Original Research Article

Antiatherosclerotic activity of methanolic extract of *Woodfordia fructicosa* flowers

ABSTRACT:

In the present investigation the methanolic extract of *Woodfordia fructicosa* flowers at the doses of 100, 200 and 400 mg/kg was investigated for antiatherosclerotic against high fat diet induced atherosclerosis and triton induced atherosclerosis. In high fat diet induced atherosclerosis several parameters of lipid profile such as total cholesterol (TC) and triglycerides (TG), lipoprotein profile such as low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc) and high density lipoprotein cholesterol (HDLc), atherosclerotic markers such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) and atherogenic index parameters such as TC/HDLc, LDLc/HDLc were determined and found to significantly altered in induction control group treated with high fat diet. The histopathological studies of liver and heart tissue were also performed wherein high fat diet showed toxic effects on cardiac and hepatic tissue. Similarly, in triton induced atherosclerosis parameters of lipid profile such as total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein levels were determined and were found to be significantly increased in induction control group. methanolic extract of *Woodfordia fructicosa* flowers showed protection against the atherosclerosis by bringing back the altered parameters to normal I both the models and showing ameliorating effects against high fat diet induced hepatic and cardiac damage. The multistep putative action of methanolic extract of flowers of *Woodfordia fructicosa* is attributed to the prominent phytoconstituents namely ellagic acid estimated through HPTLC analysis of the extract. Thus the study exhibited the protective effect of methanolic extract of flowers of *Woodfordia fructicosa* against atherosclerosis.

KEYWORDS: Atherosclerosis; Cardioprotection; High fat diet; Triton

INTRODUCTION:

Cardiovascular disease (CVD) is the leading cause of mortality and is a growing health challenge in developing countries. CVD is not a single disease, but a cluster of diseases and

injuries that affect the cardiovascular system usually affecting in later stage of life. This peculiarity is responsible to disturb productive to dependant ratio [1]. Atherosclerosis is a chronic condition in which arteries harden through build-up of plaques. It is the predominant contributor to mortality and serious morbidity in the Western world. Westernization of the life-style, including diet, may account for the increased incidence of coronary and cerebral artery diseases. The classical risk factors for atherosclerosis include dyslipoproteinaemia, diabetes, cigarette smoking, hypertension and genetic abnormalities. The end result of the stenosis caused by the atherosclerotic plaques are the terminal events-acute coronary syndrome, myocardial infarction, fatal arrhythmias, sudden cardiac death [2].

Large number of medicines with different mechanisms like HMG-COA reductase inhibitors, bile acid sequestrants, triglyceride lowering drugs, HDL enhancing therapies etc. are available however none of them have been reported to be ideal especially on long term and with respect to occurrence of side effects [3].

On the other hand, published literature states that a large number of plants in the traditional (Herbal/Alternative) systems of medicine are providing comprehensive relief to the people suffering from cardio-vascular diseases including ischemic heart disease [4,5].

Woodfordia fructicosa, is one of such herbal medicines can be effective against atherosclerosis. *Woodfordia fructicosa* L., is a traditional medicinal plant belonging to the family Lythraceae. Its therapeutic potential has been well documented in traditional, complementary, and alternative medicine for more than a century. Several pharmacological investigations have revealed that *Woodfordia fructicosa* possesses antitumor, antioxidant, immunomodulatory, antidiabetic, antiproliferative, anihyperlipidemic, antimicrobial, heppatprotective and anti-ulcer properties [6,7]. *Woodfordia fructicosa,* has been traditionally claimed to possess antiatherosclerotic and cardioprotective properties but hasn't yet been explored scientifically till now to best of our knowledge [8]. In light of this, the investigation of anticonvulsant effect of methanolic extract of flowers of *Woodfordia fructicosa* against high fat diet induced atherosclerosis and triton induced atherosclerosis model was carried out.

MATERIAL AND METHODS

Plant material collection and authentication:

Flowers of *Woodfordia fructicosa* were procured from local market. The plant materials were taxonomically identified and authenticated.

Preparation of extract:

The powdered drug (100 gm.) was extracted successively using a Soxhlet extractor with 200ml each of methanol and ethyl acetate. Extract was filtered, concentrated and after complete solvent evaporation, each of these solvent extract was weighed and preserved at 5°C in an airtight bottle until further use.

Preliminary phytochemical screening of extract

The methanolic extract of *Woodfordia fructicosa* flowers were analyzed for the presence of phytochemical constituents such as terpenoids, alkaloids, quinines, flavonoids, saponins, steroids and phenolic compounds using the standard qualitative phytochemical methods [9].

Drugs and chemicals

All the drugs and chemicals of AR grade were procured from local vendor.

Animals

Swiss albino mice (20-40gm) and Wistar albino rats (180-220gm) of either sex were procured from Local vendor and were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle) at animal house. The animals had free access to food and water throughout study. Institutional Animal Ethical Committee approved the protocol. All the experiments were carried out between 9:00- 16:00 hour.

Ethical clearance:

Institutional Animal Ethical of D. Y. Patil College of Pharmacy, Akurdi, Pune approved the protocol

Preliminary acute toxicity test

Healthy adult male Swiss albino mice (18-22 g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic cooperation and development (OECD-2000). The mice were observed continuously for 2 h for behavioural and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days [10].

Antiatherosclerotic activity

a) High fat diet induced atherosclerosis model:

48 rats (180-220gm) of either sex were divided into 08 groups, each consisting of 06 rats. All these rats except rats of Group-I (normal group) were fed with especially ordered high fat diet (90% standard chow, 08% saturated fat i.e. groundnut oil, 2% cholesterol and 0.1% calcium) for the period of 30 days, while normal rats fed with standard chow pellet.

All these rats received respective treatments from 1st to 30th day. The food intake and weight of rats was measured on 10th, 20th and 30th day.

On 31st day, the blood samples were collected from retro orbital sinus and following various parameters were estimated:

•Lipid profile: total cholesterol (TC) and triglycerides (TG)

•Lipoprotein profile: low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc), high density lipoprotein cholesterol (HDLc).

•Atherosclerotic markers: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine phosphokinase (CPK).

•Atherogenic index: TC/HDLc, LDLc/HDLc

On 31st day, one hour after estimation of blood, representative rat from each group was sacrificed; heart and liver were isolated, weighed and subjected to histopathological investigations [11].

b) Triton induced atherosclerosis model:

Preselected healthy 36 rats were weighted and numbered. They were randomly divided into 06 groups each consists of 06 rats, and labelled as normal control, induction control, test(s) and reference stand. The respective treatment was given for the period of 07 days (During this period rats had free access to food and water). On 7th day; 01 hour prior to administration of respective treatment, triton WR 1339, 100 mg/kg was injected intraperiotneally. 24 hours, after the administration of respective treatment, blood sample was collected, and allowed to coagulate for 30 minutes at room temperature. After 30 minutes, coagulated blood sample was centrifuged at 2500 rpm for the period of 10 minutes. The separated serum was collected in fresh container. Total cholesterol triglyceride LDL, VLDL levels were estimated using diagnostic kits [12].

Statistical analysis:

Results were expressed as mean + SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by One-way analysis of variance

(ANOVA) followed by Bonferroni multiple comparisons test. P > 0.05 were considered insignificant.

High Performance Thin Layer Chromatography (HPTLC) studies of extract

Instrumentation

HPTLC system of CAMAG, Muttenz, Switzerland, Anchrom Enterprises (I) Pvt. Ltd, Mumbai, consisting of sample applicator (Linomat 5), Twin trough chamber with lid {10×10 cm, CAMAG, Muttenz, Switzerland), UV cabinet (Aetron, Mumbai) with dual wavelength (254/366 nm) and the HPTLC photodocumentation (Aetron, Mumbai) was used for study. FT-IR, NMR were recorded at Department of Chemistry, North Maharashtra University, Jalgaon. LC-MS was carried out at Venture Centre, Pune. IR spectra was recorded using KBr on "Shimadzu FT-IR IR Spirit" by KBr method. 1H-NMR spectra was recorded in CDCl3 solution on "FTNMR VARIAN MERCURY YH-300" using tetramethyl silane (TMS) as internal standard.

Purity checking and Mass Spectra recording was carried by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI/MS) with accurate mass measurements upto four decimal. It was recorded on Agilent LC-MS Q – TOF (6200 series TOF/6500 series) (5301 Stevens Creek Blvd, Santa Clara, CA 95051, United States) equipped with a dual AJS ESI with improved sensitivity [AJS – ESI: Agilent Jet Stream Electro Spray Ionizer] and Q-TOF B.05.01software version.

Chromatographic conditions

The sample was spotted in the form of bands of width of 6 mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F254 (5 cm ×10 cm) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using n-Hexane: Ethyl acetate (4: 6 v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 15 min. The length of chromatogram run was 8 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier.

Sample Preparation

10 mg of methanolic extract of *Woodfordia fruticosa* flower was dissolved in 10 ml of Methanol. 10 µl volume of clear supernatant sample was applied on the TLC plate.

Calculation of Rf Values:

Plate was observed in the daylight, under UV light (254 and 366 nm). Retention factor (Rf) was calculated by following formula (Chatwal and Anand, 2004; Sethi and Charegaonkar, 1999).

Rf = A/B

A = distance between point of application and central point of spot of material being examined.

B = distance between the point of application and the mobile phase front [13].

Results:

Preliminary phytochemical screening

The results of preliminary phytochemical screening of methanolic extract hydroalcoholic extract of flowers of *Woodfordia fructicosa* (MWF) showed the presence of steroids, triterpenoids, alkaloids, glycosides, proteins and carbohydrates.

High fat diet induced atherosclerosis model:

Estimation of lipid profile

Table No. 1. Effect of extract on the total cholesterol (TC) levels

Experimental Groups	**TC (mg/dl) (Mean±SEM)**	
	Day 1	**Day 31**
Normal Control	69.16± 2.35	69.83±2.61
Positive Control	74.33± 2.47	154.33±2.67***
MWF 100	71.16± 2.63	142.16±2.46$^{\#}$
MWF 200	70.66±2.23	140.16± 2.89$^{\#\#}$
MWF 400	71.50±2.99	137.50±2.18$^{\#\#\#}$
FF65	69.50 ±2.70	97.66±3.92$^{\#\#\#}$

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test $^{***}P<0.001$ as compared to normal control. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ as compared to positive control

The results of the study suggested that there was significant (P<0.001) increase in the total cholesterol levels on 31st day in high fat diet induced positive control group as compared to normal control group. The treatment with MWF showed significant and dose dependent inhibition of increasing level of total cholesterol by high fat diet at the doses 100 mg/kg (P<0.05), 200 mg/kg (P<0.01) and 400 mg/kg (P<0.001). Reference standard (FF65) showed highest activity in this regards.

Table No. 2. Effect of extract on the triglycerides (TG) levels

Experimental Groups	**TG (mg/dl) (Mean±SEM)**	
	Day 1	**Day 31**
Normal Control	48.83±3.85	57.50±3.06
Positive Control	50.66±3.21	149.50±3.10***
MWF 100	49.33±3.64	138.00±2.93#
MWF 200	47.00±3.63	137.50±3.41#
MWF 400	45.50±3.99	133.3±2.66##
FF65	52.00 ±4.04	97.50±2.71###

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test $^{***}P<0.001$ as compared to normal control. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ as compared to positive control.

The results of the study revealed significant (P<0.001) increase in the TG levels on 31st day in high fat diet induced positive control group as compared to normal control group. The treatment of MWF at 200 mg/kg ad 400 mg/kg showed significant (P<0.01 and P<0.01

respectively) reduction in the TG levels. Reference standard (FF65) was found to be more effective in this regards.

Estimation of lipoprotein profile

Table No. 3. Effect of extract on the low density lipoproteins (LDL) levels

Experimental Groups	**LDL (mg/dl) (Mean±SEM)**	
	Day 1	**Day 31**
Normal Control	27.33±1.66	31.33±1.62
Positive Control	26.16±1.77	99.00±3.15***
MWF 100	26.83±2.05	97.83±3.31
MWF 200	26.00±1.80	86.33±3.49##
MWF 400	26.16±1.35	72.83±1.85###
FF65	26.16 ±1.66	61.83±2.12###

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test ***P<0.001 as compared to normal control. #P<0.05, ##P<0.01, ###P<0.001 as compared to positive control

The results of the study indicated that there was a significant (P<0.001) increase in the LDL levels on 31st day in high fat diet induced positive control group as compared to normal control group. The administration of MWF significantly reduced the elevated levels of LDL in dose dependent manner at doses 200 mg/kg (P<0.01) and 400 mg/kg (P<0.01). The lowest dose of MWF was found to be ineffective.

Table No. 4. Effect of extract on the very low density lipoproteins (VLDL) levels

Experimental Groups	**VLDL (mg/dl) (Mean±SEM)**	
	Day 1	**Day 31**
Normal Control	13.50±1.33	17.00±1.65
Positive Control	14.33±1.45	32.66±1.83***

MWF 100	15.33±1.08	28.33±0.80
MWF 200	18.33±1.80	27.00±0.87$^{\#\#}$
MWF 400	15.66±2.40	26.83±0.73$^{\#\#\#}$
FF65	14.00 ±1.88	25.50±1.05$^{\#\#\#}$

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test ***P<0.001 as compared to normal control. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 as compared to positive control.

The results of the study indicated that there was a significant (P<0.001) increase in the VLDL levels on 31st day in high fat diet induced positive control group as compared to normal control group. These increased levels were reduced by MWF. The lowest dose of MWF was insignificant which is on similar lines of VLDL. The treatment of MWF showed significant effect at higher two doses i.e. 200 mg/kg (P<0.01) and 400 mg/kg (P<0.001) on 31st day. The reference standard (FF65) was most potent and efficacious in this regards.

Estimation of atherosclerotic markers:

Table No. 5. Estimation of alanine transaminase (ALT)

Experimental Groups	**ALT (U/L) (Mean±SEM)**	
	Day 1	**Day 31**
Normal Control	76.66±1.80	80.83±1.74
Positive Control	83.33±3.60	125.00±2.26***
MWF 100	79.00±1.59	123.16±2.30
MWF 200	85.83±2.71	120.33±1.70
MWF 400	89.50±1.45	113.83±2.17$^{\#\#}$
FF65	82.83 ±2.06	102.33±2.15$^{\#\#\#}$

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test ***P<0.001 as compared to normal control. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 as compared to positive control.

There was significant increase (P<0.001) in the ALT levels on 31st day in high fat diet induced positive control group as compared to normal control group. These elevated levels of ALT were significantly (P<0.01) reduced by the treatment with MWF but only at highest doses (400 mg/kg). MWF showed equipotent effect. The lower of the MWF extract was ineffective. Reference standard was most significant (P<0.001) in this regards.

Table No. 6. Estimation of alanine transaminase (AST)

Experimental Groups	**AST (U/L) (Mean±SEM)**	
	Day 1	**Day 31**
Normal Control	93.66±2.59	98.00±2.03
Positive Control	94.16±3.08	136.80±2.60***
MWF 100	95.50±2.36	131.83±3.01
MWF 200	95.00±2.33	135.00±2.12
MWF 400	99.33±1.66	125.83±2.54#
FF65	98.83 ±1.53	123.16±2.27##

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test ***P<0.001 as compared to normal control, #P<0.05, ##P<0.01, ###P<0.001 as compared to positive control.

The results of the study showed that there was significant (P<0.001) increase in the AST levels on 31st day in high fat diet induced positive control group as compared to normal control group. These levels were significantly (P<0.05) reduced by the treatment with MWF extract but only at highest doses (400 mg/kg). The lower dose of MWF extract was ineffective. Reference standard was the most significant (P<0.01) in the regards.

Estimation of lactate dehydrogenase (LDH)

Table No. 7. Estimation of alanine transaminase (LDH)

Experimental Groups	**LDH (U/L) (Mean±SEM)**	
	Day 1	**Day 31**

Normal Control	240.66±2.45	239.83±2.02
Positive Control	236.83±3.00	538.83±4.15***
MWF 100	239.33±2.99	538.66±2.29
MWF 200	238.16±2.66	532.16±3.99
MWF 400	236.50±2.17	518.00±3.75##
FF65	237.83±3.38	447.83±4.58##

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test ***P<0.001 as compared to normal control, #P<0.05, ##P<0.01, ###P<0.001 as compared to positive control.

The results revealed that there was significant (P<0.001) increase in the LDH levels on 31st day in high fat diet induced positive control group as compared to normal control group. These levels were significantly (P<0.05) reduced by the treatment with the extract but only at highest dose (400 mg/kg). The extract was ineffective at lower dose. Reference standard FF65 was the most significant (P<0.01) in the regards.

Estimation of atherogenic index: TC/HDLc, LDLc/HDLc

Table No. 8. Estimation of atherogenic index: TC/HDLc, LDLc/HDLc

Experimental Groups	**TG/HDL (Day 1)**	**TG/HDL (Day 31)**	**LDL/HDL (Day 1)**	**LDL/HDL (Day 31)**
	(Mean±SEM)			
Normal Control	0.81± 0.06	0.94± 0.04	0.45±0.05	0.51±0.06
Positive Control	0.84±0.08	2.63±0.03***	0.43±0.04	1.74±0.05***
MWF 100	0.87± 0.05	2.31±0.02	0.47±0.06	1.63± 0.02
MWF 200	0.82±0.07	2.32±0.06	0.45±0.02	1.45±0.08
MWF 400	0.86±0.02###	2.07± 0.06##	0.49±0.03	1.13±0.05##
FF65	0.84±0.08	1.48±0.03###	0.42±0.05	0.94±0.01###

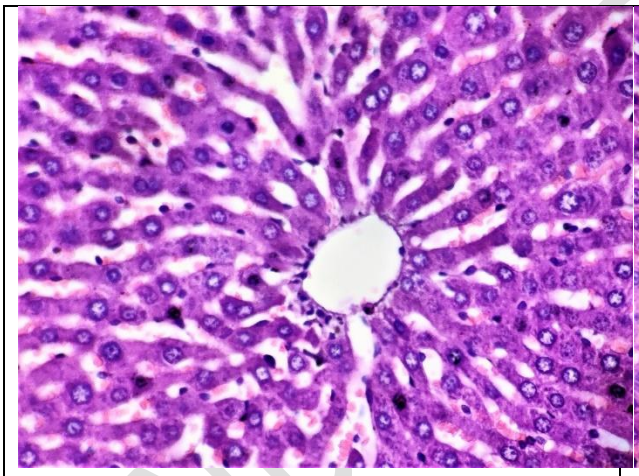
Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance

(ANOVA) followed by Bonferroni multiple comparisons test $^{***}P<0.001$ as compared to normal control, $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ as compared to positive control

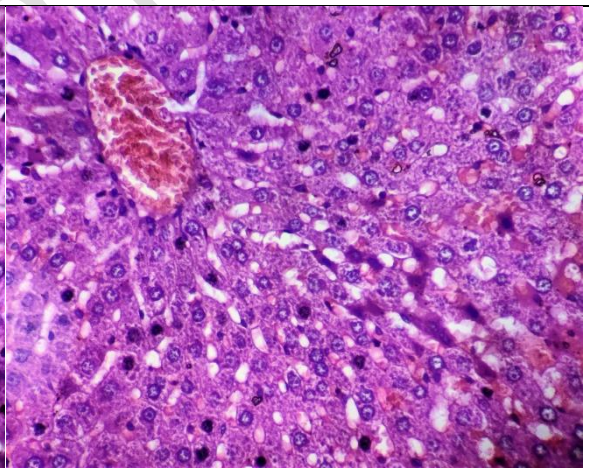
The atherogenic indices TC/HDLc, LDLc/HDLc were found to be significantly increased on 31st day in high fat diet induced positive control group as compared to normal control group. These indices were significantly ($P<0.01$) reduced by the treatment with MWF extract but only at highest doses (400 mg/kg). The lower of MWF was found to be ineffective. Reference standard FF65 was found to be most significant ($P<0.01$) in the regards.

Histopathological investigations of liver

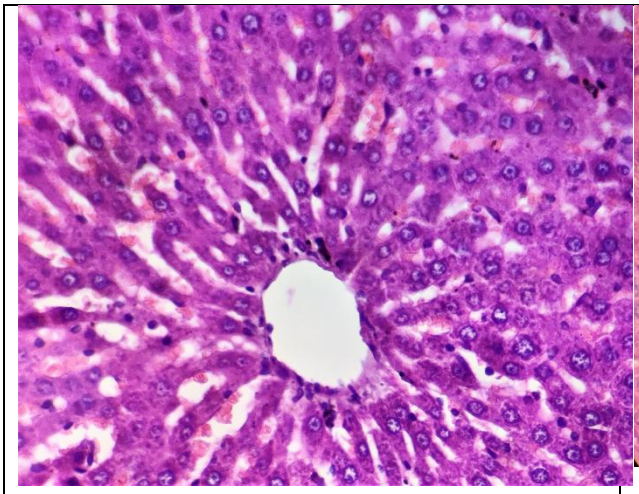
High fat diet showed minimal focal bile duct proliferation, minimal focal sinusoidal congestion along with vacuolisation and inflammatory infiltration, congestion of central vein and periportal scattered inflammation in the liver tissue. Pretreatment with extract MWF 400 and standard FF65 effeciently amleliorated these alterations showing protective activity against high fat diet induced hepatotoxicity.

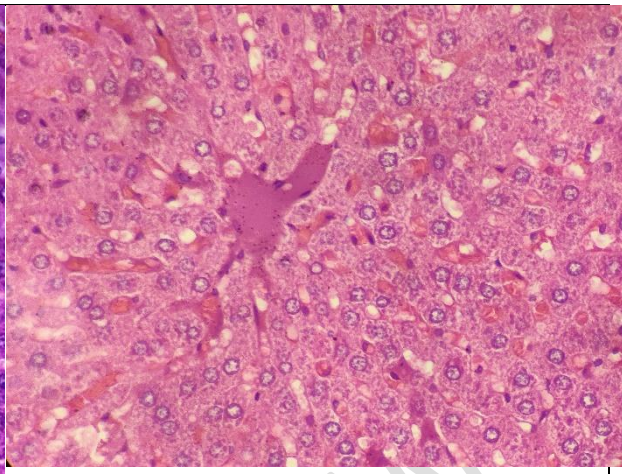


A. Normal Control



B. Positive Control





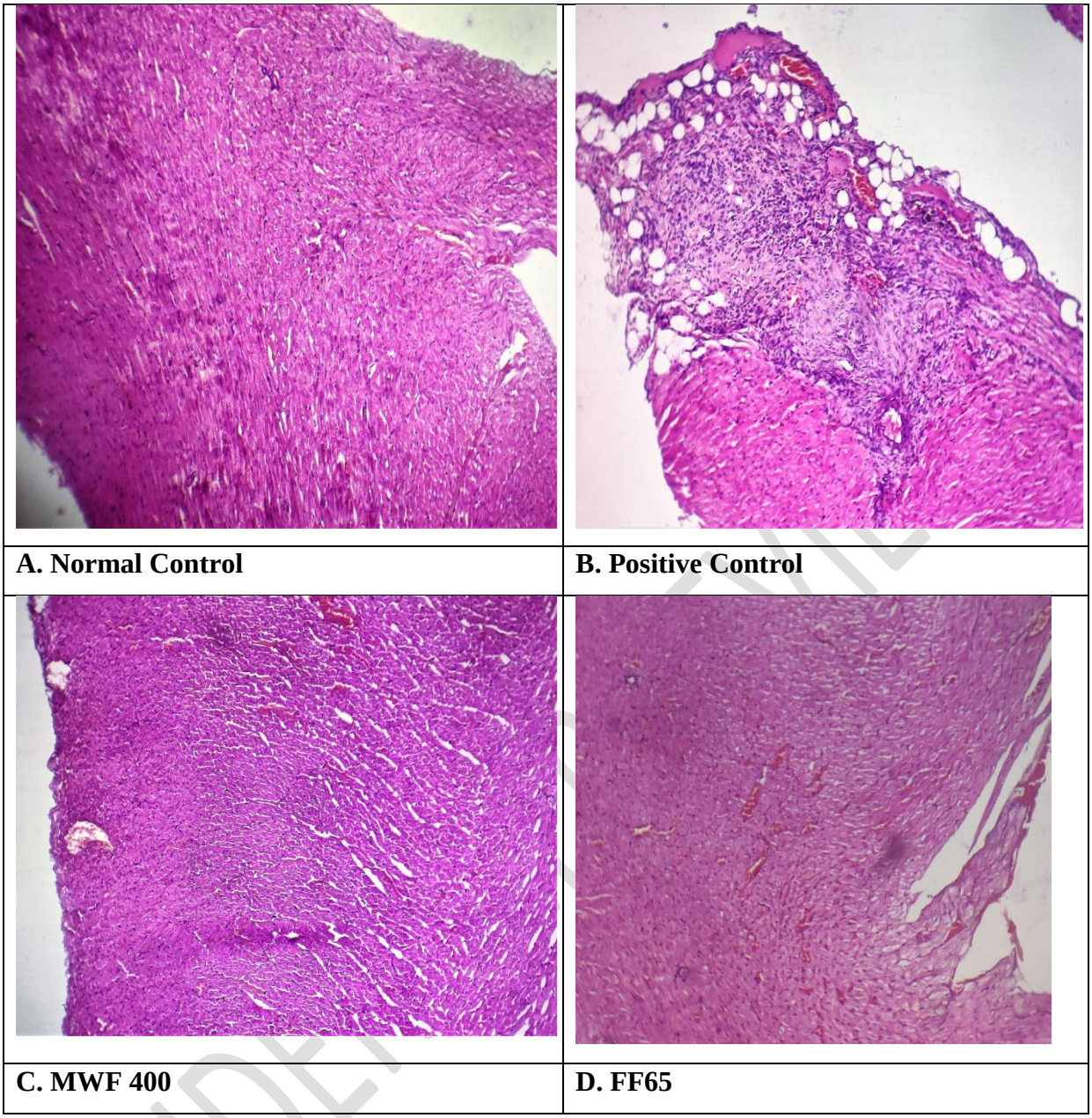
C. MWF 400	D. FF65

Figure 1. Effect of methanolic extract of flowers of *Woodfordia fructicosa* (MWF) on high fat diet induced changes in the liver tissue

Representative photomicrographs (H & E stain) of liver tissue (A) Normal control (B) Positive control (C) MWF 400 (D) FF65. Rats subjected high fat diet showed minimal focal bile duct proliferation, minimal focal sinusoidal congestion along with vacuolisation and inflammatory infiltration, congestion of central vein and periportal scattered inflammation. Pretreatment with extract MWF 400 and standard FF65 effeciently amleliorated these alterations.

Histopathological investigations of heart

High fat diet produced inflammatory cells infiltration, fibrous proliferation, blood vessel congestion, higher collagen deposition and hypertrophied cardiomyocytes in heart tissue. Pretreatment with extract MWF 400 mg/kg and standard FF65 effeciently amleliorated these alterations showing protective activity against high fat diet induced cardiotoxicity.



A. Normal Control	B. Positive Control
C. MWF 400	D. FF65

Figure 2. Effect of methanolic extract of flowers of *Woodfordia fructicosa* (MWF) on high fat diet induced changes in the heart tissue

Representative photomicrographs (H & E stain) of liver tissue (A) Normal control (B) Positive control (C) MWF 400 (D) FF65. Rats subjected high fat diet showed produced inflammatory cells infiltration, fibrous proliferation, blood vessel congestion, higher collagen deposition and hypertrophied cardiomyocytes in the heart tissue. Pretreatment with extract MWF 400 and standard FF65 effeciently amleliorated these alterations.

Triton induced atherosclerosis model:

A) Estimation of lipid profile

Table No. 9 Effect of extract on the lipid levels

Experimental Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
	(Mean±SEM)			
Normal Control	158.01± 2.66	202.51± 4.67	75.88±1.25	125.81±1.61
Positive Control	196.75±3.18***	280.35±3.22***	57.75±1.64***	174.20±1.65***
MWF 100	195.44± 2.54	280.30±1.62	55.88±1.06	173.86± 2.11
MWF 200	188.15±2.07	273.34±1.66	64.39±1.32##	162.84±2.08##
MWF 400	176.26±1.59###	266.47± 3.60##	64.93±1.37##	157.90±2.85###
FF65	165.53±1.38###	231.67±2.34###	70.83±1.56###	144.96±1.99###

Results were expressed as mean ± SEM (n=6). Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test ***P<0.001 as compared to normal control. #P<0.05, ##P<0.01, ###P<0.001 as compared to positive control

The results of the study suggested that there was significant (P<0.001) increase in TC, TG and LDL levels while HDL levels were found to be significantly reduced in triton induced control group as compared to normal control group. MWF extract was found to be significant (P<0.001) in this regards at higher dose only (400 mg/kg). Reference standard showed highest activity in this regards.

High Performance Thin Layer Chromatography (HPTLC) studies of methanolic extract of *Woodfordia fruticosa* flowers

A

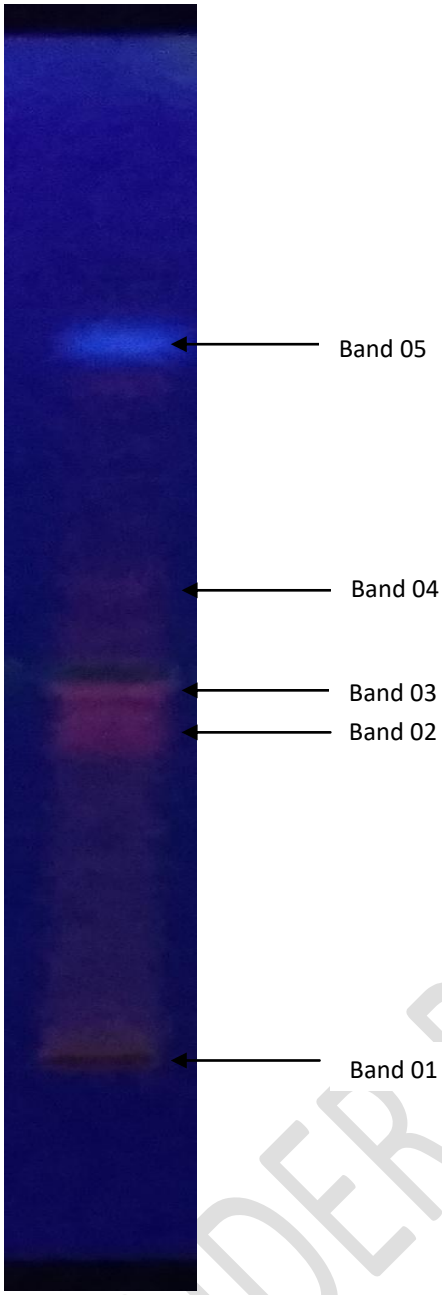


Figure 3. Methanolic extract of flowers of *Woodfordia fruticosa* at 366 nm, Volume applied 10 µl.

The Band 2 at Rf Value 0.47 was scratched and subjected to structure elucidation.

B

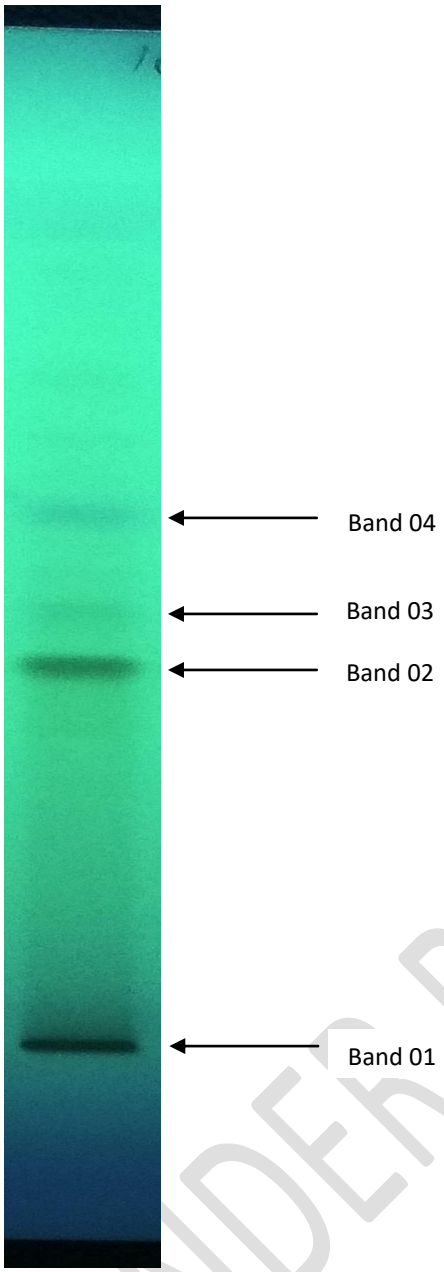


Figure 4. Methanolic extract of flowers of *Woodfordia fruticosa* at 254 nm, Volume applied 10 µl.

The Band 2 at Rf Value 0.47 was scratched and subjected to structure elucidation.

C

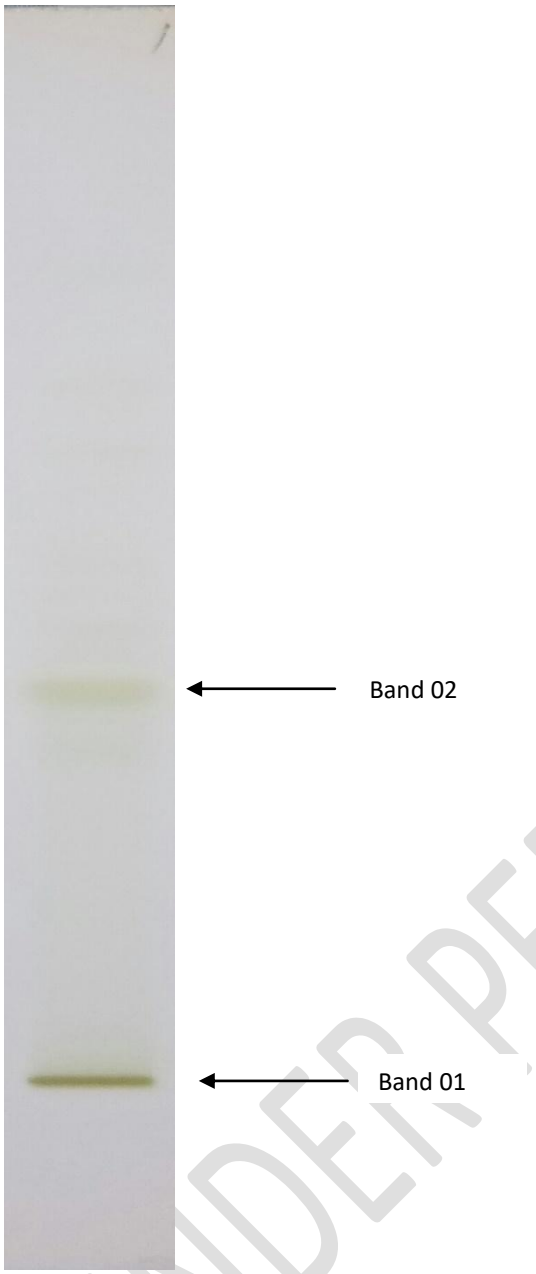


Figure 5. Methanolic extract of flowers of *Woodfordia fruticosa* at Visible light, Volume applied 10 μl.

The band at Rf Value 0.47 was scratched, extracted with methanol and evaporated to dryness (The process required semipreparative TLC to achieve sufficient amount) for further analysis by IR, NMR and Mass Spectrometry.

Spot at Rf Value – 0.47

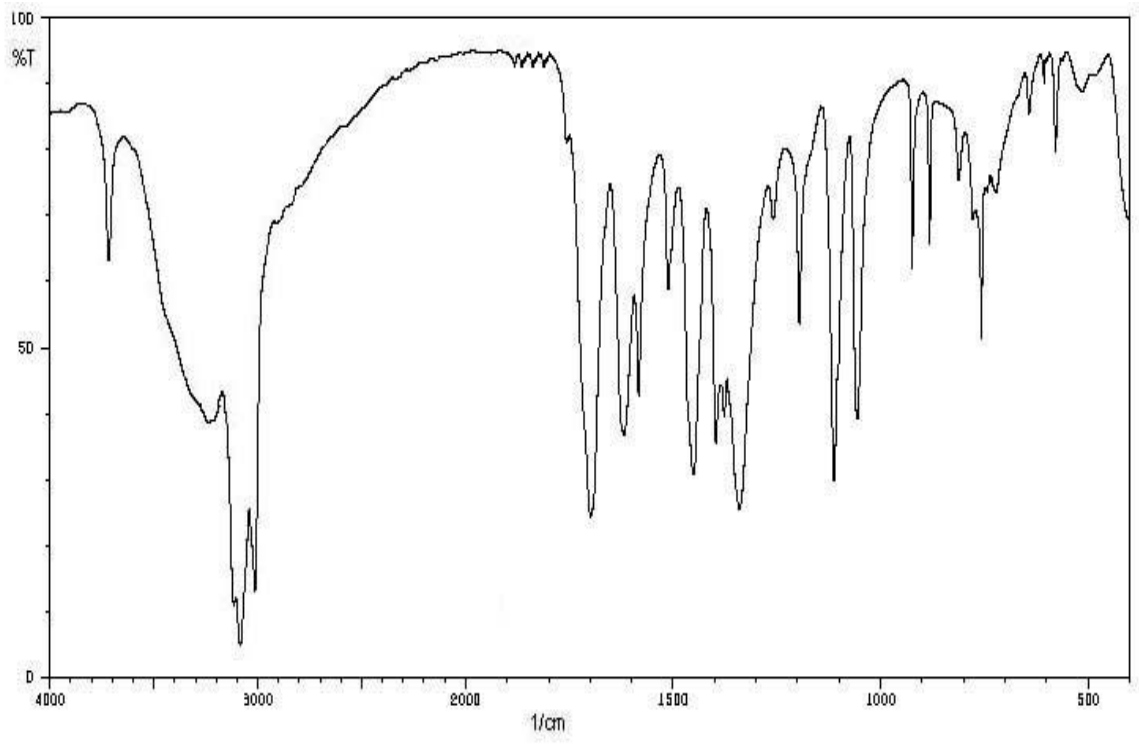


Figure 6. FT-IR Spectrum of compound at Rf – 0.47

Table 10. Interpretation of FTIR spectrum of compound

Sr. No.	**Part of molecule**	**Vibration**	**General Range (Cm^{-1})**	**PI 36**
1	Ar Rings	a) C=C stretch b) C-H stretch δ (ppm) c) C-H bend d) Overtone	1500-1650 3000-3100 740-762 1700-2000	1649 3092 758 1700-2000
2	-O-H	O-H stretch	3200-3600	3231
3	C=O	C=O stretch	1650-1750	1708

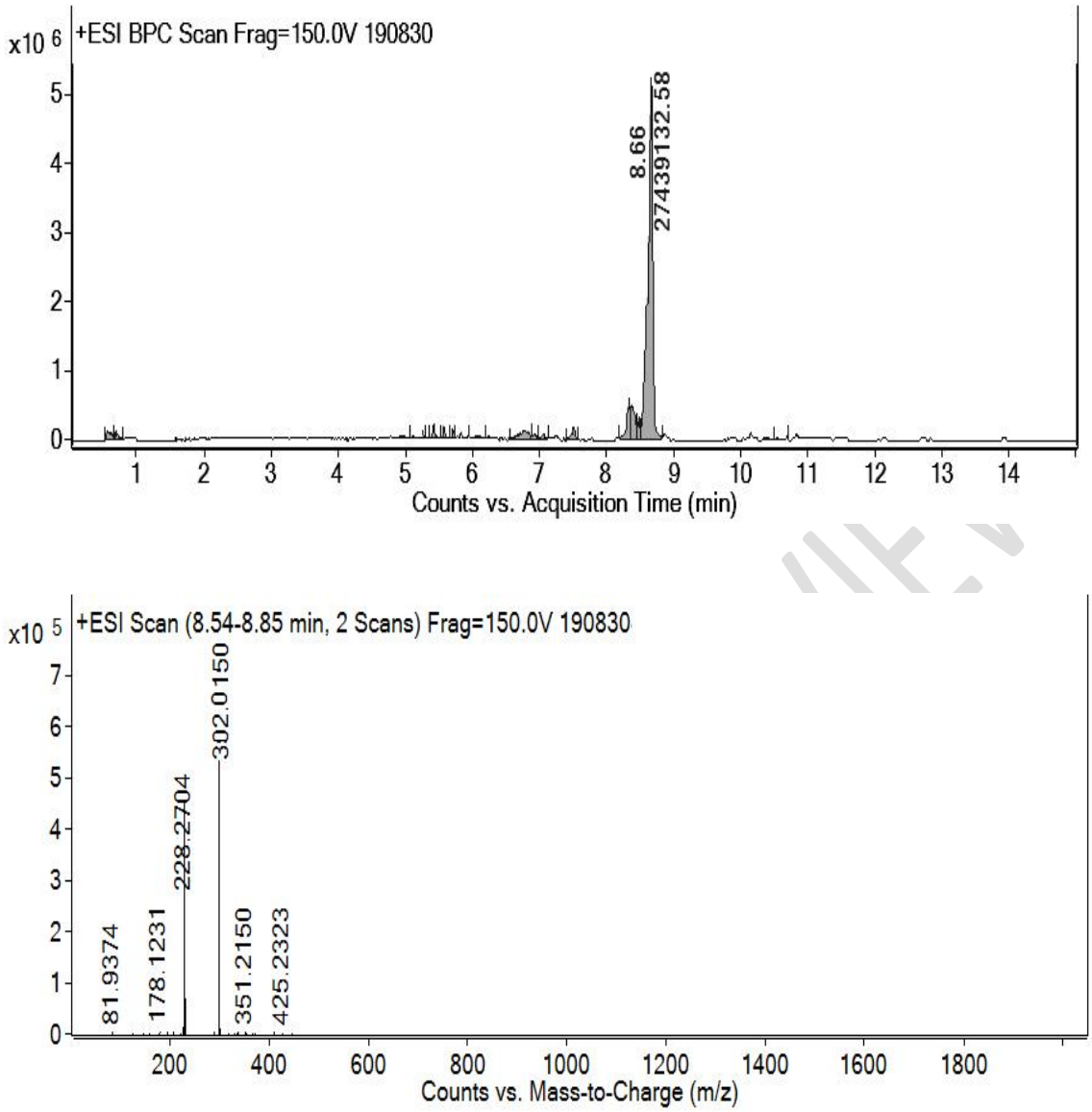


Figure 7. LC-MS A) chromatograph and B) Spectrum of compound at Rf – 0.47

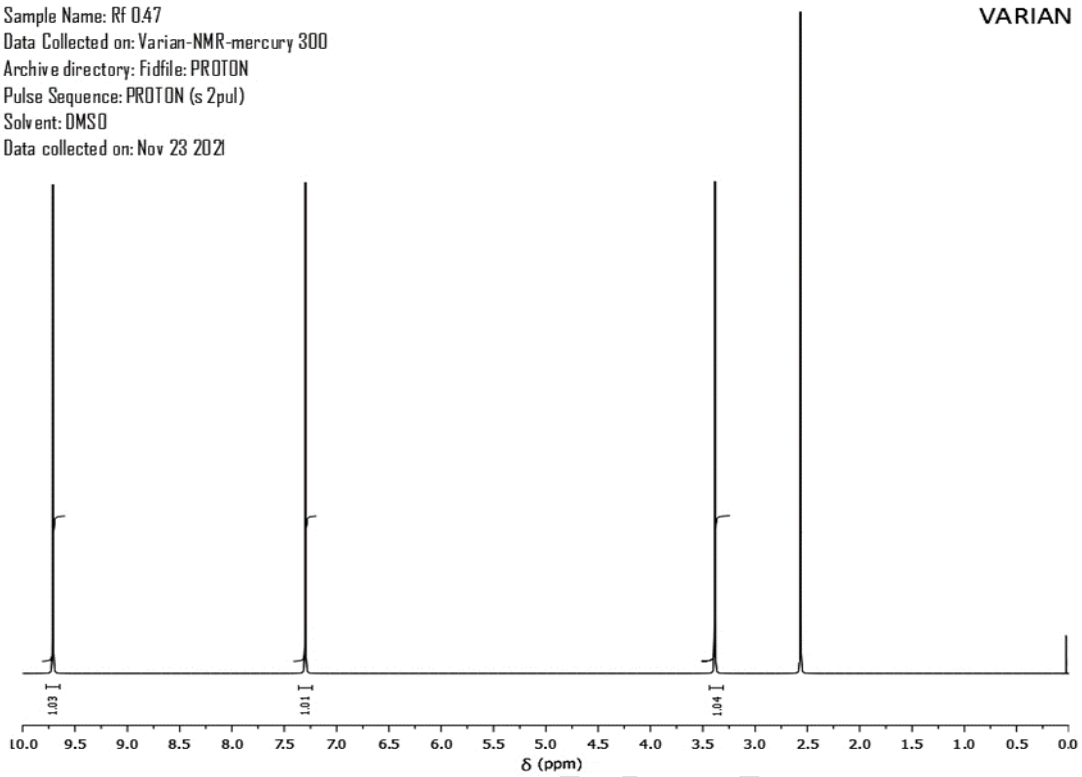


Figure 8. NMR Spectrum of compound at Rf – 0.4

Sr No.	δ	No of Protons	Multiplicity	Type
1	3.378	2 H	s	02 OH Protons
2	7.309	2 H	s	02 Aromatic protons
3	9.716	2 H	s	02 OH Protons

Table 11. Interpretation of NMR spectrum

Probable structure of the isolated compound is as below

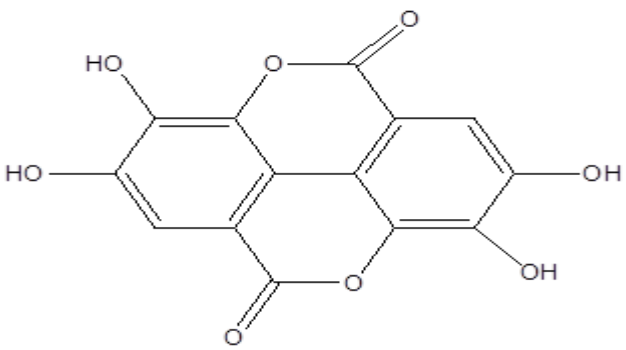


Figure 9. Structure of ellagic acid

Discussion

Cardiovascular diseases (CVDs), principally ischemic heart disease (IHD) and stroke are the leading cause of global mortality and a major contributor to disability [14]. Atherosclerosis is a complex disorder that displays many of the characteristics of a chronic inflammatory process [15]. Though several conventional medications in the form of various formulations acting through different mechanism of action are available in the market as conventional formulations for the treatment of CVDs, they are still far away from ideal category especially because of their low safety, limited biological efficacy, adverse effects, chronic and dose dependent toxicities and tendency to produce drug resistance [16]. Thus the current scenario advocates a need for novel agents with higher cardioprotective efficacy with minimal adverse effects and drug interactions [17,18].

Many plant derivatives as have been reported to be useful drugs and playing significant role in health-care systems around the globe.

In light of this, the preliminary phytochemical analysis of methanolic extract of flowers of *Woodfordia fructicosa* was carried out. The methanolic extract of flowers of *Woodfordia fructicosa* (MWF) showed the prominent presence of such as steroids, triterpenoids, alkaloids, glycosides, proteins and carbohydrates. After the confirmation of phytochemical profile and its pharmacological importance, then toxicity profile testing becomes highly essential to confirm its safety before going for actual exploration of its pharmacological activity. Toxicity profile testing is critically important for any therapeutic medication for confirmation of the extent of its therapeutic utilization (OECD, 2000) [10]. The results of acute oral toxicity studies of MWF revealed that the extract was safe up to 2000 mg/kg which is highest prescribed limit as per this test.

Based upon these findings and available literature, the three different doses i.e. 100, 200 and 400 mg/kg of each extract were selected for the further preclinical investigations.

In the present study, the antiatherosclerotic actions of the extract was evaluated using widely used preclinical screening models i.e. high fat diet induced atherosclerosis, triton induced atherosclerosis. These models are known to be of highly predictive relevance with respect to the clinical spectrum of activity [19,11].

The administration of high fat diet induces extreme hypercholesterolemia and associated features of the metabolic syndrome such as obesity, hypertriglyceridemia, and hyperinsulinemia. These features overcome the normal modulating impact of immune functions and hence often used as experimental model i.e. “high fat diet induced atherosclerosis model’ for evaluation of anti-atherosclerotic drug which in turn termed as cardioprotective activity [11].

The high cholesterol levels exhibit wide pathological outcome including neuronal vascular blockade, memory loss, chest pain, abdominal pain, gall stones. It increases the risk of stroke and heart attack which is increased by multiple folds in case of diabetic patient, patient with immunocompromised status etc. [20].

The consumption of excess of saturated fat, deprived sleep and almost exposure to continuous stress which has become almost integral part of modern lifestyle are prominent reasons for hypercholesteremic condition [21]. It is also projected that; these conditions are going to be increased with advancement of lifestyle hence has become matter of concern [22]. The currently available antihyperlipidemic drugs are associated with variety of side effects but some of them like elevated blood glucose level, memory loss, hepatic damage, muscular atrophy etc. worsen the condition more than the disease [23].

On this background, the current study results into significant reduction in the total cholesterol and low density lipoproteins are the promising outcomes. Simultaneously significant modulation in atherosclerotic markers like ALT, AST, LDH and improvement in the atherosclerotic index supporting findings for its antiatherosclerotic potential [22]. This multiple action is add-on benefit over modern medicines which are usually eliciting specific action. Hence the patient with complex hypercholesteremic conditions can be better treated with the extract which otherwise need polypharmacy in modern medicine. Simultaneous multiple action of extract can be complimentary to the patient over modern medicine [24]. *Woodfordia fruticosa* is documented for hepatoprotective activity hence can be better used in patient with hepatic disorders having hypercholesteremia while the statins, the first line modern antihypercholesteremic drugs aggravate existing liver damage. Similarly, inflammation and high cholesterol level has close relationship. Sustained inflammation may lead to the lowering of HDL and raised levels of LDL. Moreover, elevated cholesterol can also set of inflammation which in turn demand for anti-inflammatory drugs [25]. In such patients, regular use of NSAIDs to combat inflammation which has hepatic damage as major side effect can invite more complication. In such patient, use of these extract which have anti-

inflammatory potential to control elevated cholesterol level provide additional protection against inflammation and can avoid NSAID induced liver damage [26].

Biochemical alterations are kind of primary indicators which are later on usually correlated with histopathological changes. Alterations in the normal histology is usually a result of chronic biochemical alterations [27]. Hence to confirm the results of biochemical parameters the histopathological studies of heart and liver tissues were done.

The study reported significant improvement in the histopathological score suggesting that treatment not only modulate the biochemical indicators but also halt its adverse impact of architecture of related organs. This is one of the precious outcomes of the study. It is well reported that, histopathological alterations is the initial step of complications which if left unattended may lead to lethal outcome [28].

It is documented that if approximately 25 % of patient die due to high fat diet induced liver and kidney damage respectively. Even long term administration of atorvastatin like drug may lead to jaundice and easy acquiring of UTI which may further worsen the condition [29]. On this background, this multistep action of extract which may be due to multicomponent approach can provide remedial protection against the wide range of conditions ranging from the uncomplicated and complicated hypercholesteremia. These characteristics are providing upper hand to these extract over modern medicine [30].

Stanley in his study suggested that intravenous or intraperitoneal injection of Triton increases hepatic cholesterol synthesis by increasing HMG CoA reductase activity, the first committed enzyme of the HMG-CoA reductase pathway in rodents within 24 hours which results in the increased levels of plasma cholesterol and triglycerides and concentrations of low density lipoproteins [31]. Our results are in collaboration with that of previous reports (Mitropoulos et al., 1994 and Souliman et al., 2009) [31,32]. Triton causes structural modifications in the circulatory lipoproteins and suppress the action of lipases especially lipoprotein lipase activity and as a consequence block the uptake of circulating lipids by extra hepatic tissues, and, in turn, resulting in increased blood lipid concentration. Significant increase in the level of cholesterol in the triton-induced animals might also be due to the increased activity of HMG CoA reductase [12].

Triton induction was also reported to interact preferentially with HDL, changing the size and density of lipoproteins which used as substrates for the enzyme LCAT, enzyme activity decreased in parallel to the displacement of apo A-1 [33]. In the present study, reduction in HDL levels in triton-induced animals is in agreement with the earlier statements [32]. In our

study, the changes in plasma cholesterol, triglycerides, low density and high density lipoprotein levels induced by triton resisted were brought back to normal by extract pretreatment indicating anti-atherosclerotic and cardioprotective activity which may be due to the inhibition of the activity of HMG CoA reductase and the stimulation of LCAT activity (Bazzano et al., 2011; Ashen and Bluementhal, 2005) [34,35].

The complicated hypercholesteremia involves alterations in lipoproteins, triglycerides, plasma cholesterol which often need individual drug from the modern medicine. As a result of such polypharmacy, some other untoward effects may be observed over a period of treatment and many times physicians left with no options. It is documented that 60 % of patients die due to the cardiac complications having such complex biochemical pattern [25]. In this study, the extract showed improvement in almost all related parameters which is a most valuable outcome.

The HPTLC analysis of MWF was carried out to identify the phytoconstituent for the anti-atherosclerotic activity. The results of phytoextract revealed the prominent presence of a compound namely ellagic acid and 2-(3,5-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol respectively as pharmacologically important phytoconstituents. The isolation of these phytoconstituents is an important concluding result of the study and cardioprotective activity of the two extract may be attributed to these phytoconstituents [36].

Conclusion

The present study documented the antiatherosclerotic activity of methanolic extract of flowers of *Woodfordia fructicosa* using preclinical models namely high fat diet induced atherosclerosis and triton induced atherosclerosis. Both the extract offered wide range of protection via control of heamodynamic parameters, modulation of various markers imparting antioxidant action and thereby improvement in histopathology as well. The multistep putative action of methanolic extract of flowers of *Woodfordia fructicosa* is attributed to the prominent phytoconstituents namely ellagic acid.

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