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RESEARCH ARTICLE

Preparation of Methanolic Extracts of Plant from Dracocephalum Species and Evaluation for Cardiac Protective Activity in Experimental Animals

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ABSTRACT

Major complication of hyperlipidemia are atherosclerotic heart disease, heart attack and heart stroke, but atherosclerosis is primary cause of death. Developing countries are reliant on medicinal plants as their main source of treatment for diseases. As Dracocephalum rupestre have the native habitat the production is more so it is locally available cost effective with no side effects. As Dracocephalum rupestre is cost effective and beneficiary in metabolism of cholesterol, so it has been taken in to consideration in order "To evaluate cardio protective activity of Methanolic Extracts of Dracocephalum rupestre in triton X - 100 induced hyperlipidemic rats and also to evaluate the Anti-hyperlipidemic activity of the respective extracts. And the final results showed that this plant shows the hyperlipidemic activity and also shows minimal side effects towards the liver and cardiac muscle tissues.

Keywords: Dracocephalum rupestre, hyperlipidemic activity, cardio protective

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1. Introduction

Cardio vascular disease are leading cause of death in both Industrialized and developing Nations³. Therefore it is very important to pay attention to early stage prevention and Asian Journal of Medical and Pharmaceutical Sciences

control of Hyperlipidemia in a comprehensive way¹. Among this hyper cholesterolemia and hyper triglycemia are closely related to ischemic heart disease². Reduction in serum cholesterol level reduces the risk for coronary heart

disease (CHD)³. The main aim of treatment of patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease⁴. The mainstay of treatment for hyperlipidemia is dietary and lifestyle modification, followed by drug therapy, as necessary. Hyperlipidemia should not be considered refractory to dietary treatment if the therapeutic regimen included animal products or more than minimal amounts of vegetable oils. Such diets do not lower LDL cholesterol concentrations as effectively as high-fiber, low-fat diets that exclude animal products. Medicinal plants play a major role in Antihyperlipidemic Activity⁵. Some of the natural medicine used as anti hyperlipidemic agents are *Camellia sinensis* (L.) Kuntze (Theaceae), *Chlorella pyrenoidosa* Chick. (Oocystaceae), *Citrus aurantium* L. (Rutaceae), *Garcinia cambogia* L. (Clusiaceae), *Lagerstroemia speciosa* (L.) Pers. (Lythraceae), *Panax ginseng* C.A. Meyer (Araliaceae), *Salix matsudana* Koidzumi (Salicaceae), *Nelumbo nucifera* Gaertn. (Nymphaeaceae) and *Stellaria media* and *Clerodendrum phlomis*¹⁰. Developing countries are reliant on medicinal plants as their main source of treatment for diseases. As *Dracocephalum rupestre* have the native habitat the production is more so it is locally available cost effective with no side effects.

Plant Profile

Dracocephalum Rupestre

Dracocephalum rupestre is a plant species in the genus *Dracocephalum*, endemic to China. The specific epithet, "rupestre", is derived from Latin, and pertains to the plant growing among rocks.

Scientific classification

Kingdom: Plantae
 (unranked): Angiosperms
 (unranked): Eudicots
 (unranked): Asterids
 Order: Lamiales
 Family: Lamiaceae
 Genus: *Dracocephalum*
 Species: *D. rupestre*

Binomial name: *Dracocephalum rupestre*



Fig 1: Dracocephalum rupestre

Chemical constituents: The moisture content (6.13%), total ash (2.18%), extractives (35.25%) and quantitative and qualitative analysis of biologically active constituents of aerial parts of *Dracocephalum nutans* were determined.

Their relative contents were determined by area normalization in which 45 volatiles were identified.

Medicinal Uses

It is said that Dragonhead tastes like lemon balm (only stronger), and like lemon balm tea, dragonhead tea uplifts the spirit, lightens heavy emotions and gives the heart courage. Dragonhead tea is astringent, tonic and anti-oxidant. It keeps its scent when dried.

2. Experimental

Collection and Authentication of Plant Material

The whole plant of *Dracocephalum rupestre* for the study were procured and authenticated.

Extraction of Plant Material

The plant flowers are grinded in to a coarse powder with the help of suitable grinder.

Cold Extraction (Methanol Extraction)

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum decicator for 7 days.

% Yield value of Methanol Extract from Aerial Parts of *Dracocephalum rupestre* Plant.

Powder taken for extraction = 200gm
 Weight of the empty china dish = 53.70gm
 Weight of the china dish with extract = 123.24gm
 Weight of the extract obtained = (123.24-53.70) g = 69.54 gm
 % yield of methanol extract = (weight of extract)/(powder taken for extraction) × 100
 = 69.54/200 × 100 = 34.7 %.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *Dracocephalum rupestre* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, and Flavonoids as per the standard methods⁴⁰.

Animals: Healthy Adult Male rats of 5 weeks old with Average weight in the range of 100-150gms were selected. Animals are housed 4 per cage in temperature controlled (27 °C ± 3 °C) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *Ad libitum*. The guidelines of committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Govt of India were followed and prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Experimental Animal Protocol:

Experimental rats, starved for 18 hr, were provided water *ad libitum*. The rats were divided into six groups containing four animals in each group.

Group – I: Normal Control. (Normal saline 10ml/kg orally for 7 days)

Group – II: Hyperlipidemic control, (Triton x 100.)

Group – III: Hyperlipidemic rats treated with MEDR at dose of 400mg/kg for 7days.

Group – IV: Hyperlipidemic rats treated with MEDR at dose of 500mg/kg. For 7days.

Group – V: Hyperlipidemic rats treated with Atorvastatin at 10 mg/kg for 7days.

All the groups receives single i.p. injection of Triton X-100 at dose of 100mg/kg, simultaneously with Group- II, Group – III, Group – IV, Group – V, except Group – I (Normal control). After 72 hours of Triton X-100 injection. The Group – V receives Atorvastatin at dose of 10 mg/kg, was prepared by suspending bulk Atorvastatin in aqueous 0.5% methyl cellulose⁴² for 7 days. The Group– III, receive MEBM, at daily dose of 500mg/kg orally for 7 days and Group – IV, Group – V receives MEDR at daily dose of 400mg/kg and 500mg/kg orally for 7 days.

Method of Induction: The systemic administration of the surfactant Triton X-100 to rats results in a biphasic elevation of plasma cholesterol and triglycerides. Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100mg/kg) in physiological saline solution after overnight fasting for 18 h.

Acute toxicity studies

The Acute Toxicity Studies was performed using female rats as per OECD Guideline No.423 (Short term toxicity). Male rats were selected of weight around 50 ±10 gm for main test. Single animals are dosed in sequence usually at 48 h intervals. A Dose Progression Factor of 3.2 is used. Using the default dose progression factor, doses would be selected from the sequence (1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000). However, the time intervals between dosing are determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose should be delayed until one is confident of survival of the previously dosed animal. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. The toxicological effects were observed in terms of mortality expressed as LD₅₀. The number of animals dying or surviving during a period was noted.

Blood Sample Collection and Analysis

The rats are anesthetized by ether and then Blood samples were collected on 0th and 8th day¹³ from retro-orbital plexus of rat using micro capillary technique from rats of all the groups, and centrifuged at 3000 rpm for 15 min so as to get serum. The serum is analyzed for total cholesterol, triglycerides and HDL levels using biochemical kits (diagnostic kit.). Serum LDL- Cholesterol concentration was calculated According to the equation of Fried and Wald.

$$\text{LDL-Cholesterol} = \text{Total Cholesterol} - (\text{HDL-Cholesterol} + \text{TG}/5)$$

Where, VLDL-C = TG/5

Measurement of blood pressure

Before the systolic blood pressure measured by the tail cut method using Power Lab data acquisition systems (AD Instruments, Bella Vista, NSW, Australia), the rats were warmed for 10 min. Five readings were obtained from each rat and averaged.

Experimental design for cardio protective activity

The rats will be divided into six groups (6rats in each group). All animals were deprived of food and water 2hours before, and 2hours after administration of different doses of the methanolic extract of *Dracocephalum rupestre* leaves for period of 14days.

After the experimental regimen, all the animals were sacrificed by cervical dis location under mild, chloroform anesthesia. Blood was collected in to clean centrifuge tubes by carotid bleeding and allowed for clotting. Then these rum was separated by centrifugation at 3000rpm for 15minutes and was kept at 4°C to assess the activities of serum enzymes. The heart was excised immediately, rinsed in ice-cold saline and used for biochemical assays. A portion of heart tissue was fixed in 10% buffered neutral formalin solution for Histopathological studies.

Assay of hematological parameters

Hemoglobin content, red blood cells (RBC), white blood cells (WBC), differential count, total count, and platelet count were assayed in the blood as per the standard methods.

Estimation of Hemoglobin

Reagents: Drabkin's solution, Cyanmethemoglobin standard: 65 mg/dl

Procedure: Pipetted out 0.02 ml of serum and 5.0 ml of Drabkin's solution into a test tube. Simultaneously, a blank was set up with Drabkin's solution and distilled water. Mixed well the above tubes and allowed to stand at room temperature for 5 minutes. Measured the absorbance of test at 546 nm. Take the absorbance of cyanmethemoglobin standard was taken directly without adding working reagent against blank at 546 nm. The results were expressed as g/dl in serum.

Estimation of Red Blood Cells

Reagents: RBC diluting fluid: (Trisodium citrate-3g, distilled water~99ml, and formalin-1ml).

Procedure: The whole blood was taken into the RBC pipette exactly up to the 0.5 mark (Thoina pipette mark 101) and the diluting fluid (formal citrate solution) was immediately drawn up to the mark 101. The pipette was rotated between the thumb and the forefinger. This gave a dilution of 1:200. The cover glass was placed in position over the ruled area using gentle pressure. The suspension was mixed thoroughly by rotating the pipette for about a minute, holding it in horizontal position, and finally shook it sidewise. The fluid was expelled from the stem of the pipette and filled the chamber immediately by holding the pipette at an angle of 45° and slightly touching the tip against the edge of the cover glass. There should not be any bubbles under the cover glass. Then the red corpuscles were allowed to settle for 2 to 3 minutes. The number of RBCs was counted in 180 small squares (4 squares of 16 at each

four corners and one of 16 at centre). The cells touching the lower and right hand lines were not counted, but the cells touching the upper and left hand lines were counted. The cells counted are expressed as million cells/rnmj blood.

Calculation: Number of RBCs/mm³ = Number of cells counted in 5 squares x 10000.

Estimation of White Blood Cells

Reagents: WBC diluting fluid (Turk solution): (Acetic acid-3ml, distilled water-97ml).

Procedure: The whole blood was taken up to the mark 0.5 in WBC pipette and diluted up to the mark 11 with WBC fluid as described in RBC counting and filled the counting chamber in the same manner. Then the cells area was allowed to settle for 3 minutes. The Neubauer counting chamber was used to count the cells in the four corners and each of these 4 sq mm. areas is subdivided into 16 squares by using the low power objective and a medium ocular. While counting, the cells included were those touching the lines on the left and bottom. The difference between the two squares millimeter area as thousand cells /mm blood.

Calculation: Number of WBCs/mm³ = Number of cells counted x 50.

Estimation of Lactate Dehydrogenase

Reagents: Glycine buffer, 0.1 M, pH 10: 7.505 g of glycine and 5.85 g of sodium chloride were dissolved in 1 litre of water.

Buffered substrate: 125ml of glycine buffer and 75ml of 0.1N NaOH were added to 4 g of lithium lactate and mixed well.

Nicotinamide Adenine Dinucleotide: 10mg of NAD was dissolved in 2ml of water.

2,4-Dinitrophenyl hydrazine: 200 mg of DNPH was dissolved in 100ml of 1N HCl. 0.4 N NaOH. Standard pyruvate, 10^{−6} mol/ml:

11 mg of sodium pyruvate was dissolved in 100ml of buffered substrate (1 μ mole of pyruvate /ml), NADH solution, 10^{−6} mol/ml: 8.5 mg/10ml buffered substrate.

Procedure:

Placed 1.0ml buffered substrate and 0.1ml sample into each of two tubes. Added 0.2ml water to the blank. Then to the test added 0.2ml of NAD. Mixed and incubated at 37°C for 15 mins. Exactly after 15 mins, 1.0ml of dinitrophenyl hydrazine was added to each (test and control). Left for further 15mins. Then added 10ml of 0.4N Sodium hydroxide and the color developed was read immediately at 440 nm. A standard curve with sodium pyruvate solution with the concentration range 0.1 -1.0 μ mole was taken. LDH activity in serum was expressed as μ moles of pyruvate liberated / L and in liver homogenate as nmoles of pyruvate liberated / minute / mg protein.

Estimation of Creatine Kinase

Reagents: Tris-HCl buffer: 0.1 mM, pH 9.0, ATP: 18.5 mM in Tris HCl buffer, Magnesium-cysteine reagent: 24.65mg magnesium sulfate and 15.76 mg cysteine HCl were dissolved in 10 ml of distilled water, Creatine: 240 mM

Procedure: The incubation mixture containing 0.75ml of double distilled water, 0.05ml of serum, 0.1ml of ATP solution, 0.1ml of magnesium-cysteine reagent and 0.1ml of creatine was incubated at 37°C for 20mins. The tubes were centrifuged and the supernatant was used for the estimation of phosphorus. The enzyme activity is expressed as IU/L.

Estimation of Creatine Kinase- MB Activity

Reagent: CK-MB Reagent: Creatine Phosphate 30mM Adenosine-5-Phosphate 2mM NAD 2mM Hexokinase (Yeast) >3000U/LG-6-PDH (Bacterial) > 2000U/L Anti Human CK-M antibody (Goat)-sufficient amount to inhibit up to 1500 U/L of CK-MM.

Procedure: To the test tubes added 1000 μ l of the reagent and 50 μ l of the sample. The mixture was mixed and incubated at 37°C. The absorbance was measured after 300 seconds. Two additional absorbance was taken at 1 minute interval. The mean absorbance change/minutes (AA/min) was calculated. The change in absorbance/ minute was multiplied by factor 3376 that is equal to CK-MB.

DPPH Free Radical-Scavenging Activity

The methanolic solution of DPPH (0.1 mM, 1 ml) was incubated with 3 ml of different concentrations of the root extract ranging from 10-100 μ g/ml. Incubation was carried out at room temperature (25°C) for 30 min. For each concentration, the assay was run in triplicate. At the end of the incubation period, the optical density of each sample was determined at 517 nm. Ascorbic acid solution was used as a standard. EC₅₀ values (concentration required to scavenge 50% of the free radicals) for both ascorbic acid and the root extract were determined. The radical scavenging activity of the tested sample was expressed as an inhibition percentage (IP).

$$\text{DPPH Scavenged (\%)} = \left(\frac{A_{\text{DPPH}} - A_{\text{test}}}{A_{\text{DPPH}}} \right) \times 100$$

Where,

A_{DPPH} is the absorbance of the 0.1 mM of DPPH solution and

A_{test} is the absorbance in the presence of the extract or ascorbic acid.

IC₅₀ value was determined from the graph obtained using standard ascorbic acid by using the “y = mx + c” formula from the slope of the graph.

Statistical Analysis: Results are expressed as Mean \pm S.D. All the results were compared with control subject one-way analysis of variance (ANOVA), followed by the Dunnett t-test using Graph Pad Prism Software 6 version. P Values < 0.05 were as considered statistically significant.

3. Results and Discussion

Table 1: Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	-
Alkaloid	+
Tannin	+
Carbohydrate	-

Phenol	+
Flavonoid	+
Saponin	+
(+) Present.	(-) Absent

Table 2: Lipids Levels Obtained on 8th Day (After Treatment)

Sl.NO	Groups	TC	TG	HDL	LDL	VLDL
I	Normal Control	64.03 ± 1.45	82.66 ± 2.46	38.91 ± 2.33	8.45 ± 3.43	16.53 ± 0.49
II	Hyperlipidemic Control	192.47 ± 5.05	168.9 ± 5.28	21.86 ± 2.74	136.82 ± 7.00	33.79 ± 1.05
III	MEDR 400mg/kg.	121.74 ± 7.74*	107.93 ± 6.67*	31.04 ± 4.32**	69.11 ± 10.51***	21.58 ± 1.33***
IV	MEDR 500mg/kg.	112.97 ± 5.25*	103.55 ± 4.2*	33.15 ± 2.51**	59.1 ± 6.89*	20.71 ± 0.84***
V	Standard Atrovastatin10mg/kg	92.29 ± 5.63*	102.26 ± 7.68*	39.18 ± 3.14**	32.91 ± 7.61*	20.44 ± 1.53**

All the data are expressed as MEAN ± S.D (n=4), *P = < 0.001, **P = < 0.01, ***P = < 0.05. vs GROUP I. TC: Total Cholesterol ; TG: Triglycerides ; HDL-C : High Density Lipoprotein cholesterol; LDL-C : Low Density Lipoprotein-cholesterol ; VLDL-C : Very Low Density Lipoprotein ; MEDR: Methanolic Extract of *Dracocephalum rupestre*.

Table 3: Effect of methanolic extracts on hematological profile in blood of control and experimental rats

Parameters	Group I	Group II	Group III	Group IV	Group V
Hemoglobin (g%)	10.99 ± 0.22 ^a	13.8 ± 0.6	12.32 ± 0.09 ^C	12.3 ± 0.04 ^d	11.82 ± 0.07 ^e
RBC (X10 ⁶ /l)	3.83 ± 0.31 ^a	4.60 ± 0.09	4.09 ± 0.20 ^o	4.01 ± 0.14 ^d	3.93 ± 0.1 ^e
PCV	32.21 ± 0.11 ^a	41.4 ± 0.10	36.93 ± 0.06 ^C	35.1 ± 0.6 ^d	34.5 ± 0.03 ^C
MCV (fl)	84.04 ± 1.04 ^a	90.01 ± 1.01	87.08 ± 0.17 ^C	89.01 ± 0.03 [^]	86.40 ± 0.99 ^e
MCHC (g/dl)	32.62 ± 0.25 ^a	33.30 ± 0.30	34.20 ± 0.01 ^C	34.82 ± 0.41 ^d	33.00 ± 0.50 ^e
MCH (Pg)	28.00 ± 0.06 ^a	30.00 ± 0.02	28.00 ± 0.6 ^C	29.2 ± 0.4 ^d	29.1 ± 0.2 ^e
PLT (k/ul)	4.48 ± 0.88 ^a	15.17 ± 1.99	7.75 ± 0.21 ^C	4.25 ± 0.03 ^d	3.21 ± 0.04 ^e

Values are expressed by mean ± SD (n = 6)

Statistical comparisons:

a-Group I is compared with Group II b-Group III is compared with Group II

c-Group IV is compared with Group II d-Group V is compared with Group II

e-Group VI is compared with Group II a, b, c, d and significant at P < 0.05

Table 4: Estimation of blood pressure and heart rate

S.No	Groups	Blood Pressure (mmHg)		Heart Rate/Min
		Mean-BP	Systolic-BP	
I	Normal Control	122 ± 0.68	121 ± 0.38	335 ± 0.84
II	Hyperlipidaemia Control	149 ± 0.35	151 ± 0.74	275 ± 0.68
III	MEDR 400mg/kg	140 ± 0.68	139 ± 0.29	321 ± 0.64
IV	MEDR 500mg/kg	132 ± 0.87	130 ± 0.15	325 ± 0.68

Table 5: Activities of liver marker enzymes in serum and liver of Control and experimental rats

Sl.NO	Groups	LDH	
		Serum	Liver
I	Normal Control	73.58 ± 0.68	6.12 ± 0.75
II	Hyperlipidemic Control	73.70 ± 0.50	6.35 ± 0.84
III	MEDR 400mg/kg.	73.66 ± 0.38	6.00 ± 0.8
IV	MEDR 500mg/kg.	73.69 ± 0.40	6.23 ± 0.74

Table 6: Activities of creatine kinase (CK) and creatine kinase MB (CK-MB) in Serum and heart of control

and experimental rats

Parameters	CK		CK-MB	
	Serum	Heart	Serum	Heart
Normal Control	250.79 ±2.27	181.85 ± 1.14	41.5415 ±0.82	106.29 ±2. 45
Hyperlipidemic Control	490.83 ±2.59	1 14.00 ± 1.14	103.97±0.82	62.15 ± 1.96*
MEDR 400mg/kg.	363.00 ±2.88	159.09 ±0.93	76.02 ±0.27	81.68±0.93
MEDR 500mg/kg.	325.08±2.68	165.67 ±1.40	58.54 ±0.67	86.47 ± 1.83

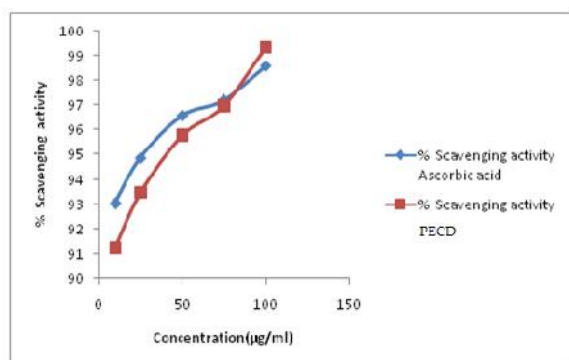
Values are expressed by mean +SD (n=6)

Units: CK, CK-MB (Heart)-p.mol of phosphorus liberated/min/mg protein

CK, CK-MB (Serum)-IU/L

Table 7: Showing % scavenging activity of Ascorbic acid and MEDR

Concentration(µg/ml)	%Scavenging activity	
	Ascorbic acid	MEDR
10	93.05	91.23
25	94.87	93.45
50	96.58	95.78
75	97.2	96.98
100	98.58	99.34
IC₅₀(µg/ml)	43.137µg/ml	41.024 µg/ml

**Fig 2:**Anti-oxidant activity of MEDR& ascorbic acid

Discussion

The present study was designed to investigate the cardio protective activity of *Dracocephalum rupestre* extract in Triton X-100 induced hyperlipidemic rats. Phytochemical Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Methanolic Extract of *Dracocephalum rupestre* while only Phenol were present in Methanolic Extract of *Dracocephalum rupestre*. %Yield value of Methanolic Extract from Aerial Parts of *Dracocephalum rupestre* was found to be 34.75 % Yield value of Methanolic Extract from Aerial Parts of *Dracocephalum rupestre* was found to be 8.6 %. Administration of Triton-X-100 (100mg/kg) to all the fasted rats caused an elevation of TC, TG, VLDL and LDL and reduction in HDL levels. After 72 hrs of induction of Triton X-100 results in hyperlipidemia which is compared with normal control group .which results in significantly increased serum lipid levels in hyperlipidemic group. The change in lipid levels in group number III to VI, were comparable with group of Hyperlipidemic control (i.e Triton X-100 ,Group- II) . The Standard group (i.e Atorvastatin group) significantly lowers the serum lipid level (P<0.001).

The results of the study clearly indicate that MEDR Extract and MEDR Extract at a dose of 500 mg/kg & 400 mg/kg significantly lowered serum lipid levels (P<0.01). MEDR Extract at a dose of 500 mg/kg significantly lowered serum lipid levels, (P<0.001) i.e. antihyperlipidemic activity which was found to be more effective in higher dose of MEDR as compared to MEDR and lower dose of MEDR when administered orally in triton induced hyperlipidemic models. MEDR Extract having very low hypolipidemic activity. MEDR Extracts showed a dose dependent decrease in the levels of cholesterol, Triglyceride, LDL-C and VLDL-C level. Among three groups (i.e. group number III-V), Group number- V reduced the elevated lipid levels more significantly than the other Groups.(P<0.001) Flavonoids have exhibited a variety of pharmacological activities, including the antiatherogenesis and antioxidant effect⁴⁸. Thus the present result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of Tannins, Phenols, and Flavonoids in the Extracts. The changes in HB content, RBC, platelets, and differential count of WBC due to Doxorubicin administration were found to be reversed in the rats pretreated with different doses of ethanolic extracts which indicates that ethanolic extracts possessed protective action on the haemo poietic system. The effect of the extract on the levels of cardiac marker enzymes CK, CK-MB, troponin-T, AST, ALT, LDH, and ALP in both serum and heart was assessed. In case of marker enzymes, DOX was showing significant increase in the activities of CK, CK-MB, and troponin-T, AST, ALT, LDH and ALP (important markers of myocardial infarction) in serum with subsequent decrease activities of CK, AST, ALT, and LDH in heart. In heart tissue, the levels of CK-MB and ALP were found to be increased, in DOX induced rats when compared with control rats. Increase in the activities of these enzymes in serum could be due to the leakage of these enzymes from the heart as a result of DOX-induced necrosis. The changes

in the activities of these enzymes were reverted back to near normal in the rats pretreated with different concentrations of ethanolic extracts.

4. Conclusion

Natural products extract of therapeutic relevance are of paramount importance as reservoirs of structural and chemical diversity. A recent review on national pharmacopoeias from several countries reveals at least 120 distinct chemical substances from different plants that have utility as life saving drugs. This has been achieved through chemical and pharmacological screening of only 6% of the total plant species. Untapped, hidden wealth in the flora needs to be unearthed and explored to cure diseases like heart disease, cancer, diabetes, AIDS etc. Most countries face high and increasing rates of cardiovascular disease. Combating these cardiovascular diseases is of a paramount importance today. Ischemic heart disease leading to myocardial infarction (MI) is a major clinical concern and remains as a clinical challenge and a problem of great importance despite considerable advances in therapy and management that have been made over the past three decades. MI continues to be a major public health problem, not only in western countries but also increasingly in developing countries and makes significant contribution to the mortality statistics. The results concluded that MEDR (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemic model and which is equipotent activity when compared with Atorvastatin treated groups. And also proved to be cardio protective in the respective concentrations further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

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