AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

A COMPARATIVE ANALYTICAL AND INVIVO EVALUATION OF *MUSTA* (*CYPERUS ROTUNDUS* LINN.) AND *SHATAVARI* (*ASPARAGUS RACEMOSUS* WILD.) FOR ANTI ATHEROSCLEROTIC ACTIVITY

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Article info

Article History:
Received: 18-07-2022
Revised: 06-08-2022
Accepted: 27-08-2022

KEYWORDS:
Musta, Shatavari, Antiatherosclerotic activity, New Zealand White rabbits.

ABSTRACT

Obesity and hyperlipidemia are predisposing factors for major number of cardiovascular diseases, especially Atherosclerosis which has become the major cause for death. *Sthoulya* is a *Medo pradoshaja vikara*. Hyperlipidemia and Atherosclerosis can be correlated to *Rasa pradoshaja vikara* and *Dhamani Prathichaya* respectively. The treatment advocated for all the three conditions is *Kaphahara* and *Medohara*. Out of the big list of *Kaphamedohara dravyas*, *Musta* and *Shatavari* are two *Dravyas* with dissimilar *Rasapanchakas* that needs to be evaluated for the specific activity and indication. Analytical studies of the drugs were carried out as per the standard protocol. Invivo evaluation of *Churna*, aqueous and methanolic extracts of both the drugs in high fat diet induced Atherosclerosis were carried out on New Zealand White rabbits in both preventive and curative aspects. Though both the drugs were effective, *Shatavari* showed significant result in reducing blood glucose, Total Cholesterol, Triglycerides, LDL and VLDL in comparison with *Musta*. *Churna* and aqueous extract of *Shatavari* were effective than methanolic extract. *Musta* was found better in reducing the body weight in post treatment group than *Shatavari*. *Churna* and methanolic extract of *Musta* were effective than aqueous extract in the reduction of body weight, total cholesterol, triglycerides, LDL and VLDL. Except body weight in pre-treatment, the drugs were effective in both preventive and curative aspects. *Musta* is drug of choice in obesity associated with hyperlipidemia where as *Shatavari* would be effective in hyperlipidemia associated with *Rasa kshaya lakshanas*. Both *Musta* and *Shatavari* have potential anti-atherosclerotic activity.

INTRODUCTION

Cardiovascular diseases are the leading cause of mortality worldwide, accounting to 31% of global deaths[1]. Atherosclerosis is a chronic arterial disease and one of the major predisposing factors for CVD. Fatty streaks in arterial walls gradually develop into atheroma and characteristic plaques. The acute rupture of these atheromatous plaques cause local thrombosis, leading to partial or total occlusion of the affected artery, resulting in heart attack and stroke.

Access this article online	
Quick Response Code	
	https://doi.org/10.47070/ayushdhara.v10i4.1001

Its major clinical manifestation includes ischemic heart disease, ischemic stroke and peripheral arterial disease[2]. In Ayurveda, this condition refers to *Kapha medo dushti* causing *Sanga* or obstruction. The main treatment principle advocated in such condition is *Deepana, Kaphahara, Medohara, Upalepahara* and *Srothoshodhana karma* which refers to the scraping action of the disproportionately accumulated *Kapha* and *Medas*[3].

In Ayurvedic literature, around 160 drugs have been identified to have *Kapha-medohara karma*[4]. Even though these drugs have same action, they differ in their *Rasapanchaka*. This provides an excellent opportunity for condition specific drug selection in tackling the disease. *Musta* (*Cyperus rotundus* Linn.) and *Shatavari* (*Asparagus racemosus* Wild.) are two such drugs having *Kapha-medohara karma* with dissimilar *Rasapanchaka*. This calls for an experimental

study to compare the efficacy of *Musta* and *Shatavari* in performing *Medohara karma* with special reference to Anti atherosclerotic activity.

OBJECTIVES OF THE STUDY

- Analytical evaluation of *Musta* and *Shatavari*
- Invivo evaluation of anti-atherosclerotic activity of *Musta*
- Invivo evaluation of anti-atherosclerotic activity of *Shatavari*
- Comparative evaluation of anti-atherosclerotic activity of *Musta* and *Shatavari*

Review of Literature

Musta

Musta botanically identified as *Cyperus rotundus* Linn., belonging to Cyperaceae family finds its reference from *Veda kala* to *Adhunika kala*. It is a very common drug with extensive usage in Ayurveda and other traditional systems of medicine.

The word *Musta* means that which destroys the *Rogas* like *Kapha-pitta-rakta vikaras, Trushna, Jvara* etc[5]. It bears various synonyms as that of clouds which throws light on its hydrophytic nature, habit, *Guna* and *Karma.* [6]

Table 1: Synonyms of *Musta*

Synonyms of clouds	*Ambudhara, Megha, Ghana, Varaha, Abda, Jeemuta, Jalada, Ambodha, Varidanama*
Synonyms indicating habit	*Pinda Musta, Pitara, Vrushadhwankshi, Paripelava, Kolakasheruka*
Synonyms indicating habitat	*Gangeya, Gundra, Kaivartha, Kuruvinda, Prachya, Gonarda, Poorvakosta, Nagara Musta*
Synonyms indicating *Guna*	*Hima, Sugandhi*
Synonyms indicating *Karma*	*Musta, Bhadra Musta, Bhadramsha, Mustaka, Vara musta*

Musta is mentioned in *Lekhaniya, Stanyashodhana, Trishnanigrahana, Tritptighna, Kandugna, Ghana* of *Charakoktha dashemani*[7]. *Acharya Sushruta* mentioned *Musta* under *Mustadi* and *Vachadi varga*[8]. The varieties[10] of *Musta* mentioned in different *Nighantus* can be compared to following four species;

Table 2: Varieties of *Musta*

Varieties of *Musta*	**Botanical identification**	**Description**
Musta	*Cyperus rotundus* (Nut grass)	Round, thick rhizomes
Nagara Musta	*Cyperus scariosus* (Nut sedge)	Elongated, thin rhizomes
Kaivarta Musta (*Jala Musta*)	*Cyperus esculentus* (Earth almond)	-
Kshudra Musta (*Kaseru*)	*Scirpus kyssor* (Water chestnut)	Rhizomes are as big as J*aiphala*

Musta possess *Tikta-Kashaya-Katu Rasa, Laghu-Ruksha guna, Sheeta virya, Katu vipaka* and *Kaphapitta hara karma*. It is attributed with *karmas* like *Lekhana, Deepana, Pachana, Medohara, Kaphaghna* etc[6,7,8,9]. *Musta* is one of the ingredient in *Kapuradhyarka* and *Vyoshadi guggulu* which are indicated in the treatment of *Medoroga, Hrudroga, Kapharoga, Amavata*. It is also a component in *Yogaraja guggulu, Amrutharishta*, *Soubhagya shunti, Mustakarishta* etc. [9,11-14]

Musta (*Cyperus rotundus* L) is a perennial herb, stolons are elongated, slender, 10-20cm long and, 0.8 to 2.5cm diameter bearing hard, ovoid, tunicate, black fragrant tubers with flexuous hairy root fibres. Rhizomes/tuberous roots are having dark brown to black colour externally and creamish yellow internally[15]. It is found throughout India up to an altitude of 1,800mtrs, from Kashmir to Shimla, Gahwal to Khasia Hills. In Karnataka *Musta* (*Cyperus rotundus* L) is found in North Canara, Shivmoga, Dharwad, Hassan, Kolar, Mysore Bangalore[16]. Part used are rhizomes/tuberous roots. Dosage of *Musta churna* is 3 to 6gm and *Kashaya* is 50 to 100ml[17].

Cyperus scarious R Br and *Cyperus arundinaceum* Barker of the family Cyperacea are used as a substitute for *Cyperus rotundus* Linn. *Musta* itself is a substitute for *Ativisha* (*Aconitus heterophylum)* [17,18].

The major chemical constituents of *Musta* are Cyperene-1, Cyperene-2, β-selinene, Cyperenone, α-cyperone. And essential oils from tubers are Eugenol, Cyperol, Isocyperol, α-and β-rotunol, Cineol[17,19].

It is proved for its various pharmacological activities like anti-obesity, anti-inflammatory, wound healing, anti-microbial, anti-convulsant, hepato-protective, and anti-diabetic activity[17].

Shatavari

Shatavari, botanically identified as *Asparagus racemosus* Wild belonging to Asperagacea family, finds wide usage from *Veda kala* to *Adhunika kala*

Shatavari has synonyms based on morphology like *Adhahkantaka, Phanijihvika, Shatapadi, Shatamuli,*

Bahusuta, Bahuputri, Bheeru, Sukshmapatra, Bahumoola, Abhiru, Atmashalya, Keshika and synonyms based on properties and actions like *Vari, Variyasi, Shatahva, Shataveerya*[5,10, 20-24].

Shatavari is mentioned in *Balya, Vayahsthapana, Prajasthapana Ghana* of *Charakoktha dashemani* and *Varunadi gana* of *Susruta Samhita* and *Astanga Hridaya. Nigantukaras* have mentioned *Maha Shatavari* as a type of *Shatavari* which is botanically identified as *Asparagus sarmentosus* Linn[5,7,8,9, 20-24].

Shatavari possess *Madhura-Tikta Rasa, Snigdha-Guru guna, Sheeta virya, Madhura Vipaka,* and *Tridoshahara karma*. It is attributed with pharmacological actions like *Hridhya, Balya, Medhya, Pushtida, Rasayana, Shukrala, Kaphamedohara* etc[5,7,8,9,20-24]. *Shatavari* is a prime ingredient in various formulations like *Varunadi Kashaya, Vajeekaran Gritham, Shatavari kalpa, Shatavari guda*, *Phalagritha, Narayana Churna*, *Narayana Taila* etc[7,8,9,11-14].

Asparagus racemosus Wild is commonly seen throughout Sri Lanka, India and the Himalayas. It grows one to two metres tall and prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1300-1400m elevation. It is a woody climber growing to 1-2m in height. The leaves are like pine needles, small and uniform; flowers are white and have small spikes. Tuberous roots are 10-30cm in length and 0.1-0.5cm thick, tapering at both ends with longitudinal wrinkles; colour is cream; sweetish taste[15]. It is also distributed in Africa, throughout South Asia or China, South Malaysia and North Australia[16]. Part used are rhizomes/tuberous roots. Dosage of *Shatavari churna* is 3 to 6gm and *Kashaya* is 50 to 100ml[17].

Shatavari roots contain mainly 4 steroidal Saponins known as *Shatavarins* I to VI, Sitosterol, Benzaldehyde and trace minerals found are zinc, manganese, and copper, cobalt along with calcium, potassium zinc, and selenium. It is proved to have many pharmacological activities like hypolipidemic, antidepressant, anti-ulcerogenic, adaptogenic, anti-diarrhoeal and analgesic, antibacterial, aphrodisiac, anti-hepatocarcinogenesis, and galactogogue and so on[17].

Atherosclerosis

Atherosclerosis is a chronic disease condition characterized by the deposition of lipids and inflammatory responses in the wall of the arteries. The accumulation and deposition of oxidised cholesterol in a gradual series of events gives rise to atherosclerotic plaques due to which the arteries get blocked and narrowed over time. Consequently the arteries get blocked completely and may lead to myocardial infarction. The principal risk factors of AS is hypercholesterolemia, dyslipidemia which is mainly influenced by various causes like hereditary, sedentary lifestyle, consumption of high cholesterol foods, and diabetes. [25]

The atherosclerotic lesions consist of 3 major components; 1. Cellular components like smooth muscle cells and macrophages. 2. The connective tissue matrix and extracellular lipids 3. The intracellular lipids that accumulates within the macrophages which gets converted to foam cells. The appearance of atherosclerotic lesions is a result of cascade of events like inflammatory stimuli, release of cytokines, proliferation of smooth muscle cells, synthesis of connective tissue matrix and the accumulation of lipids within macrophages. [26]

Etiology: AS begins with the damage of the intimal layer of the artery may be caused by hypertension, hyper-triglyceredemia, hyper- cholesterolemia, tobacco smoking, diabetes/insulin resistance, obesity, inflammatory disease conditions like arthritis, infections, inflammations of other causes. The most common symptoms include chest pain (angina), arrhythmia, shortness of breath, fatigue and muscle weakness[27,28]. The treatment includes lifestyle changes such as eating a healthy diet and exercising, medication and surgical procedures. In medical management, routinely used drugs are Statins (Atorvastatin), Fibric acid derivatives, Bile acid sequestrants, cholesterol absorption inhibitors, nicotinic acid derivatives. The common adverse effects of statin are myopathy, flushing, palpitations and gastrointestinal disturbances. [29]

In Ayurveda, atherosclerosis can be correlated to *Dhamani Pratichaya,* a *Kapha nanatmaja vikara.* [9]

Dhamani Pratichaya

Dhamyate iti, 'dham'- 'dhmane' The word *Dhamani* is derived from '*Dham*' *Dhatu*. "*Dhmanaat damanyaha*". The act of blowing or piping. (Monier - william's). *Dhamani* can be defined as the vessels carrying *Rasa* and *Rakta dhatu* [essential nutrients and blood]. *Pratichaya*- "*pratikoolam cheeyate iti*" which means unwanted excessive accumulation. "*Dhamani pratichayo dhamnyupalepaha, Dhamanyupalepena dhamaninam pushtata*" (*Gangadharaha*). *Dushita meda* (increased lipids) while circulating in *Dhamanis*, may get deposited leading to *Upalepa* (coating) and thereby thickening of the *Dhamanis*. "*Dhamaninam pratichayaha atipuranam*" (*Yogendranathasen- Cha.Su.* 20/20). *Pratichaya* means '*Atipurana*' i.e., coating/ filling within the vessels. The *Lakshanas* of *Dhamani Pratichaya* are not mentioned in the texts.

As *Dhamani Pratichaya* is *Kaphaja nanatmaja vyadi, Guru- Apatarpana* and *Kaphahara chikitsa* should be adopted[9].

MATERIALS AND METHODS

A comparative analytical and Invivo evaluation of *Musta* (*Cyperus rotundus* Linn.) and *Shatavari* (*Asparagus racemosus* Wild.) for anti-atherosclerotic activity. The study was carried out in three phases:

1. Procurement, authentication and preparation of trial drugs
2. Analytical study of trial drugs
3. Experimental study.

Procurement, Authentication and Preparation of Trial Drugs

- Dried tubers of *Musta* and *Shatavari* were procured from Sri Gurukrupa Ayurveda & Homoepathy, Bangalore and powdered after authentication.
- The trial drugs were authenticated as *Musta* tubers and *Shatavari* tubers by Head Botanist, Centre for Herbal Gardens, Institute of Ayurveda and Integrative Medicine, FRLHT, Yelahanka, Bengaluru.
- Extraction of the trial drugs by Soxhlet apparatus were carried out at In vivo Biosciences, Bangalore and stored in air tight plastic containers

Analytical Study of Trial Drugs

Pharmacognostic, physicochemical and phytochemical analysis of the trial drugs were carried out at Drug Testing Laboratory, Govt. Central Pharmacy, Jayanagar, Bengaluru.

- **Pharmacognostic Evaluation:** The organoleptic characters (sensory evaluation) of the trial drugs wereobserved. Powder microscopy of the trial drugs were carried out at G.A.M.C, Bangalore. Pinch of fine powder of the crude drugs were soaked in water and 2-3 drops of chloral hydrate was added. This was put on the slide, a drop of 1% Safranin stain was added, mounted with glycerine and powder characters were observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.
- **Physicochemical Evaluation:** The tests to determine foreign matter, moisture content, total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and pH values were carried out as per the standard procedures[19].
- **Phytochemical Evaluation:** Qualitative chemical tests for the detection of organic constituents like alkaloids, glycosides, flavonoids, saponins, tannins, phenolic compounds, steroids, triterpenoids, carbohydrates, proteins, starch and resins were carried out as per the standard procedures. Qualitative chemical tests for the detection of inorganic constituents like calcium, magnesium, sodium, potassium, iron, sulphate, chloride, phosphate, carbonates and nitrates were conducted as per the standard methods[19].

Experimental Study

Objective of the Experimental Study

- To evaluate the anti-atherosclerotic activity of *Musta*.
- To evaluate the anti-atherosclerotic activity of *Shatavari*
- To compare the anti-atherosclerotic activity of *Musta* and *Shatavari*.

Parameters Selected

1. Body weight
2. Blood parameters- Glucose, total cholesterol, triglycerides, HDL, LDL, & VLDL
3. Histopathology- Histopathological study of aorta was carried out.

Methodology

Locale of Work: The Experimental study was carried out at Invivo Biosciences, Bengaluru.

Ethical Clearance: As per the protocol outlined in publication of the Committee for the Purpose of CPCSEA and approval was obtained from Institutional Animal Ethics Committee (IAEC) with reference No. Invivo/101/2020.

Test System

Animal model: Healthy adult New Zealand White Rabbits of either sex, weighing between 1.5 to 2kg wereselected as per the literature survey[30].

Identification: Animals were identified by specific body markings with crystal picric acid colour. The cages were labelled with the number of animals and dosage groups.

Acclimatization: 5 days

Number of groups: 16

Number of animals per group: 06

Husbandry: Animal house conditions

- Animals were housed under standard air-conditioned laboratory conditions.
- **Temperature:** Maximum: 24°C and minimum 23°C
- **Relative humidity:** Maximum: 63% and minimum 48%
- 12 h light and 12 h dark cycle.

The maximum and minimum temperature and relative humidity in the experimental room was recorded once daily.

Housing: Polypropylene cages with provision for water bottle holder and feed hopper with corn cobs asbedding material.

Diet: ad libitum, Pelleted feed from VRK nutrition solutionNormal diet: Animal chaw food pellets

High fat diet: Standard laboratory rabbit diet supplemented with 0.25% w/w of cholesterol dissolved in 3% w/w peanut oil and 3% w/w coconut oil, mixed with apple sauce to enhance palatability.

Water: ad libitum, Aqua guard water in polypropylene bottles

Feeding Schedule: The rabbits were provided with 40gm of high fat diet at approximately 12 hours intervals. Pre-treatment group was given high fat diet along with treatment for 45 days. Post-treatment group was fed with high fat diet for first 30 days and then treatment along with normal diet was given for next 45 days.

Induction: Atherosclerosis induced with high fat diet.
Dose: Reference from previous research articles. [31,33,34,35] [Table no.3]

Table 3: Dosage of Standard and Trial drugs

Treatment	Dosage
Atorvastatin (STD)	2.5 mg/kg bw
Musta Churna (MC)	500mg/kg bw
Shatavari Churna (SC)	500mg/kg bw
Aqueous extract of *Musta* (AQM)	500mg/kg bw
Aqueous extract of *Shatavari* (AQS)	500mg/kg bw
Methanolic extract of *Musta* (MEM)	500mg/kg bw
Methanolic extract of *Shatavari* (MES)	500mg/kg bw

Duration: 45 days
Route of Administration: Oral
Group Allocation

Table 4: Grouping of experimental animals

Group	Group Name	Treatment
I	Normal Control	Normal diet
II	Disease Control	High fat diet (DC)
Pre-treatment (Preventive): High fat diet along with treatment for 45 days		
III	Standard	Atorvastatin (STD)
IV	Trial Drug	*Musta Churna* (MC)
V	Trial Drug	*Shatavari Churna* (SC)
VI	Trial Drug	Aqueous extract of *Musta* (AQM)
VII	Trial Drug	Aqueous extract of *Shatavari* (AQS)
VIII	Trial Drug	Methanolic extract of *Musta* (MEM)
IX	Trial Drug	Methanolic extract of *Shatavari* (MES)
Post treatment (Curative): High fat diet for 30 days, then normal diet with treatment for 45 days		
X	Standard	Atorvastatin (STD)
XI	Trial Drug	*Musta Churna* (MC)
XII	Trial Drug	*Shatavari Churna* (SC)
XIII	Trial Drug	Aqueous extract of *Musta* (AQM)
XIV	Trial Drug	Aqueous extract of *Shatavari* (AQS)
XV	Trial Drug	Methanolic extract of *Musta* (MEM)
XVI	Trial Drug	Methanolic extract of *Shatavari* (MES)

Vehicle for Administration of the Drug

The drugs were made into homogenous suspension with 0.25% w/w Sodium CMC and administered orally once a day.

Observation of the Parameters

After the dosing period of 45 days, the blood was collected from marginal ear vein of all the animals for biochemical analysis.

The body weight and the blood parameters of the pre-treated group were observed on 0th and 46th day. For post treated group they were observed on 0th, 31st (after induction of Atherosclerosis) and 76th day (after the dosing period of 45 days).

The histopathological study of aorta was carried out after the treatment period of 45 days. Cholesterol deposition in aorta is a classical

manifestation of atherosclerosis. Hence Sudanophilia test was performed wherein Sudan IV clings to cholesterol deposits resulting in red coloured patches

Aortic Sudanophilia test was performed by isolating the left half of the aorta, which was stretched and pinned on cork board, immersed, and fixed in 10% formalin. All specimens were subsequently stained with Sudan IV for macroscopic determination of Sudanophilia. Sudanophilia was confirmed by dark red regions corresponding to the uptake of Sudan IV by fat.

Euthanasia: Animals were sacrificed as per CPCSEA guidelines (overdose of anaesthesia- ether)

Macroscopic Features

Data Compilation and Statistical Analysis

Average of all the data were compiled and SEM were calculated. All the data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Values <0.05 were considered as statistically significant

RESULTS

The results of the study are presented under two headings:

- Analytical study
- Experimental study

Table 5: Organoleptic characters of the drugs

S.No.	Features	*Musta*	*Shatavari*
1	External Surface	Rough	Rough and shrinked
2	Shape	Ovoid, tapering towards both ends to a point	Tuberous, tapering towards both ends
3	Colour	Dark brown externally and creamish internally	Creamish white externally, slight yellowish internally
4	Taste	Bitter	Sweetish, bitter
5	Odour	Aromatic, pleasant	Wheatish odour
6	Fracture	Short	Short and fibrous

Table 6: Macroscopic characters of the powdered drugs

Features	*Musta*	*Shatavari*
Touch	Smooth	Smooth
Colour	Slight brownish	Cream
Odour	Characteristic	Wheatish
Taste	Bitter	Sweet, Bitter

Table 7: Observations during preparation of extracts

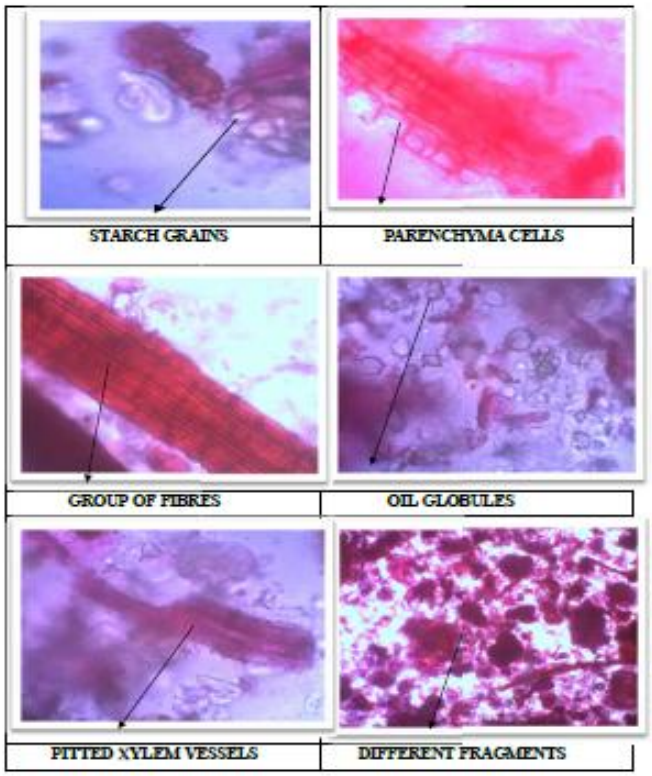
S. No	Observations	Aq. ext of *Musta*	Met. ext of *Musta*	Aq. ext of *Shatavari*	Met. ext of *Shatavari*
1.	Coarse powder of drug taken	1kg	1kg	1kg	1kg
2.	Extract	172 g	161 gms	461gms	169gms
3.	Yield obtained	17.20%	16.1%	46.1%	16.9%
4.	Colour of extract	Light brown	Dark brown	Golden	Golden
5.	Consistency	Powder	Powder	Powder	Powder
6.	Odour	Characteristic	Characteristic	Characteristic	Characteristic
7.	Taste	Slightly bitter	Bitter	Bitter	Bitter

Powder Microscopic Study

Powder microscopy of *Cyperus rotundus* Linn tuberous roots showed thepresence of;

- Fragments of abundant simple starch grains
- Compound starch grains
- Oval oil globules
- Group of fibres
- Pitted xylem vessels
- Reddish tannin content
- Parenchymatous cells

Plate No. 1: Powder microscopy of *Cyperus rotundus* Linn.



Powder microscopy of *Asparagus racemosus* Willd tuberous roots showed thepresence of;

- Fragments of lignified tracheids
- Pitted xylem vessels
- Parenchymatous cells
- Acicular crystals, Scalariform xylem vessels
- Numerous lignified, rectangular elongated stone cells.

Plate No.2: Powder microscopy of *Asparagus racemosus* Wild

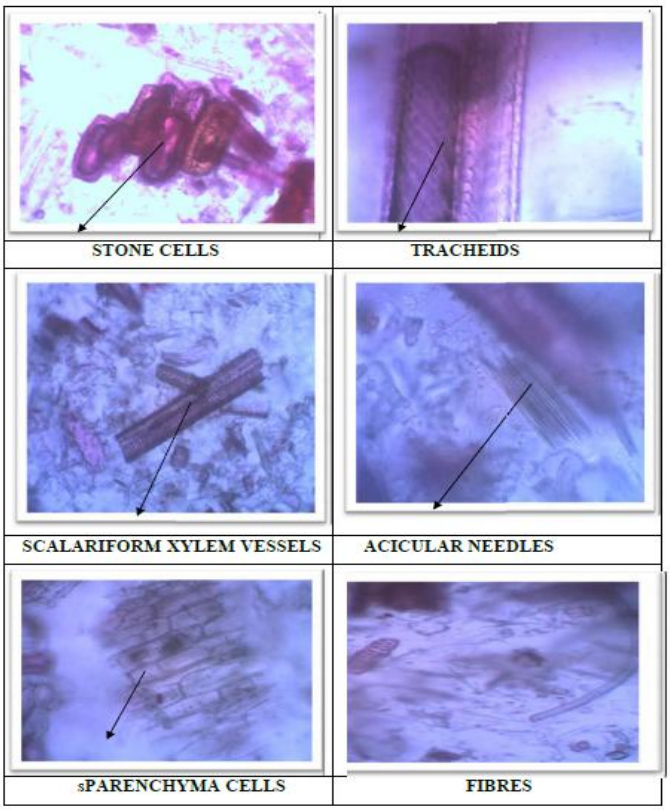


Table 8: Physico chemical constituents of *Musta* and *Shatavari*

Parameters	*Musta*		*Shatavari*	
	Results	API standards	Results	API standards
Foreign matter	0.90%	< 2%	0.70 %	< 1%
Total ash	3.9%	< 8%	3.05%	< 5%
Acid insoluble ash	2.5%	< 4%	0.60%	< 0.5%
Water soluble extractive value	18.2%	> 11%	48.1%	> 45%
Alcohol soluble extractive value	2.1%	> 5%	17.2%	> 10%
Loss on drying	9%	-	5%	-
pH value	5.5	-	6.2	-

Table 9: Organic constituents of *Musta* and *Shatavari*

Constituents	*Musta*		*Shatavari*	
	Aqueous	Methanolic	Aqueous	Methanolic
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	+	+	+
Tannins	+	+	+	+
Phenolic compounds	+	+	+	+
Proteins	+	-	-	+
Carbohydrates	+	+	+	+
Steroids	-	-	-	+
Resin	+	+	-	-
Gums	+	+	-	-

Table 10: Inorganic constituents of *Musta* and *Shatavari*

Constituents	*Musta*	*Shatavari*
Iron	+	+
Sodium	+	-
Calcium	+	+
Magnesium	+	+
Potassium	+	+
Chloride	+	-
Sulphate	+	-
Phosphate	+	-
Carbonate	-	-
Nitrate	-	-

Experimental Study

The results were statistically interpreted within the groups and the results of pre-treatment and post-treatment were compared.

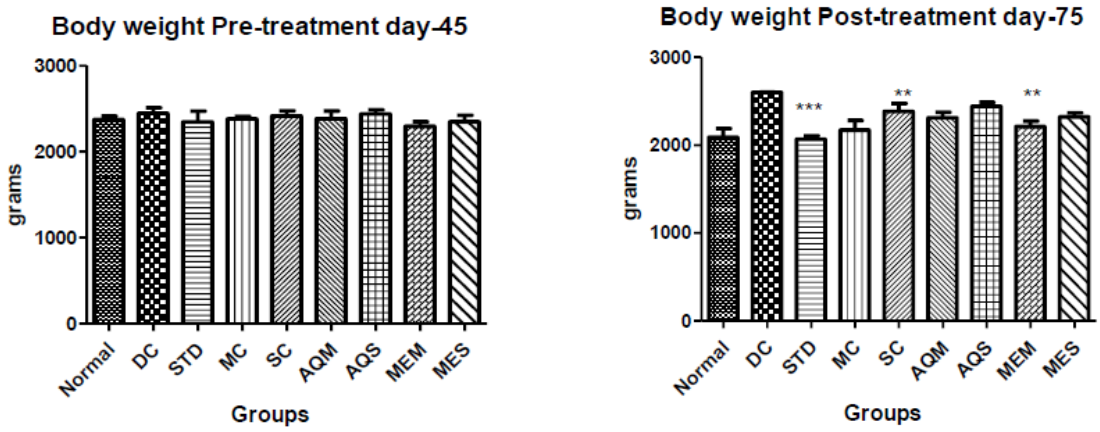
In the biochemical analysis and body weight, it was found that female animals have not been induced with atherosclerosis and weight gain both in pre-treatment as well as post-treatment groups.

In male animals, induction of atherosclerosis was confirmed by the increase in serum concentration of total cholesterol in both pre-treated and post-treated groups.

Table 11: Observation on Body Weight

Observation on Body Weight in grams					
Groups	**Pre treatment (Day 45)**		**Groups**	**Post treatment (Day 75)**	
	Female	**Male**		**Female**	**Male**
Normal	2203.33 ± 140.12	2373.33 ± 40.55	Normal	2240.12 ± 35.13	2089.67 ± 100.10
DC	2226.00 ± 107.00	2446.67 ± 63.60	DC	2283.33 ± 44.10	2598.00 ± 4.16
STD	2183.33 ± 192.21	2346.67 ± 124.54	STD	2363.33 ± 44.85	2066.67 ± 38.44
MC	2400.00 ± 125.30	2382.00 ± 29.37	MC	2206.67 ± 127.19	2170.00 ± 112.40
SC	2286.67 ± 170.23	2416.67 ± 60.09	SC	2316.67 ± 60.09	2380.00 ± 92.92
AQM	2286.67 ± 144.95	2383.33 ± 89.88	AQM	2320.00 ± 133.17	2310.00 ± 64.29
AQS	2396.67 ± 103.33	2438.67 ± 47.35	AQS	2156.67 ± 88.38	2440.00 ± 45.83
MEM	2483.33 ± 136.42	2297.33 ± 52.48	MEM	2096.67 ± 31.80	2210.00 ± 63.51
MES	2046.67 ± 78.60	2349.00 ± 74.91	MES	2346.67 ± 46.67	2320.00 ± 46.19

Graph No.1 and 2: Comparison on Body weight among the trial groups



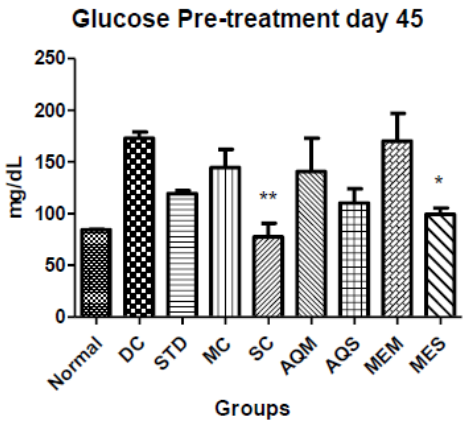
Pre-treatment (Male): There was no difference in body weights in any of the treated groups when compared against control.

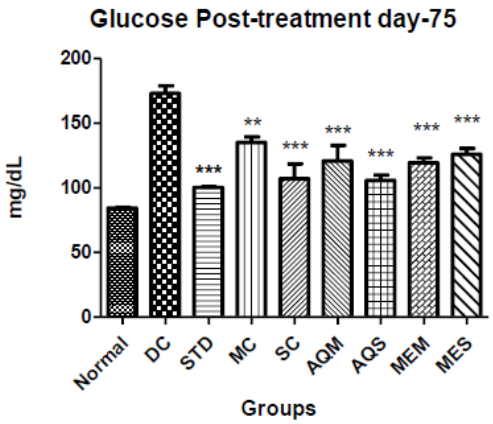
Post- treatment (Male): Statistically significant reduction in body weight was observed in STD, MEM and SC when compared to control. Post-treatment was found better than pre-treatment.

Table 12: Observation on Blood Glucose

Observation on Blood Glucose in mg/dl		
Groups	**Pre treatment (Day 45)**	**Post treatment (Day 75)**
Normal	84.47 ± 0.48	84.47 ± 0.48
DC	173.03 ± 5.95	211.67 ± 7.33
STD	119.50 ± 2.61	100.20 ± 0.70
MC	144.70 ± 17.50	135.10 ± 4.33
SC	77.60 ± 13.17	107.10 ± 11.48
AQM	140.69 ± 32.25	120.73 ± 12.13
AQS	110.40 ± 13.62	105.70 ± 4.26
MEM	170.40 ± 26.48	119.37 ± 3.93
MES	99.47 ± 6.08	125.83 ± 4.82

Graph No.3 and 4: Comparison on Blood glucose among the trial groups





Pre-treatment: The serum glucose level was decreased in SC and MES which is statistically significant and there were no significant changes observed in any other groups when compared against control.

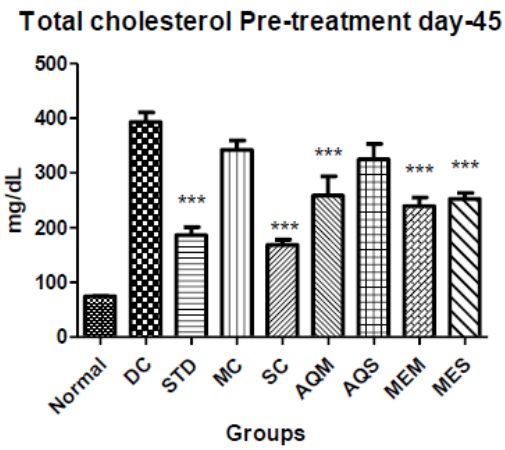
Post-treatment: Statistically significant reduction in glucose level was observed in STD, AQS, SC, MEM, AQM, MES when compared against control.

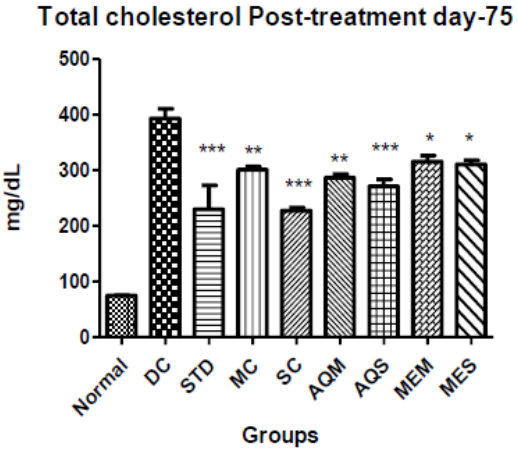
All the treated groups showed better result in post treatment in reducing the blood glucose than pre treatment.

Table 13: Observation on Total Cholesterol

Observation on Total Cholesterol in mg/dl		
Groups	**Pre treatment (Day45)**	**Post treatment (Day75)**
Normal	74.40 ± 0.47	74.40 ± 0.47
DC	393.17 ± 17.34	399.37 ± 38.73
STD	186.03 ± 15.02	229.30 ± 43.56
MC	342.17 ± 17.39	300.93 ± 5.58
SC	168.47 ± 9.53	227.27 ± 5.53
AQM	258.67 ± 35.12	286.43 ± 6.62
AQS	324.67 ± 28.63	270.83 ± 12.74
MEM	239.17 ± 15.91	315.80 ± 10.83
MES	252.10 ± 11.61	310.57 ± 6.99

Graph No.5 and 6: Comparison on Total Cholesterol among the trial groups





Pre-treatment: The serum total cholesterol level decreased significantly in SC, STD, MEM, MES, AQM when compared against disease control.

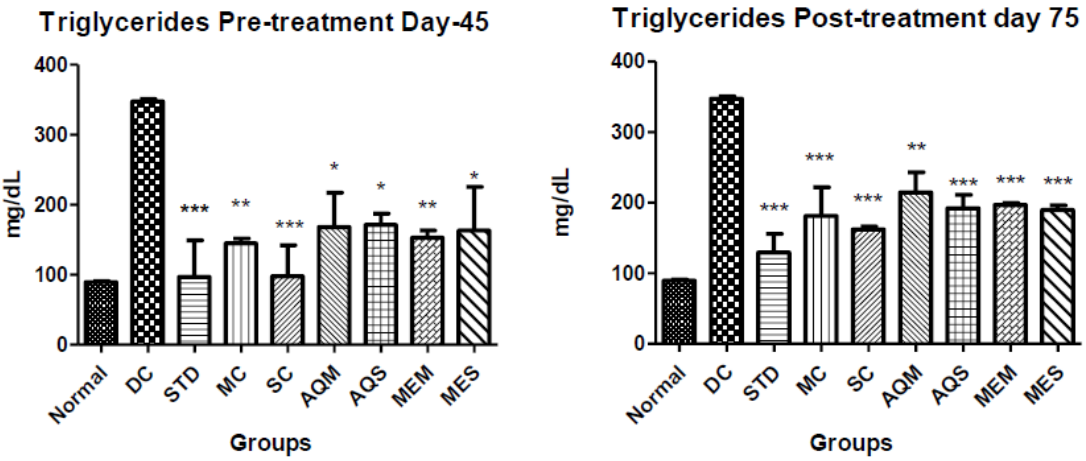
Post-treatment: Statistically high significant reduction in total cholesterol was observed in SC, STD, AQS, AQM, MC when compared against atherosclerotic control.

Shatavari churna in pre-treatment showed better result in reduction of total cholesterol than post-treatment.

Table 14: Observation on Triglycerides

Observation on Triglycerides in mg/dl		
Groups	**Pre treatment (Day 45)**	**Post treatment (Day 75)**
Normal	89.63 ± 1.07	89.63 ± 1.07
DC	347.36 ± 3.50	347.36 ± 3.50
STD	96.67 ± 52.47	129.45 ± 26.38
MC	144.87 ± 7.11	181.20 ± 40.80
SC	97.98 ± 44.27	162.05 ± 4.24
AQM	168.00 ± 49.24	214.57 ± 28.56
AQS	171.23 ± 15.79	192.13 ± 18.94
MEM	152.70 ± 10.49	196.70 ± 2.77
MES	163.08 ± 62.54	189.47 ± 6.59

Graph No.7 and 8: Comparison on Triglycerides among the trial groups



Pre treatment: Highly significant result was observed in STD, SC, MC, MEM in reducing triglyceride level when compared to atherosclerotic control.

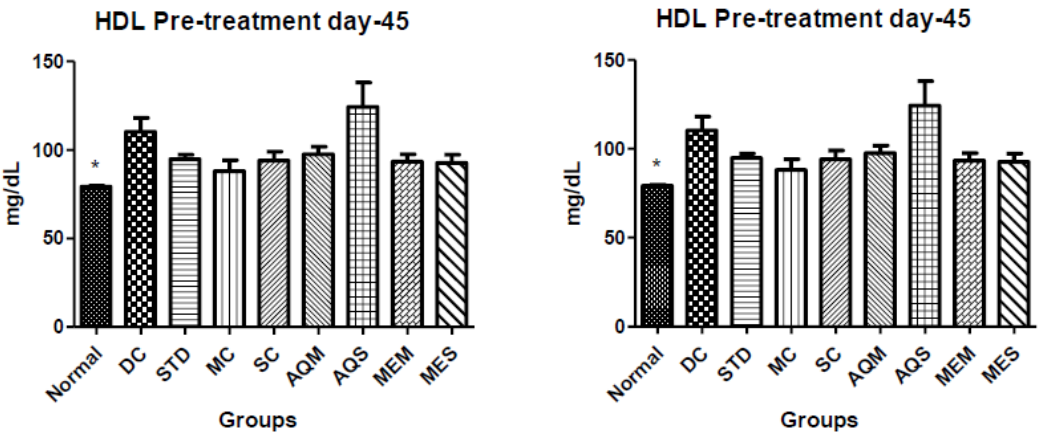
Post treatment: Highly significant result was observed in STD, SC, MC, MES, AQS, MEM in reducing triglyceride level when compared to atherosclerotic control.

Standard, *Shatavari churna* and *Musta churna* in Pre treatment showed better result in reduction of triglycerides than post treatment.

Table 15: Observation on HDL

Observation on HDL in mg/dl		
Groups	**Pre treatment (Day 45)**	**Post treatment (Day 75)**
Normal	79.20 ± 0.61	78.80 ± 0.44
DC	110.29 ± 7.74	116.47 ± 6.09
STD	94.78 ± 2.49	101.80 ± 3.37
MC	88.07 ± 6.11	102.31 ± 5.58
SC	94.02 ± 5.11	103.85 ± 4.23
AQM	97.57 ± 4.19	99.50 ± 0.36
AQS	124.33 ± 13.78	107.05 ± 9.68
MEM	93.40 ± 4.19	108.10 ± 4.45
MES	92.63 ± 4.70	99.27 ± 1.12

Graph No.9 and 10: Comparison on HDL among the trial groups



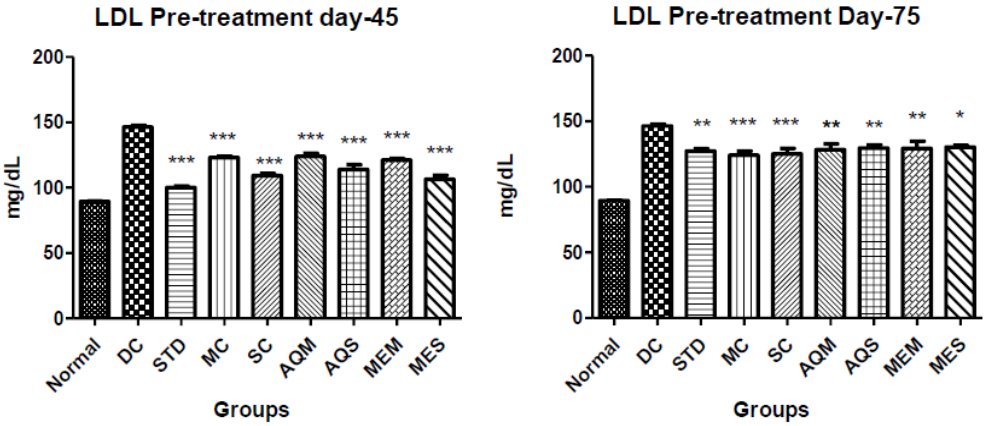
Pre-treatment: There were no significant changes observed in serum HDL level cholesterol in any of the treated groups when compared against control.

Post-treatment: There were no significant changes observed in serum HDL level cholesterol in any of the treated groups when compared against control.

Table 16: Observation on LDL

Observation on LDL in mg/dl		
Groups	**Pre treatment (Day 45)**	**Post treatment (Day 75)**
Normal	89.43 ± 0.35	89.43 ± 0.35
DC	146.40 ± 1.41	146.4 ± 1.41
STD	100.10 ± 1.18	127.20 ± 1.82
MC	123.13 ± 0.96	124.17 ± 3.02
SC	109.20 ± 1.73	125.17 ± 4.10
AQM	123.93 ± 2.28	128.20 ± 4.47
AQS	113.93 ± 3.69	129.67 ± 2.31
MEM	121.07 ± 1.24	129.33 ± 5.49
MES	106.40 ± 3.10	99.27 ± 1.12

Graph No.11 and 12: Comparison on LDL among the trial groups



Pre-treatment: Highly significant result was observed in STD, MES, SC, AQS, MEM, MC, AQM in reducing LDL level when compared to atherosclerotic control.

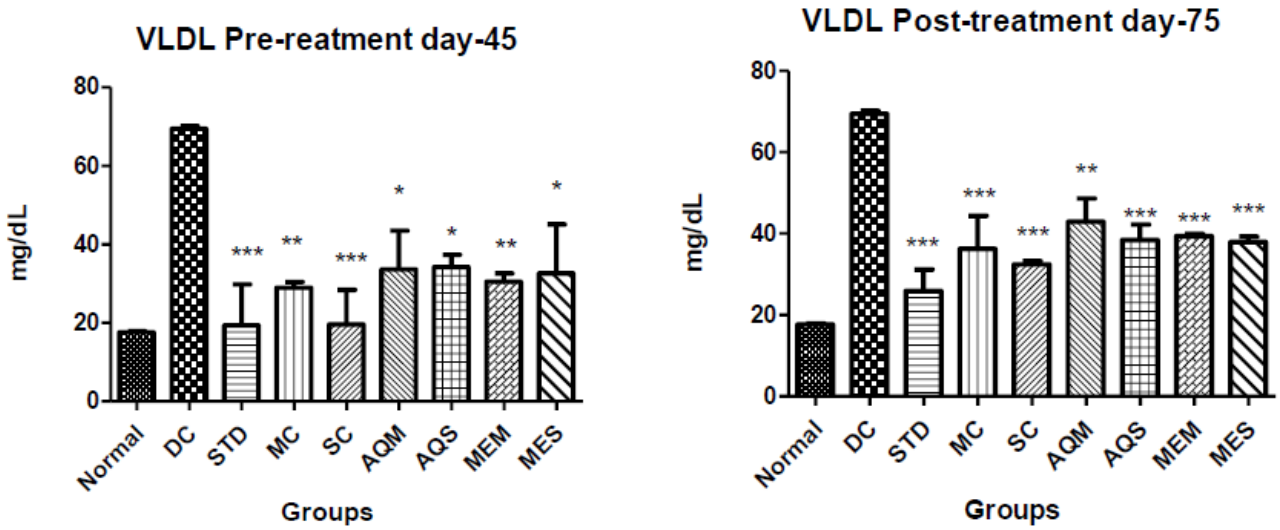
Post-treatment: Highly significant result was observed in MC, SC, STD, AQS, MEM inreducing LDL level when compared to atherosclerotic control.

All the treated groups in pre- treatment showed better result in reduction of LDL than post-treatment.

Table 17: Observation on VLDL

Observation on VLDL in mg/dl		
Groups	**Pre treatment (Day 45)**	**Post treatment (Day 75)**
Normal	17.64 ± 0.25	17.64 ± 0.25
DC	69.47 ± 0.70	69.47 ± 0.70
STD	19.33 ± 10.49	25.89 ± 5.28
MC	28.97 ± 1.42	36.24 ± 8.16
SC	19.60 ± 8.85	32.41 ± 0.85
AQM	33.60 ± 9.85	42.91 ± 5.71
AQS	34.25 ± 3.16	38.43 ± 3.79
MEM	30.54 ± 2.10	39.34 ± 0.55
MES	32.62 ± 12.51	37.89 ± 1.32

Graph No.13 and 14: Comparison on VLDL among the trial groups



Pre treatment: Highly significant result was observed in STD, SC, MC, MEM, in reducing VLDL level when compared to atherosclerotic control.

Post treatment: Highly significant result was observed in STD, SC, MC, MES, AQS, MEM in reducing VLDL level when compared to atherosclerotic control.

All the treated groups in Pre treatment showed better result in reduction of VLDL than post treatment.

Aortic Sudanophilia Test

Sudanophilia was confirmed by dark red regions corresponding to the uptake of Sudan IV by fat. In this test it was observed that *Shatavari churna* and *Musta churna* has shown decreased dark red spot which is the indication of less Sudanophilia.

Histopathology of Aorta

Normal Control: A section of aorta showed intact intimal layer. Elastic fibres and smooth muscle make up the tunica media.

Atherosclerosis control: A section of aorta in the atherosclerotic group showing absence of intimal corrugation and bulge formation. Vacuolated cells in the sub-endothelial layer (foam cell) were observed. There is apparent increase in the thickness of both tunicae intima and media.

Standard (Atorvastatin): A section of aorta in the standard group showing intact tunica intima. Elastic fibres and smooth muscle make up the tunica media. The outermost layer is the tunica adventitia which made of loose connective tissue.

***Musta Churna*:** A section of aorta in the *Musta churna* treated group showing intact tunica intima. The tunica media is made of smooth muscle and elastic fibers. The tunica adventitia formed of loose connective tissue. The thickness of both tunicae intima and media is noted.

***Shatavari Churna*:** A section of an aorta in the *Shatavari churna* treated group showing intact tunica intima with thin wavy corrugated endothelium and thin sub-endothelial layer. Elastic fibres and smooth muscle make up the tunica media.

Aqueous extract of *Musta*: A section of aorta in the aqueous extract of *Musta* treated group showing the tunica media is made of smooth muscle and elastic

fibers. Vacuolated cells (foam cells) are in the sub-endothelial layer. The tunicae intima is thickened.

Aqueous extract of *Shatavari*: A section of an aorta in the aqueous extract of *Shatavari* group showing adhesions of RBCs to the surface of the intima. There is an apparent increase in the thickness of the sub-endothelial layer.

Methanolic extract of *Musta*: A section of aorta in methanolic extract of *Musta* treated group showing prominent intact internal and external elastic laminae. Tunica media looks rich in parallel elastic fibers. Some areas still show loss of elastic fibers.

Methanolic extract of *Shatavari*: A section of aorta in the Methanolic extract of *Shatavari* treated group showing intact tunica intima with thin wavy corrugated endothelium. There is an apparent increase in the thickness of the sub-endothelial layer Elastic fibers and smooth muscle make up the tunica media.

Plate No.3: Histopathological Findings of Pre-treatment group

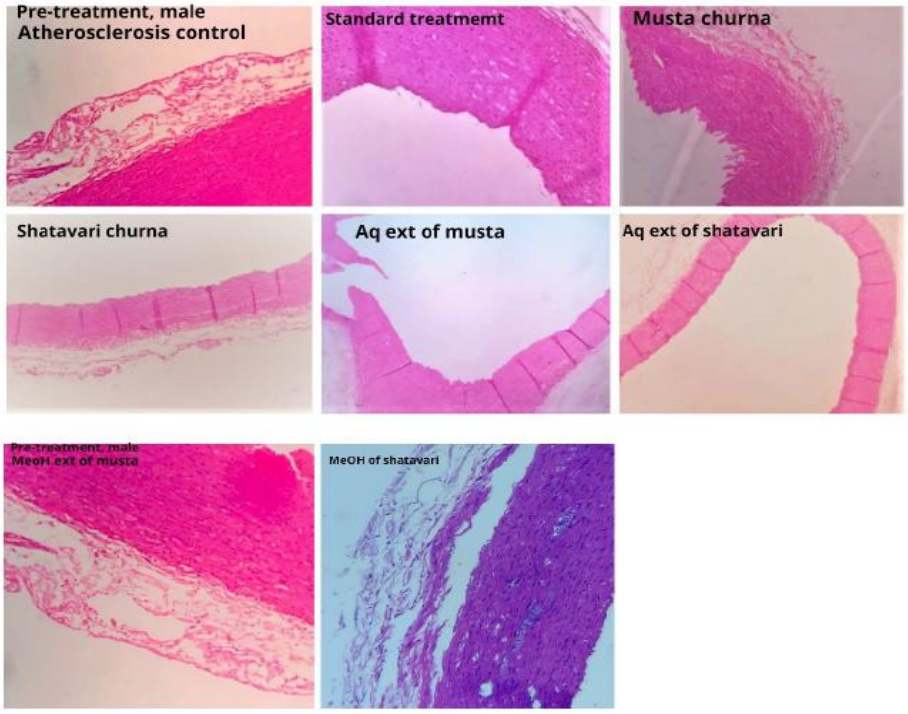
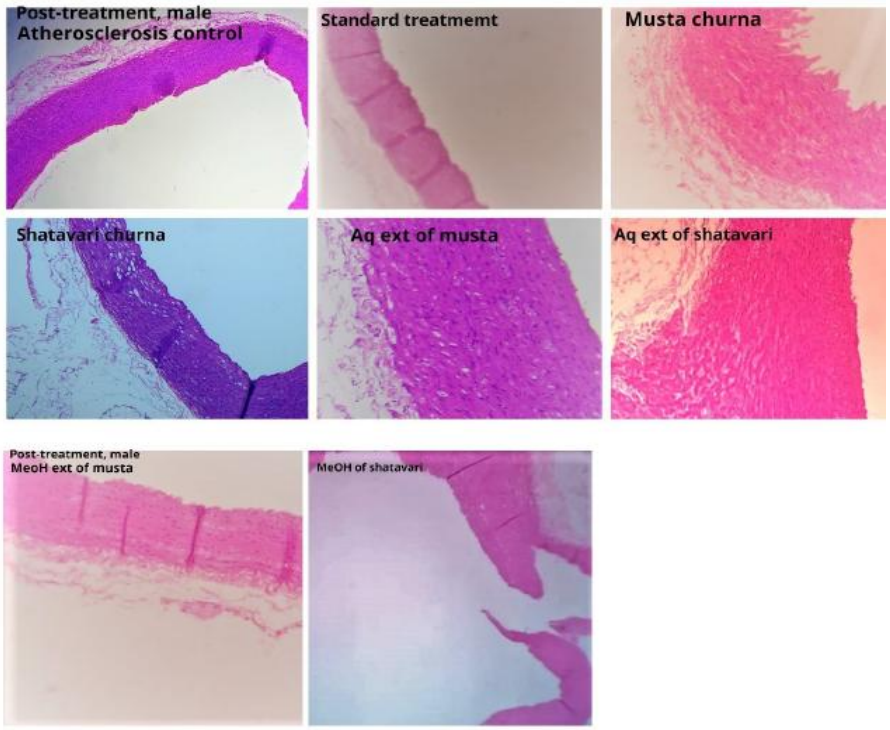


Plate No.4: Histopathological Findings of Post-treatment group



DISCUSSION

Cardiovascular diseases are group of problems that occur when the heart and its blood vessels fail to work. According to WHO, these take the lives of 17.7 million people every year and 31% of all global deaths due to CVD. 80% of its death is due to heart attacks and stroke. Obesity and hyperlipidemia are predisposing factors for major number of cardiovascular diseases, especially Atherosclerosis which has become the major cause for death.

Obesity is a *Medo pradoshaja vikara* and hyperlipidemia is a *Rasa pradoshaja vikara*. *Medas* refers to lipids in the plasma in the context of hyperlipidemia and adipose tissue in context of obesity. The *Malas* produced during the *Dhatu parinama* gives us a lead to understand that *Kapha* as the *Mala* of *Rasa* present in plasma can possess the *Lakshanas* of *Medas* also. This can be justified by *Ashraya ashrayi bhava*. When this becomes abnormal qualitatively and quantitatively, it can lead to hyperlipidemia. This is detected through serological investigations because lipids are the components of plasma. This hypothesis can be strengthened by the principle of treatment adopted in both the condition is *Kaphahara* and *Medohara* which can reduce both hyperlipidemia as well as obesity. Thus, hyperlipidemia correlating with *Rasa pradoshaja vikara* can be justified. Atherosclerosis can be compared to *Dhamani Prathichaya*

Around 160 are said to possess *Kaphamedohara karma*. *Musta* and *Shatavari* are two drugs having dissimilar *Rasapanchakas*. When two drugs are attributed with same activity possess either identical *Rasapanchaka* or disparity in the same needs to be evaluated for the specific activity and indication. Thus, the present study was carried out to compare *Musta* (*Cyperus rotundus* Linn) and *Shatavari* (*Asparagus racemosus* Wild) for anti-atherosclerotic activity invivo.

The parts used in both the drugs are *Kanda* which were authenticated. The pharmacognostic characters of both *Musta* and *Shatavari* were as per the API standards, thus proving their identity. Physico chemical values of both the drugs were within the normal limit which confirms the genuinity. The pH value of *Musta* and *Shatavari* are 5.5 and 6.2 respectively. The acidic nature of *Musta* may be attributed to *Katu Vipaka* and *Deepana- pachana karmas*. *Shatavari* is slightly acidic which may be due to *Madhura Vipaka* and *Deepana karma*. Phytochemical screening of both the drugs showed the presence of alkaloids, glycosides, flavanoids, saponins, tannins, phenolic compounds, protiens and carbohydrates. Additionally, steroids were present in *Shatavari* (methanolic extract) and resins-gums in *Musta* (both extracts). Both the drugs have iron, calcium, magnesium, potassium. *Musta* additionally contains sodium, chloride, sulphate, phosphate.

In the experimental study, New Zealand White rabbits were taken as the animal model because of a unique feature of lipoprotein metabolism like human but unlike rodents.[30] Atherosclerosis was induced by administration of Atherogenic diet, suggested by previous research works. Dietary fat is one of the most important factors associated with cardiovascular disease. High levels of cholesterol and saturated fat in diets have shown to promote atherosclerosis. The standard drug taken was Atorvastatin, which is a HMG CoA reductase inhibitor (substances which helps in metabolism of cholesterol of the blood) effectively used in lowering plasma LDL, cholesterol and triglycerides.[31] The adverse drug reactions are severe liver injuries, haemorrhagic stroke, arthralgia, diarrhoea, and nasopharyngitis. Dosages of 2.5mg/kg bw of Atorvastatin, 500g/kg bw of *Churna*s of *Musta* and *Shatavari*, 500mg/kg bw of aqueous and methanolic extracts of *Musta* and *Shatavari* were taken based on the previous research works. The parameters of the study were body weight, lipid profile and histopathological study of aorta.

In the biochemical analysis and body weight, the female animals did not get induced with atherosclerosis and weight gain in both pre treatment as well as post treatment groups which again supports previous research work. Cholesterol levels show greater seasonal variations in female than in males and are lower in pregnant and lactating females. Owing to these features, males are used more often than females.[31] In male animals, atherosclerosis was confirmed by increase in total cholesterol in both pre-treated and post-treated groups.

Body Weight: Standard, methanolic extract of *Musta*, *Shatavari Churna* showed significant reduction in body weight in post treated group. Both *Musta* and *Shatavari* contain flavanoids and phenolic compounds (polyphenols) which have potential effect in modulating obesity by increasing lipid metabolism, suppression of fat absorption from the gut and by stimulating catabolic pathways in adipose tissues. *Musta* is proved to have effective adipolytic activity.[32]

Blood Glucose: All the post treated groups showed highly significant reduction of glucose. *Churna* and aqueous extract of *Shatavari* showed better result than *Musta*. The anti-hyperglycemic activity of *Musta* may be due to the presence of stilbene dimmer compounds which are considered as potent inhibitors of alpha-glucosidase. [37]

Total Cholesterol: *Shatavari churna* showed highly significant result equivalent to standard in reducing total cholesterol. Aqueous and methanolic extracts of *Musta* and *Shatavari* also exhibited significant result.

Anti hypercholesterolemic activity of *Shatavari* may be due to the presence of phytosterols, saponins, flavonoids, poly phenols, ascorbic acid, essential fatty acid- gamma linolenic acid which could be responsible for increased bile acid production and elimination of excess cholesterol.[38] Sesquiterpenes in *Musta* exhibit effective lipid peroxidation.

Triglycerides: *Churna*s of *Shatavari* and *Musta* have shown better result than their extracts. *Shatavari churna* exhibited result equivalent to standard in reducing triglycerides. Both *Musta* and *Shatavari* contain Saponins which are reported to lower the triglycerides by inhibiting pancreatic lipase activity. [38,39]

HDL: Significant results were not observed in any of the treated groups.

LDL: All the trial groups significantly reduced LDL in pretreated groups. *Shatavari churna* and *Musta churna* were effective in post treated group. Lipophilic phenolic compounds and steroidal saponins present in *Shatavari* helps in reducing the formation of lipid peroxidation.[40] *Musta* helps in lowering the plasma LDL. [31]

VLDL: *Churna* and both extracts of *Shatavari*, *Churna* and aqueous extracts of *Musta* showed significant reduction of LDL in post treatment. *Shatavari churna* exhibited similar result as that of standard in pre-treatment. *Musta* and *Shatavari* have flavonoids which act as anti-oxidants due to their free radical scavenging activity and protect tissue against free radicalmediated lipid peroxidation. They aid in vasodilation and regulate the programmed cell death process in the endothelium (the inner lining of blood vessels).

Histopathology: *Shatavari churna* and *Musta churna* have shown less dark red spots (indication of less sudanophilia) than other treated groups. This supports *Hrudya karma* of *Shatavari* and *Lekhana karma* of *Musta*. Sitosterols present in both *Musta* and *Shatavari* are recommended for the prevention of different cardiovascular diseases. It prevents the absorption of cholesterol by displacing it from micelles, thereby decreasing the amount in plasma.

Probable Mode of Action

***Deepana*:** Both *Musta* and *Shatavari* are attributed with *Deepana karma*. Hyperlipidemia is abnormal increase of *Rasa mala* i.e., *Kapha* and *Sthoulya* is *Medo dhatu vruddhi*. Both the conditions are tackled upon by the rectification of respective *Agnis*. *Tikta rasa* in both drugs does A*mapachana*, thus correcting the *Agni.*

***Kaphahara, Medohara*:** *Tikta rasa* of *Shatavari*, *Tikta-kashaya rasa* of *Musta* helps to reduce *Kapha* and *Medas.* [9] Both drugs possess *Sheeta veerya*, because of which inspite of *Kaphamedo Shoshana*, there will be no exhaustion (*Prahladana karma* of *Sheeta veerya*). In addition, the presence of carbohydrates, starch and proteins help in promoting energy. Presence of saponins, tannins and phenolic compounds act as hypolipidemic.

***Dhamani Upalepahara*:** This refers to deposition of lipids (*Kapha*) in the form of *Mala* responsible for sclerotic changes in the arteries. *Upalepahara karma* is achieved by *Tikta- Kashaya-Katu rasa, Laghu-Ruksha guna, Katu vipaka* and *Lekhana karma* of *Musta*. Though *Shatavari* has *Madhura-Tikta Rasa*, *Guru-Snigdha Guna, Madhura Vipaka,* it acts as *Upalepahara* which may be due to its *Hrudya karma*. "*Hrudayaya hitam priyam yat tat Srotah shudyartham deyam*"- One which is *Hita* to *Hrudaya* and does *Srothoshodana* is *Hrudya. Hrudaya* and *Dasha Dhamanis* are the *Sthanas* for *Rasa. Srothoshodhana* in *Dhamanis* can be correlated to *Upalepahara.*

Thus, the present study reveals that *Musta* and *Shatavari* have effective anti-atherosclerotic activity, the latter being comparatively better.

CONCLUSION

Both *Musta* and *Shatavari* fall under the framework of potential anti-atherosclerotic drugs. *Churna* and aqueous extract of *Shatavari* were effective than methanolic extract. *Churna* and methanolic extract of *Musta* were effective than aqueous extract. Both the drugs were effective in both preventive and curative aspects. *Musta* is drug of choice in obesity associated with hyperlipidemia. *Shatavari* is potent drug in hyperlipidemia associated with *Rasa Kshaya Lakshanas*

ACKNOWLEDGEMENT

This research work is financially supported by Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka [Project grant number: 19AYU136].

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Cite this article as:
Dharani, Savita B Sutagatti, Ahalya S, Lalitha B.R. A Comparative Analytical and Invivo Evaluation of Musta (Cyperus Rotundus Linn.) and Shatavari (Asparagus Racemosus Wild.) for Anti Atherosclerotic Activity. AYUSHDHARA, 2022;9(4):1-17.
https://doi.org/10.47070/ayushdhara.v10i4.1001
Conflict of interest: None Declared

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