al*., 2000). Even though these synthetic drugs help treat hypertension, they have associated side effects which have also been reported. For instance, dry cough is a common side-effect of ACE inhibitors and a major limiting factor of their use (Sara *et al*., 2015). Again, despite the availability of synthetic drugs, treatment is often ineffective, mainly as a consequence of the patient's lack of compliance with the medicinal administration (WHO, 2003). Moreover, because of the limited resources majority of the hypertensive patients are unable to afford synthetic drugs due to higher costs.

WHO encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of how easier and cheaper way to obtain them, than the synthetic medicines and their fewer or no-side effects reported (Maiha *et al*., 2009).

Types of hypertensions

Hypertension is generally divided into two main categories including; primary (essential) hypertension and secondary (non-essential) hypertension.

Primary hypertension

The most prevalent form of hypertension affects 90 to 95% of patients diagnosed with hypertension and does not have an established etiology that is identifiable. It differs mainly from secondary hypertension, where elevation of blood pressure occurs due to another known cause (Eckman and Kirk, 2013). The specific cause of primary hypertension explanation is unknown and yet no specific cause has been identified. However, this phenomenon could be aided by several genetic and environmental or lifestyle factors. The pathogenesis of genetic and environmental or lifestyle factors is believed to be (Fagard, 2005). In addition, environmental and behavioral factors have been reported to explain high blood pressure involving elevated sodium, alcohol, and caloric intake, stress, and physical inactivity (Adeniyi, 2012). Primary hypertension has no definite cause or specific etiology that distinguishes it from secondary hypertension.

Secondary hypertension

The other 5-10% of cases of hypertension are listed as secondary hypertension (Eckman and Kirk, 2013). The cause may be a particular pathophysiology or condition resulting in hypertension, or the development of high blood pressure may result from the ingestion of certain drugs, food, or chemicals (Eckman and Kirk, 2013).

Stages of hypertension

Table 1 Stages of hypertension (Source: Whelton *et al*., 2018)

Category	Systolic		Diastolic
Normal	<120	And	<30

Prehypertension	120-139	Or	80-89
High Blood Pressure/Hypertension			
Stage 1 Hypertension	140-159	Or	90-99
Stage 2 Hypertension	>160	Or	>100

Causes of hypertension

The underlying cause of hypertension is often unknown but mostly it is an effect of a health condition or medication previously diagnosed. Chronic kidney disease is an underlying condition causing high blood pressure in most cases (Jha *et al*., 2013). The kidneys cannot absorb liquids under these conditions, resulting in excess fluids in the body. Then the blood pressure is raised (Jha *et al*., 2013).

The use of alcohol and tobacco is a significant factor that contributes to the risks of hypertension. Large amounts of alcohol consumption and tobacco smoking frequently raise an individual's blood pressure (Ohayon *et al.,* 2000).

Other risk factors include age. Hypertension is found to be widespread in individuals over 60 years of age. When age increases, the elasticity of the blood vessels decreases and becomes steeper. There is also an accumulation of plaques over time, resulting in the narrowing of the blood vessels. Weight and size also play a key role in increasing hypertension chances. Excess weight gain, particularly in the case of increased visceral adiposity, is a major cause of hypertension, accounting for 65% to 75% of the risk of primary (essential) hypertension in humans (John *et al.,* 2015). People who are overweight or obese are susceptible to hypertension. Hypertension contributes to other factors such as physical inactivity and improper diet (Neter *et al.,* 2003).

Factors affecting the regulation of blood pressure

High blood pressure is a mechanism caused by environmental or genetic factors, either in adults or young people. Systolic blood pressure is the pressure at which the heart pumps oxygenated blood to the entire system, and diastolic blood pressure is the pressure at which the natural cycle of filling the heart with blood. When blood pressure rises or falls, the body system controls the impact to restore it to its normal state.Increased blood pressure may be caused by multiple factors such as increased vascular resistance and initial volume increase. Neurogenic and humor stimuli stimulate blood vasoconstriction and cause retention of renal volume, resulting in increased cardiac output, blood flow of tissue, and vascular resistance, resulting in increased blood pressure. Increasing blood volume also helps in blood pressure vascular resistance (Navar *et al*., 1997).

Renin Angiotensin Aldosterone System (RAAS)

Renin Angiotensin Aldosterone System (RAAS) is part of a complex feedback system that plays a key role in maintaining mammalian homeostasis of blood pressure, fluid, and salt balance (McAlister *et al*., 2001; Lee *et al*.,2010). It is known as a hormonal regulator of blood pressure along with sodium, potassium, and water balance, as it requires both short-term and long-term control of blood pressure (Sharifi *et al*., 2004). The growing operation of the RAAS hormonal system has been related to the development and maintenance of hypertension pharmacological treatment involving the RAAS (Igic *et al*., 2007; Starr *et al*., 1994). The dysfunction of RAAS can affect heart, blood vessel, and kidney functions as it is an essential regulator of blood pressure and electrolyte balance (Igic *et al.,* 2007).

In the regulating system, the kidney secretes renin that acts on a bloodstream protein (angiotensinogen) to form angiotensin I, a relatively inactive substance that can be transformed into the most powerful blood vessel constrictor, angiotensin II (Hock *et al*., 1995). Angiotensin II (angiotensin II-octapeptide) is formed by Kinase II, an angiotensin-converting enzyme (ACE) following the removal of dipeptide Hos-Leu from angiotensin I in the lung. Angiotensin II is then transported in circulation to different target sites of the effectors, which are the blood vessels, kidneys, and the adrenal gland, to interact with particular receptors to exercise its function. Angiotensin II interacts with the receptors of

angiotensin I (AT 1) to increase the synthesis and release of aldosterone, which in effect works primarily on proximal nephrotic tubules to excrete potassium and hydrogen into the tubule and increase sodium reabsorption (Skotnicka, 2003).

Adrenal Steroids

Blood pressure is increased by minerals and glucocorticoids. Retention of sodium and water (mineralocorticoids) or increased vascular reactivity (glucocorticoids) mediates this effect. Therefore, glucocorticoids and mineralocorticoids improve the vascular tone by increasing the receptors of pressure hormones such as angiotensin I (Foëx & Sear, 2004).

Sodium and Water Excretion

Retention of sodium and water is associated with higher blood pressure. It is assumed that sodium causes an increase in intracellular calcium in the vascular smooth muscle, resulting in increased vascular tone, through the sodium calcium exchange mechanism. The primary cause of sodium and water retention can be an abnormal relationship between pressure and sodium excretion due to reduced renal blood flow, reduced nephron mass, and increased angiotensin or mineralocorticoids (Foëx & Sear, 2004).

Endothelial Mechanisms

Nitric oxide (NO) mediates the vasodilation of acetylcholine, bradykinin, nitroprusside sodium, and nitrates. Endothelial-derived relaxation is impaired in hypertensive patients. The endothelium synthesizes the strongest vasoconstrictor endothelin. Endothelin-1 generation or tolerance in hypertensive subjects is no greater than in normotensive subjects. Nonetheless, endothelin-1's deleterious vascular effects may be accentuated by reduced nitric oxide generation caused by hypertensive endothelial malfunctions (Foëx & Sear, 2004).

Autonomic Nervous System

Accordingly, the autonomic nervous system plays an important role in maintaining normal blood pressure, including physiological responses to posture changes and physical and emotional activity (Beevers *et al*., 2007). Sympathetic nervous system stimulation can cause arteriolar constriction and dilation of the arteriolar system. Such changes mediate short-term channels after stress and physical exercise in blood pressure (Beevers *et al*., 2007).

TREATMENT FOR HYPERTENSION

Most patients are treated to lower systolic blood pressure below normal levels. High blood pressure treatment involves changes in lifestyle and drug therapy.

- ***Physical activity***
- **Lifestyle modification**
- ***Salt restriction***
- ***Limited alcohol intake***
- ***A diet with increased fresh fruits and vegetables and reduced saturated fat content***
- Other lifestyle modifications may include; calcium (Reid *et al*., 2002) and magnesium supplements, limitations on the intake of caffeine (Noordzij *et al*., 2005), avoiding smoking, and a wide range of techniques for relieving stress (Sharma and Golay, 2002) through massage or relaxation therapy.

Drug Therapy

Drug treatment typically starts in patients with blood pressure greater than 140/90mmltg and lifestyle modifications have been unsuccessful. It is also given if the severity of hypertension exceeds stage 1 or if the doctor feels it is appropriate to start immediately after diagnosis (Progrars, 2014). Some commonly used drugs for the treatment of hypertension may include;

- ***Beta-blockers***
- ***Calcium channel blockers***
- ***Angiotensin-converting enzyme inhibitors***
- Other drug therapy management of hypertension may include; angiotensin receptor blockers, alpha blocker, thiazide diuretics, central adrenergic inhibitors, natriuretic peptides, etc. The drug therapy turns to cause undesirable side effects to the individual. About 8% of hospital admissions in the United States of America are due to adverse or side effects of synthetic drugs (Karimi *et al*., 2015).

Table 2. Some Antihypertensive Drugs and Their Side Effects (Sitbon *et al*., 2018).

Class	Common generic drug names	Side effects
Thiazide or thiazide-like diuretic	Chlorthalidone Hydrochlorothiazide Indapamide , Metolazone	Hypokalemia, hyponatremia, hyperuricemia, hypocalciuria, rash
Loop diuretic	Bumetanide , Ethacrynic acid Furosemide ,Torsemide	Hypokalemia, hyperuricemia, rash
ACE inhibitor	Benazepril ,Captopril Enalapril , Lisinopril Quinapril , Ramipril	Cough, hyperkalemia, increased SCr
Calcium channel blocker	Amlodipine , Diltiazem Nifedipine , Verapamil	Edema, fatigue
Beta-blocker	Atenolol, Carvedilol Labetalol, Metaprolol Nebivolol	Bronchospasm, fatigue, depression, impotence

Medical plants with antihypertensive effects

Traditional Medicinal Plants

Using traditional medicine as a therapy mechanism is growing rapidly around the globe. A large population in many developing countries relies on traditional practitioners and their medicinal plant armamentariums to meet their basic healthcare needs (Jafari *et al*., 2014).

Although modern medicine exists side by side with such traditional practices, for historical and cultural reasons, traditional medicines have often retained their popularity. Perhaps the affordability and accessibility of these traditional medicines have led to their widespread use. They include the use of medicinal plants, parts of animals, minerals, and non-medicinaltreatments including religious therapies. This includes knowledge and experience of health behaviors from generation to generation exposed to different cultures.

Herbal Medicine

Herbal medicine, also called botanical medicine or photo medicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes (Opara *et al*., 2014).

The use of herbs began long ago with the pharmacological treatment of disease (Schulz *et al*., 2001). Secondary metabolites are known to be the chemical compounds found in herbs that do not contribute directly to their growth. They are responsible for herbal medicinal properties. Plants use secondary metabolites as a defense system against infectious diseases and pest-like intruders (Patil *et al*., 2011). These compounds contain alkaloids, terpenes, and phenolic compounds, and have biological properties used for a wide variety of diseases including cardiovascular diseases, atherosclerosis, chronic inflammation, and lesions and wounds. Some important species with antihypertensive properties are shown in the table below.

Table 3: Some Antihypertensive Plants Commonly Used

Active compound	Plant source	Plant part	Type of metabolite	References
Reserpine	*Rauwolfia serpentine*	Roots	Alkaloid (indole alkaloid)	(Al Disi *et al*., 2016; Samaha *et al*., 2019)

Andrographolide	*Andrographis paniculata*	Leaves	Terpenoid (diterpene)	(Al Disi et al., 2016; Jayakumar, Elizebeth, Yen, & Sheu, 2014)
Kaempferol	*Allium cepa*	Bulb	Flavonoid	(Hamzalioğlu & Gökmen, 2016; KrisEtherton *et al*., 2002; Liu *etal*., 2017)
Lutenolin	*Bidens pilosa*	Leaves	Flavonoid	(Gavamukulya, Wamunyokoli, & Elshemy, 2017; Hamzalioğlu & Gökmen, 2016; Kris- Etherton et al., 2002)
Quercetin	*Allium sativum*	Bulb	Flavonoid (flavonol)	(Al Disi *et al*., 2016; Arranz & Eder, 2017; Hamzalioğlu & Gökmen, 2016; Kris-Etherton *et al*., 2002; Liu *et al*., 2017; Samaha *et al*., 2019)
Anomurine	*Annona muricata*	Leaves	Alkaloid (isoquinoline)	(Gavamukulya *et al*., 2017)
Magniferin	*Mangifera indica L.*	Leaves	Polyphenol	(Yang *et al*., 2018)
Forskolin	*Coleus forskohlin*	Root	Terpenoid	(De Souza *et al*., 1983)

2. PLANT PROFILE

Adina cordifoliasynonym, ***Haldina cordifolia***, is a flowering plant in the family Rubiaceae. It is native to southern Asia, from India east to China and Vietnam and south to Peninsular Malaysia.

Botanical description

Botanical Name: Adina cordifolia (Roxb.)
Family: Rubiaceae
Synonym: Haldina Cordifolia (Roxb.)
Local name : Kadami, Haldu
English name: Yellow Teak, Saffron Teak.

Figure No.1: Adina cordifolia

History of Adina Cordifolia:

Adina cordifolia's illustrious past dates back to the time of the Vedas, Puranas, and Samhita.

The roots and stems of the Adina cordifolia plant are mentioned in many Vedic period texts. Dantadhavana took advantage of it. Adina is referenced in several ancient texts, including the paraskaraGuhyasutra 1/21, the Atharvaparishista 26/5/1–4, the Yajnavalkyashiksha 34, the Mandukishiksha 4/1, and others. PanineeyaAsthadhyayi, PathanjaliMahabhashya, Gubhilagruhya sutra, and Shulwa Prathishakhya all refer to it by its Sanskrit name, Nipa.

Distribution:

This species is native to Southeast Asia. It may be found in the following countries: India, Burma (Myanmar), Sri Lanka, Bangladesh, Nepal, Thailand, South China, Bhutan, Myanmar (Myanmar), Vietnam, and Malaysia. All across India (save for the desert districts of Rajasthan), it inhabits deciduous woods up to an altitude of 900 m in the sub-Himalayan tract. It may also be found in the South Indian jungles. It thrives on well-drained soil at a low altitude (between 300 and 1000 meters). Soil pH between 5.5 and 6.5 is ideal. The ideal annual temperature range is between 250 and 350 degrees Celsius, while the ideal annual rainfall range is between 1,000 and 2,000 millimeters. It can't handle cold weather. Up to an altitude of 1000 meters above sea level (MSL), the tree may be found growing on a wide variety of geological substrates.

TABLE NO.4: TAXONOMIC CLASSIFICATION

Kingdom	***Plantae***
Class	***Magnoliopsida***
Sub-class	***Asteridae***
Superorder	***Gentiananae***
Order	***Gentianales***
Family	***Rubiaceae***
Subfamily	***Cinchonoideae***
Genus	***Adina***
Specific epithet	***Cordifolia***
Botanical name	***Adinacordifolia(*Roxb.)Benth&Hook.F.**
Synonym	***HaldinaCordifolia*(Roxb.)**

Plants of the family Rubiaceae produce secondary metabolites that are used in medicine.The family Rubiaceae has the most members of any family in the genus Sagittaria. This branch of the Gentianales family tree is also the oldest. Trees, shrubs, and, very rarely, herbs7-10 are all included in the roughly 450 genera and 6500 species that make up the family Rubiaceae. The Adina Cordifolia plant is a member of the Rubiaceae family. **Genus:** Adina cordifolia, is the sole species in the genus Haldina.

Uses:

- Bark and leaves are used for cholera, cold cough, fever, headache, Scars and skin yellowish body, and urine complaints.
- The fresh bark is ground with brown sugar and taken internally for stomach ache.
- Laves are used on cough and cold, applied over the swollen portion to remove pain and swelling.
- Fresh stem bark juice is taken in rheumatism.

- Latex is applied on aching tooth.
- Stem bark used on fever.
- Bark is used as an antibacterial, for eczema, Scabies, Bark paste is applied to eczema, Scabies, or bacterial infections on the skin

Chemical Composition of "Adina Cordifolia":

In addition to tannins, the yellow naphthaquinone pigment adinin was extracted from the heartwood. Adifoline was formerly known as adinin, but after more research, it was shown to be an alkaloid of the Bcarbolineseries, and the name was changed to reflect this. Cordifoline, benzoic acid, B-sitosterol, and umbelliferone were also found to be present. 7,4-dimethoxy-5hydroxyflavanone and 5,7-dimethoxy-4-hydroxyflavanone were found to be the flavanones extracted from the heartwood. In addition to b-sitosterol, the heartwood provided many saturated aliphatic hydrocarbons. These included nheneicosane, n-tricosane, n-pentacosane, and n-pentatriacontane. The incision of the trunk yields theoleoresin that contains between 5.2% and 6.8% essential oil. The presence of alkaloids in the stem bark was discovered in an initial chemical analysis. A novel coumarin glycoside, adicardin, identified as 7-apiglucoside of umbelliferone, was isolated from an ethanolic preparation of root bark.

Pharmacology

There is a wide variety of medical uses for Adina cordifolia. It possesses antimicrobial, anti-inflammatory, anti-aging, anti-pain, and anti-fertility properties. The chemical composition, pharmacological investigations, and biological research on Adina cordifolia plants were given the most attention in this study. Since this kind of medicine is controversial and has little study, this dissertation will help encourage scientists to coordinate their efforts.

3. MATERIALS AND METHODS

Study site

The study was carried out at Shriram College of Pharmacy, Banmore. The laboratory work such as phytochemical screening was performed at the Pharmacognosy laboratory, department of pharmacy, and the antihypertensive test was conducted at the Pharmacology department of SRCP.

Sample collection

The fresh leaves of *Adina cordifolia* will be collected from the local gardens of Gwalior, Madhya Pradesh. The leaves of *Adina cordifolia* will be dried in air & shade and then grind to a coarse powder**.**

Experimental Animals

From the central animals of Shriram College of Pharmacy, Banmore, Wistar rats weighing 200–250 g of either sex were acquired. They were kept in polypropylene cages on rodent pellets at a regulated temperature of 22±2°C and acclimated to a 12-hour light/dark cycle. Food and water were freely available until two hours before the trial. The animals were maintained and cared for under the "Committee for Control and Supervision of Experiments on Animals (CPCSEA)" approved rules. Two hours following the trial, food and water were made available. All animal tests were carried out in compliance with the project proposal no. SRCP/IAEC/70/20-21, which was issued by the establishment's ethical committee on animal experimentation [67].

Solvent and chemicals

Experiments were conducted using N-hexane, chloroform, methanol, ethanol, deionized water, hydrochloric acid, sulfuric acid, nitric acid, sodium carbonate, copper sulfate, potassium acetate, aluminum nitrite, acetone, sodium hydroxide, sodium chloride, quercetin, bovine serum albumin, gallic acid, anthrone reagent, Folin–Ciocalteau's phenol reagent, anthrone reagent, monosodium phosphate, disodium phosphate, 3, 5-dinitrosalicylic acid, potassium sodium tartrate, and distilled water.

Preparation of plant extract

The extract from Adina cordifolia leaves was obtained using the hot and cold extraction procedures. The Soxhlet equipment was used to perform hot extraction. The solvents—n-hexane, chloroform, and methanol—were used in ascending sequence of polarity to do the

extraction. Alcohol and distilled water, two polar solvents, were used to do cold extraction (Yadav and Agarwala, 2011).

Physicochemical analysis

Physicochemical analysis was performed on the leaves of *Adina cordifolia* with reference to the protocols of USP (2005).

Moisture content

After being weighed, two grams of powdered leaves were added to the crucible. After that, the crucible was baked for 30 minutes at 105 °C. The crucible was removed and weighed thirty minutes later. The weight decrease was noted. Until the powder's weight remained consistent, the process was repeated. The following formula (Eq. A.1, A.2) was used to calculate the moisture content:

Dry matter (%) = initial weight –final weight/weight of dried powder× 100

Ash values

Total ash

In a China dish that had been previously weighed, two grams of the powdered sample were collected. A muffle furnace was used to burn the China dish at 675 ± 25°C until the sample was carbon-free. After being removed from the furnace, it was left to cool at room temperature in a desiccator. After noting the weight, the total ash content was determined using the formula (Eq. B.1):

Total Ash (%) *=weight of Ash/total weight of powder* × 100.

Acid insoluble ash

After weighing two grams of the sample, it was put in a China dish and heated to 675°C in a muffle furnace until it was carbon-free. After cooling in desiccators, it was weighed. After being cooked for five minutes in twenty-five milliliters of 3N HCL, the resulting ash was let to cool at room temperature. Ashless filter paper was used to filter the mixture. After being cleaned with hot double-distilled water, the residue was put back in the furnace. Once the sample was carbon-free, it was cooled and weighed. The following formula (Eq. B.2) was used to calculate the proportion of acid-insoluble ash:

Acid insoluble ash (%) = weight of acid insoluble ash / total ash weight × 100

Water insoluble ash

After boiling 25 milliliters of double-distilled water for five minutes, the ash from the total ash test was run through ash-less filter paper. The residue that remained on the filter paper was put in a China dish and left to burn for 15 minutes at 450°C in a muffle furnace. After determining the weight of the China dish, the following formula (Eq. B.3) was used to evaluate the amount of water-insoluble ash:

Water insoluble ash (%) = weight of water-insoluble ash/total ash weight × 100

Sulfated ash

A pre-weighed, dried, and cleaned china dish was filled with two grams of the sample. One milliliter of strong sulfuric acid was then added. It was also cooked over a low flame until the odors subsided. After two iterations of this procedure, the China dish was heated to 600 ± 25°C for 30 minutes in a muffle furnace. After cooling, the China dish was weighed. The following formula (Eq. B.4) was used to determine the proportion of sulphated ash:

Sulphated ash (%) = weight of sulphated ash/ weight of powder ash × 100.

Extractive values

Water soluble extractive value

A conical flask filled with 100 milliliters of double-distilled water was filled with five grams of precisely weighed powder. For twenty-four hours, the flask with the magnetic stirrer was set on the hotplate to shake. After a day, the solution was filtered. 25 cc of the filtrate was transferred to a weighted China dish, which was then placed in an oven set to 105°C to evaporate it. The China dish was weighed once again following evaporation. The extractive value in comparison to the dried powder was assessed. The following formula was used to determine the percentage (Eq. C.1):

Water soluble extractive value (%) = weight of dried extract / weight of powder sample × 100

Alcohol soluble extractive value

In a conical flask, five grams of powdered Adina cordifolia leaves were combined with 100 milliliters of ethanol and shaken constantly over a hotplate. It was taken off the hot plate and filtered after twenty-four hours. The China dish with 25 mL of filtrate was then baked at 105°C. In relation to the weight of the air-dried sample, the weight of the dried material was noted. The following formula was used to determine the percentage of alcohol-soluble extractive value (Eq. C.2):

Acid soluble extractive value (%) = weight of dried extract / weight of powder sample × 100

Estimation of primary metabolites

Total protein content

With a few minor adjustments, the Lowry et al. (1951) technique was used to estimate the total protein content. Five drops of Triton X, 10 milliliters of distilled water, and one gram of powdered material were introduced to a Falcon tube and shaken at random. For ten minutes, the fluid in the Falcon tube was centrifuged at 2,700 rpm. Once the layer of supernatant was created by centrifugation, 100 µl was extracted and double-distilled water was added to get the volume up to 1 ml.

Three milliliters of reagent C and 0.2 milliliters of Folin–Ciocalteu reagent were added to this combination. 50 milliliters of reagent A and one milliliter of reagent B were combined to create reagent C. Reagent B included 0.5% copper sulfate in 1% potassium sodium tartrate, whereas Reagent A had 2% sodium carbonate and 0.1 N sodium hydroxide. For half an hour, the sample was incubated at room temperature. At 600 nm, absorbance was measured against a blank solution. Except the powdered sample, the blank solution was made in the same manner as the sample solution. The standard used to estimate the protein content was bovine serum albumin (BSA). Plotting the absorbance of several dilutions, ranging from 20 to 120 µg/ml, against the standard curve was done through linear regression.

Total lipid content

50 g of powdered material was placed in a thimble for hot extraction with n-hexane, and maceration was done in the solvent. Throughout the extraction process, the temperature was kept between 40 and 60 degrees Celsius. Following extraction, filtration was performed, and the filtrate was dried using a rotary evaporator. After that, it was moved to a glass vial that had been previously weighed. For additional drying, the vial was subsequently put in an oven set at 40°C. Following thorough drying, the total lipid content was measured, weighed, and represented in milligrams per gram of the entire sample (Besbes et al., 2004).

Total carbohydrates

The formula discussed by Al-Hooti et al., 1997 for total carbohydrate determination was used by taking the difference between total lipid and total protein content from 100 (Eq. D.1).

Total carbohydrate (%) = 100 – (Total moisture + total ash + total fats + total protein).

Estimation of secondary metabolites

Determination of total polyphenols

The phenolic content of each of the five Adina cordifolia leaf extracts was assessed using Slinkard and Singleton's (1977) procedures. Gallic acid was the standard used in this experiment to plot the calibration curve. Methanol at a concentration of 1 mg/ml was used to create the standard and stock solutions. 200 µL of the sample and reference were transferred into separate Falcon tubes. Next, the Folin–Ciocalteu (FC) reagent (200 µL) was applied. After four minutes, two milliliters of 15% sodium carbonate were added, and three milliliters of methanol were used to get the final volume. Except for the sample, a blank solution was prepared using identical reagents. The sample was then diluted at 10, 20, 40, 60, 80, 100, and 120 µg/ml. All the samples, blank and standard were incubated at room temperature for 2 h and absorbance was found using a UV–visible spectrophotometer at 760 nm. Total phenolic content was measured through a linear regression equation and expressed as mg/g.

Determination of total flavonoids

Chang et al. (2002) proposed a method to assess the amount of flavonoids in Adina cordifolia leaves. Methanol was utilized to create a standard quercetin solution. Methanol was used to create stock solutions for each of the five sample extracts at a concentration of 1 mg/ml. A variety of sample dilutions were prepared. 200 µl of the stock solution and standard solution were combined with methanol to create the working solution, which had a volume of 1 ml. Following that, 4.6 ml of double-distilled water, 100 µl of 10% (w/v) aluminum nitrate, and 100 µl of 1 M potassium acetate were added. Except for the sample, all of the reagents were present in the blank solution. All test tubes were incubated at 25°C to measure the absorbance, and absorbance was recorded using a UV spectrophotometer at a wavelength of 415 nm. Using quercetin as a reference standard, total flavonoids were then calculated.

Total polysaccharides

To determine the total polysaccharide content, the method described by Hussain et al. (2008) was applied. 200 mg of each of the five extracts were combined with 80% of 7 milliliters of heated ethanol. After running the solution on a vortex mixer for two minutes, it was centrifuged for roughly ten minutes at 2,700 rpm. Drop by drop, the residue was treated with anthrone reagent (45 mg anthrone in 100 ml cold 85% sulfuric acid), and the procedure was repeated until the solution showed no color. After drying the residue, 10 milliliters of the digestion mixture—five milliliters of 25% HCl and five milliliters of double-distilled water—were added.

For 20 minutes, the Falcon tubes were submerged in an ice bath until the temperature dropped to 0°C. The supernatant was then extracted after centrifugation for ten minutes. The entire supernatant collection procedure was carried out again. Distilled water was used to reach the final volume; 4 milliliters of anthrone were added, and the liquid was heated for 8 minutes in a water bath before being immediately chilled. The sample was absent from the blank solution, which also contained anthrone, ethanol, water, and digestion mixture. At 630 nm, absorbance was measured using glucose as a reference. For each extract, several dilutions ranging from 10 to 120 µL were made, absorbance was measured, and a standard curve was constructed. The linear regression equation was used to find out the values, and the results were multiplied by a factor of 0.9.

Total glycosaponins

According to Siddiqui et al. (2009), 50 ml of methanol was added to 1 g of extract in a 100 ml round-bottom flask. A reflux condenser was used to heat the solution for half an hour. After two iterations, it was filtered. Ten milliliters of the filtrate remained in the flask after the filtrate was run through a rotary evaporator until most of it evaporated. Ten milliliters of the filtrate were then added dropwise to a beaker containing fifty milliliters of acetone. Saponins precipitated out as a result. Until the weight remained consistent, the precipitates were dried at 100°C in an oven. The same approach was applied for the rest of the extracts.

The formula used for the estimation of glycosaponins is stated below (Eq. E.1):

glycosaponins (mg/g) = weight of precipitate/ weight of sample × 100

Experimental design

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

Grouping of animals:

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

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

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

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

Group 4: Animals received 10% fructose solution instead of drinking water, *ad libitum*, with Methanolic extract of Adina cordifolia (ME) (100 mg/kg/day, p.o.) for 21 days.

Group 5: Animals received 10% fructose solution instead of drinking water, *ad libitum,* with Methanolic extract of Adina cordifolia (ME) (300 mg/kg/day, p.o.) for 21 days Hypertension was induced in experimentally in male Wistar rats (200-250 gm) by giving 10% fructose solution to drink *ad libitum* for five to six weeks. A 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 the development of hypertension through activation of the sympathetic nervous system, increased salt r**e**tention, and enhanced renin-angiotensin.

In vitro antihypertensive activity

Preparation of reagents

- Reagent A: 300 mM sodium chloride with pH 8.3 in 100 mM sodium borate buffer
- Reagent B: Buffer substrate solution (using 5 mM hippuryl-L-histidyl–L-leucine (HHL) and reagent A)
- Reagent C: Angiotensin-converting enzyme (ACE) solution (0.1 unit/ml).

Preparation of sample and standard stock solutions

One milligram of each extract was mixed with one milliliter of sodium borate buffer (pH 8.3) to create sample solutions of n-hexane, chloroform, methanol, ethanol, and water extract. One milligram of captopril in one milliliter of sodium borate buffer (pH 8.3) was used to create the reference standard solution.

Angiotensin-converting enzyme (ACE) inhibition assay

With minor adjustments, the ACE inhibition test that Cushman and Cheung (1971) suggested was carried out. 100 µl of buffer substrate solution (reagent B), 40 µl of extract solution, and 20 µl of ACE solution (reagent C) were combined to create the sample for each extract. A mixture of 20 µl of ACE solution (reagent C), 40 µl of deionized water, and 100 µl of buffer substrate solution (reagent B) served as the control. To prepare the blank solution, 100 µl of buffer substrate solution (reagent B) and 60 µl of deionized water are needed. The standard solution was made with 100 µl of buffer substrate solution (reagent B), 40 µl of captopril solution, and 20 µl of ACE solution (reagent C). The temperature was kept at 37°C for 30 minutes while each test tube was incubated. The reaction was terminated by adding 250 µl of 1M HCl. The interaction of hippuryl-L-histidyl-L-leucine (HHL) with angiotensin-converting enzymes (ACE) results in the synthesis of this acid. One milliliter of ethyl acetate was used to extract hippuric acid, and it was vigorously stirred for fifteen seconds in a vortex mixer. All samples were centrifuged in ten minutes. After moving one milliliter of the organic layer to a test tube, it was heated to 100°C for 30 minutes to evaporate it. One milliliter of deionized water was used to dissolve the leftover material once again. The absorbance of the sample, blank, control, spectrophotometer, and standard was measured at 228 nm. The percentage inhibition was calculated by using the following equation (Eq. F.1):

Percentage inhibition (%) = test control- test solution /test control- blank control × 100

Principle of recording B.P:

After the animal was put in the NIBP restrainer, a suitable cuff with a sensor was attached to its tail and heated to between 33 and 35 °C. The Power Lab data gathering system and computer were used to record the pulse while the tail-cuff was gradually released after being inflated to a pressure far higher than the anticipated systolic blood pressure, or 250 mm Hg. Each rat's mean arterial pressure (MAP), diastolic blood pressure (SBP), and systolic blood pressure (SBP) were measured. A better measure of the perfusion of the kidneys, brain, and coronary arteries is mean arterial pressure, which is the average pressure in the arteries for a single cardiac cycle.

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

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

Results

Extraction and Proximate Analysis

Table 1 lists the findings of the Adina cordifolia proximate study. Adina cordifolia leaves have a moisture content of 9.1%. The moisture content of the powdered roots was 6.48 ± 0.45%, while the total amount of ash found in the Adina cordifolia sample leaves was 18%. 5.7% of the ash was acid-insoluble, 5.20% was water-soluble, and 21.60% was sulfated.

Table 5: Proximate analysis of extracts of Adina cordifolia leaf powder

S.No	Physicochemical parameters	Percentage content ±SD (%w/w)
1	Moisture contents	9.2±0.1
2	Total ash value	18.20±0.6
3	Acid insoluble ash value	5.70±.02
4	Water soluble ash	5.20±0.3
5	Sulfated ash	21.65±0.6
6	Alcohol soluble extractive	4.99±0.1
7	Water soluble extractive	1.1±0.5

Determination of phytochemicals

Determination of primary metabolites

Table 2 shows the resolute main metabolite content of all Adina cordifolia leaf extracts. A linear regression equation (Y = 0.0101x + 0.0296, R 2 = 0.9452) was used to determine the protein content, and a calibration curve was generated for bovine serum albumin (BSA) dilutions of 10, 20, 40, 60, 80, and 100 µg/ml.

Table 6: primary metabolites (mg/g) of powdered leaves of Adina cordifolia

S.No	**Primary metabolite**	**mg/g ±SD**
1	Total proteins	35.90±0.8
2	Total lipids	3.48±0.5
3	Total carbohydrates	11.45±1.2

Determination of secondary metabolites

Table 3 shows the resolute secondary metabolite content of all Adina cordifolia leaf extracts. Different standards for various metabolites were used in the linear regression equation analysis of all secondary metabolites. The total protein content was determined using the linear regression equation, which reads y = 0.0101x + 0.0296, R2 = 0.9452. A calibration curve was produced using bovine serum albumin as the standard. The leaf methanol extract had the highest protein content, followed by n-hexane, water, ethanol, and chloroform. The regression equation that was employed for the assessment of total polyphenols was y = 0.0037x + 0.0178, R2 = 0.9953. To find the methanol extract with the highest phenolic content, gallic acid was employed as a standard.

The total flavonoid count, calculated using the quercetin standard curve, was y = 0.0024x + 0.0147. The flavonoid content of methanol was the greatest.

Using glucose as a benchmark, the linear regression equation y = 0.0028 + 0.022, R2 = 0.9957 was utilized to estimate total polysaccharides. The findings demonstrated the high concentration of polysaccharides in the methanol extract. Our research indicates that the water extract contains the most saponins.

Estimation of ACE inhibition activity of extract of leaves of Adina cordifolia

The ability of different Adina cordifolia leaf extracts to inhibit the angiotensin-converting enzyme (ACE) at a dosage of 1 mg/ml was examined. Figure 2 displays each solution's % inhibition. The results make it clear that the highest percentage of angiotensin-converting enzyme inhibition is seen in the methanolic extract of Adina cordifolia leaf powder. As a result, the methanol extract exhibits antihypertensive properties similar to those of the common medication captopril, which inhibits ACE. On the other hand, n-hexane exhibits the least similar inhibitory impact to the reference standard, with a 35% effect. To learn more about Adina cordifolia's potential as an antihypertensive, more in vivo research with different models can be done on the plant's leaf extract.

Table 7: Secondary metabolites (mg/g) of extracts of powdered leaves of Adina cordifolia.

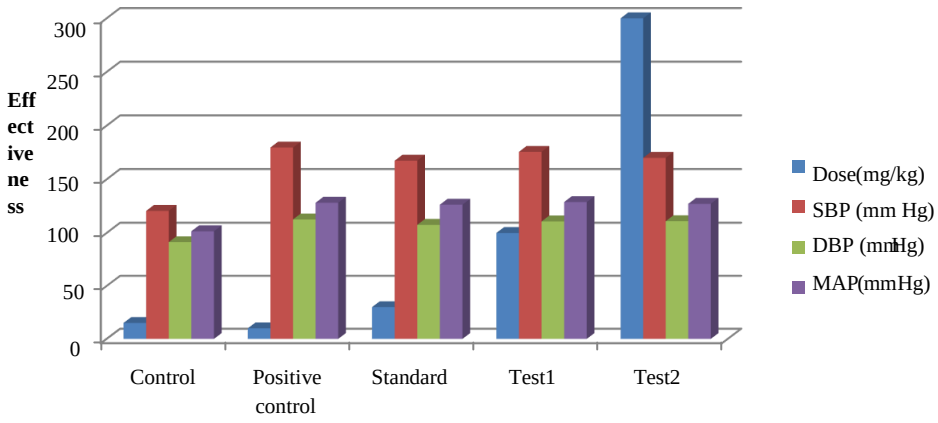
Extract	Total protein	Total polyphenols	Total flavonoids	Total polysaccharide	Total glycosaponins
n-hexane	9.78 ±1.2	15.82 ±0.7	28.54 ±0.5	21.53±0.4	Negative
Chloroform	13.68 ±1.3	74.65 ±0.7	151.93 ±1.0	26.30±0.5	Negative
Methanol	56.29 ±1.1	93.63 ±0.6	259.13 ±0.6	56.63±1.4	60.13±1.6
Ethanol	35.53 ±0.6	37.38 ±1.2	172.68 ±0.5	43.16±1.0	53.26±0.6
Water	37.18 ±0.9	46.66 ±0.5	105.08 ±0.6	19.02±0.4	70.0±1.3

Fructose-induced hypertension:

When compared to fructose-induced hypertension control groups, the methanolic extract of Adina cordifolia demonstrated a substantial ($p<0.05$) reduction in SBP, DBP, and MAP antihypertensive impact in 0 and 7 days at doses of 100 and 300 mg/kg (table 1 and table 2). Only DBP and MAP were considerably reduced by the test extracts at doses of 300 mg/kg on day 14 ($p<0.05$), and only DBP was lowered on day 21 of therapy; nevertheless, the treatment was prolonged, and no significant activity was seen for SBP or MAP in the hypertensive rats. Nevertheless, antihypertensive activity persisted for the 14th and 21st days, and it was shown that test extracts reduced every parameter in the hypertensive control group in a dose-dependent way.

Table 8: Effect of *Adina cordifolia* on blood pressure in fructose-induced hypertensive rats on 0 days.

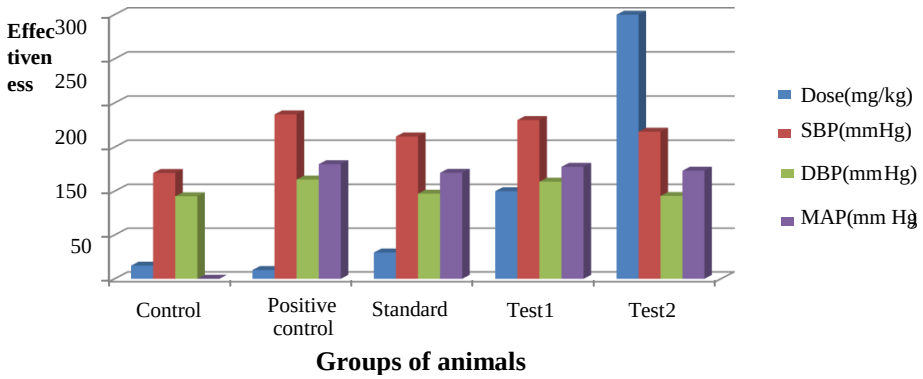
Groups	Treatment	Dose(mg/kg)	SBP(mm Hg)	DBP(mmHg)	MAP (mmHg)
1	Control	-	120.6±1.53	91.4±1.50	101.6±1.02
2	Positive control (10% fructose)	10	179.6±1.20	112.6±1.02	128.2±0.66
3	10% fructose + Captopril	10+20	167.4±1.20	107.6±1.12	126.4±0.74
4	10% Fructose 10 + Methanolic extract of Adina cordifolia	100	175.6±0.97*	110.8±1.15*	128.8±0.96*
5	10% Fructose + Methanolic extract of Adina cordifolia	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 9: Effect of *Adina cordifolia* on blood pressure in fructose-induced hypertensive rats on 7th day.

Groups	Treatment	Dose(mg/kg)	SBP (mm Hg)	DBP (mm Hg)	MAP (mmHg)
1	Control	-	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 Adina cordifolia	100	180.6±0.81*	110.8±0.96*	127.6±0.81*
5	10% Fructose + Methanolic extract of Adina cordifolia	300	167.4±0.87*	94.6±1.02*	123.2±1.01*

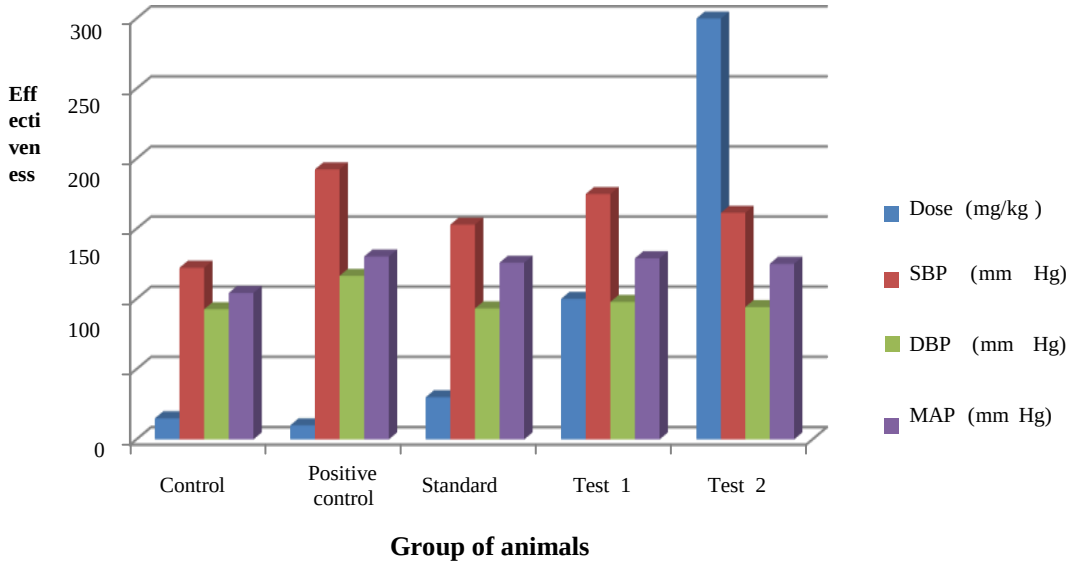


Graph 2: Effect of *Adina cordifolia* on fructose-induced hypertensive rats taking groups of animals against effectiveness of the extract. Data are expressed as mean ± SEM; n=6. One-way ANOVA followed by Tukey's multiple comparison test when compared with normal control, *P<0.05 significant

Table 10: Effect of *Adina cordifolia* on blood pressure in fructose-induced hypertensive rats on the 14th day.

Groups	Treatment	Dose(mg/kg)	SBP (mm Hg)	DBP (mm Hg)	MAP (mm Hg)

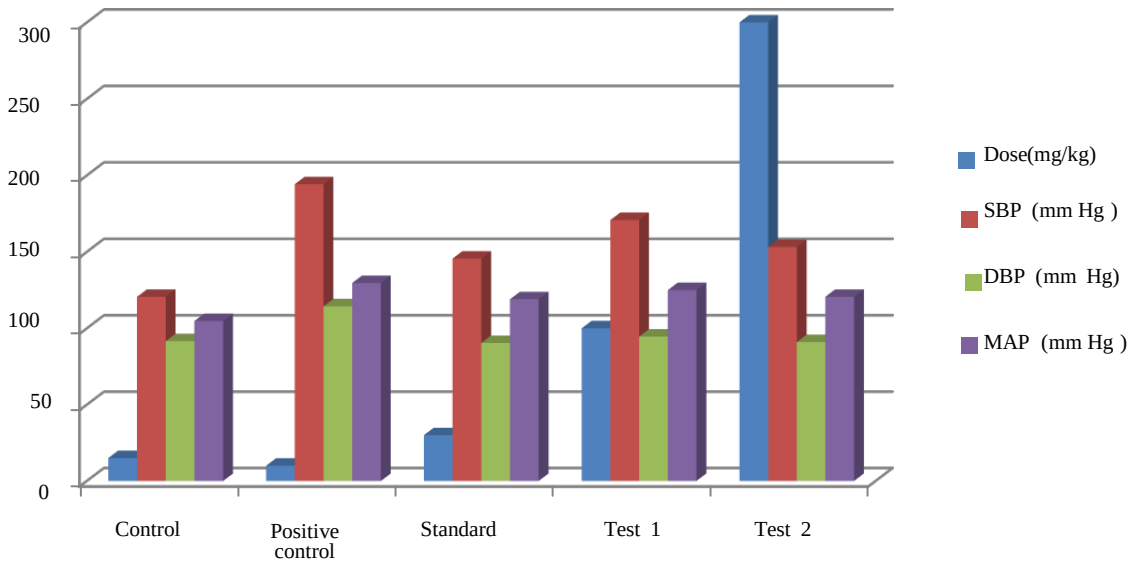
1	Control	-	122.2±1.49	92.6±1.16	104.2±1.28
2	Positive control (10 % fructose)	10	192.4±0.97	116.4±1.12	130.2±1.11
3	(10 % fructose+ captopril)	10+20	153±1.14	93.2±0.73	125.8±0.66
4	10 %Fructose 10 + Methanolic extract of Adina cordifolia	100	174.8±1.06	97.8±0.66*	129±0.99*
5	10 % Fructose + Methanolic extract of Adina cordifolia	300	161.4±0.97	94.2±0.66*	125±0.70*



Graph 3: Effect of *Adina cordifolia* 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 11: Effect of *Adina cordifolia* 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	-	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 Adina cordifolia	100	170.8±1.15	94.6±0.74*	125±0.89
5	10% Fructose + Methanolic extract of Adina cordifolia	300	153.2±1.01	91±0.99*	120.6±1.16



Graph 4: Effect of *Adina cordifolia* 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.

4. DISCUSSION

The most common clinical symptom resulting from a variety of cardiovascular diseases is hypertension. The development of acute coronary artery disease and congestive heart failure are also thought to be preceded or followed by it. A key factor in assessing cardiovascular function has been thought to be hypertension. The World Health Organization has recognized it as one of the most important risk factors for morbidity and mortality globally, and it kills almost nine million people every year. Mostly, synthetic medications are used to treat hypertension. The past four decades have seen the use of several medication classes to treat hypertension. These include of calcium channel blockers (CCBs), beta-blockers (β-blockers), diuretics, and, more recently, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers.

In this work, rats with fructose-induced hypertension were used to test Adina cordifolia's anti-hypertensive properties using a methanolic extract. Male Wister rats weighing 200–250g were used to create the hypertension using a fructose-induced hypertension model. After being ground into a powder, the plant was cold macerated using methanol and hexane. For later use, the extracted plant extract was kept at 40°C. After that, a phytochemical screening was performed on the plant extract to check for the presence of proteins, carbohydrates, alkaloids, saponins, terpenoids, glycosides, and other phytochemical elements. Every test displays potential possibilities, and the outcomes are documented.

Studies on acute oral toxicity are carried out in compliance with the 2001 OECD recommendations (AOT-423). In an acute toxicity trial, Adina cordifolia extract was found to be non-toxic at a level of 2000 mg/kg. With the exception of mild diarrhea in Adina cordifolia, no typical behavioral, autonomic, or neurological symptoms were noted. For the duration of the plant study, 100 mg/kg and 300 mg/kg of extract were chosen as the dosages. At 200 mg/kg and 400 mg/kg on the first and seventh days of therapy, the methanolic extract of Adina cordifolia significantly ($P<0.05$) reduced the SBP, DBP, and MAP in fructose-induced hypertensive rats. Nevertheless, antihypertensive activity was maintained for days 14 and 21, and it was discovered that test extracts reduced every parameter in the hypertensive control group in a dose-dependent way.

There may be a number of reasons why an ethanolic and aqueous extract of Adina cordifolia lowers blood pressure in hypertensive rats. It has been shown in earlier experiments that fructose and glucose both raise blood pressure. It is well known that an increase in sympathetic activity is one of the causes of glucose-induced hypertension. Any increase in

sympathetic activity often causes blood pressure and heart rate to rise. The extracts examined in this study were found to have a considerable hypotensive impact, which may be a major contributing factor to their antihypertensive efficacy in hypertensive rats. Oxidative stress and endothelial dysfunction are two major variables that contribute to hypertension.

It is also commonly recognized that consuming large amounts of sugar is linked to an increase in the creation of reactive oxygen forms in tissues. Furthermore, decreased antioxidant concentrations have been seen in hypertension patients. Additionally, a decrease in nitric oxide levels has been linked to elevated blood glucose levels, which in turn has led to elevated hypertension.

5. CONCLUSION

The results of this investigation demonstrate that the methanolic extract of Adina cordifolia probably includes specific active ingredients that lower blood pressure in rats given fructose. Furthermore, the current study demonstrates the safety of Adina cordifolia. The presence of Turpethosides A, B, and C in the methanolic extract of Adina cordifolia may be the cause of its anti-hypertensive properties, as they may modify the RAAS system. It's unclear exactly how it works. To identify the main molecule, separate the phytoconstituents, and confirm the precise mechanism of its antihypertensive action, more investigation is necessary.

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