RESEARCH ARTICLE

Exploring the Anti-hyperlipidemic Potential of *Rivea hypocrateriformis* (Desr) Leaf Extracts: A Study on Triton-induced Hyperlipidemia Rat Model and Oxidative Stress

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Received: 25th September, 2023; Revised: 21st January, 2024; Accepted: 22nd February, 2024; Available Online: 25th March, 2024

ABSTRACT

Introduction: Type 2 diabetes and cardiovascular disease are greatly affected by hyperlipidemia, characterized by elevated blood lipid levels. The potential adverse effects of conventional pharmacological interventions have prompted the exploration of natural drugs for combinatorial therapy of hyperlipidemia management. *Rivea hypocrateriformis* (Desr), a plant species with traditional medicinal use, has shown potential anti-hyperlipidemic effects in animal models.

Materials and Methods: The study evaluated chloroform (HEC) and ethanol (HEE) extracts of *R. hypocrateriformis* (Desr) in Triton-induced hyperlipidemia in Sprague Dawley rat model at 100 and 200 mg/kg body weight. Parameters such as body weight, lipid levels, lipid parameters, and antioxidant enzymes were assessed.

Results and Discussion: There was an increase in total cholesterol and triglycerides in the triton-induced group and a decrease in high-density lipoprotein (HDL). Reduced total cholesterol and triglyceride levels and increased HDL were observed with treatment with HEC and HEE extracts. It was also found that the extracts significantly reduced low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels, suggesting a potential reduction in atherosclerosis risks. The atherogenicity index was significantly decreased in the treatment groups, supporting their anti-atherosclerotic potential. Furthermore, the extracts enhanced antioxidant defenses, reducing oxidative stress. Histopathology of liver tissue revealed the potency of extracts in control of tissue loss, lymphocyte infiltration, and fatty accumulation by triton induction.

Conclusion: The study demonstrates the potential of *R. hypocrateriformis* (Desr) extracts as a natural therapeutic agent for hyperlipidemia management. The extracts exhibited anti-hyperlipidemic and antioxidant effects, offering a multifaceted approach to combat hyperlipidemia and related complications. Future research should focus on identifying bioactive compounds, elucidating underlying mechanisms, and conducting clinical trials for human validation. *R. hypocrateriformis* (Desr) presents a promising area for drug development and nutraceutical research, with the potential to improve hyperlipidemia management and cardiovascular health.

Keywords: Rivea, Triton, Antioxidant, Lipids, Type 2 diabetes, Anti-hyperlipidemic.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.1.28

How to cite this article: Sheeba DAH, Gandhimathi R. Exploring the Anti-hyperlipidemic Potential of *Rivea hypocrateriformis* (Desr) Leaf Extracts: A Study on Triton-induced Hyperlipidemia Rat Model and Oxidative Stress. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):178-185.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Type 2 diabetes and cardiovascular diseases are associated with hyperlipidemia (elevated blood lipid levels). Anti-hyperlipidemic drugs, particularly statins (HMG-CoA inhibitors), have been widely prescribed for managing hyperlipidemia over the past two decades. However, concerns about statin overuse and potential long-term adverse effects have emerged.[1]

Rivea hypocrateriformis (Desr), commonly known as ginseng vine or elephant creeper, belongs to the Convolvulaceae family and is a flowering plant species native to different regions worldwide, including India, Australia, and parts of Southeast Asia. It has a long history of traditional medicinal use in various cultures to treat respiratory and gastrointestinal disorders.[2] This plant has been treated with a wide range of health conditions throughout history, including rheumatic pain, fever, urogenital

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problems, snake bites, piles, malaria, and skin diseases.[2] The phytochemistry of *R. hypocrateriformis* (Desr) has identified flavonoids, alkaloids, glycosides, coumarins, stilbenes and xanthone derivatives as valuable secondary metabolites. Extracts and isolated compounds from the plant have shown promising effects on various organ system, including liver, brain, joints, cancer etc.[2] Extensive research on *R. hypocrateriformis* (Desr) has underscored its pharmacological potential for various applications. Studies have demonstrated that extracts from the plant possess significant anti-inflammatory, antioxidant, and antimicrobial properties.[3] *R. hypocrateriformis* (Desr) contains bioactive compounds, such as alkaloids and flavonoids, which exhibit antitumor and immunomodulatory effects.[4] Additionally, *R. hypocrateriformis* (Desr) extracts have shown *in-vitro* anti-urolithiatic/anti-calcification activity comparable to cystone.[5] Given its diverse range of health-promoting properties, *R. hypocrateriformis* (Desr) is a promising medicinal herb for treating hyperlipidemia. Thus, in this research, the plant extracts have been used to investigate the anti-hyperlipidemic activity on triton-induced hyperlipidemia in Sprague Dawley rats.

MATERIALS AND METHODS

Plant Extraction Process

Fresh leaves of *R. hypocrateriformis* (Desr) were collected and dried in shade for a period of 7 days. The dried leaves were then ground into a fine powder and separately subjected to extraction using chloroform and ethanol through a soxhlet apparatus until the extracts turned colorless. The obtained solutions were filtered, and the solvents were removed by vacuum suction, resulting in the formation of two thick pastes named chloroform extract (HCE) and ethanol extract (HEE). The percentage yields of these extracts were determined as 15.34 and 23.46% (w/w), respectively. Subsequently, the extracts were stored in air-tight containers at room temperature until further use.

Experimental Animals

Sprague Dawley (SD) rats between 180 to 220 gm were procured from a known breeder and supplier in Bengaluru. A stainless steel cage was used to keep them, and they were given access to food (Nutrivet Pvt Ltd) and water *ad libitum*. It was observed that the cages were kept in a 12-hour light-dark cycle at a room temperature of 23°C with 55% relative humidity. It was approved by the institutional animal ethics committee to conduct all experiments on the animals. The cages were coded using cage cards and the animals were devoid of food and water 12 hours before the experiments.[6]

Acute Toxicity Studies

The acute toxicity of HEC and HEE extracts was evaluated using the OECD 423 (Acute Toxic Class Method). Extracts were administered orally to the overnight fasted rats in the form of a suspension prepared with a 0.5% carboxy methyl cellulose (CMC) solution to achieve a 2000 mg/kg dose for both extracts. A body weight measurement was conducted after the animals received extracts and were allowed access to food and water. Throughout the study, the rats were closely monitored for any clinical signs of toxicity, including changes in the respiratory, circulatory, and nervous systems and alterations in skin, fur, eyes, and mucous membranes. The behavior patterns were observed at specific intervals, namely 3, 6, 9, and 14 days after administration of the extracts.[7] Any signs of toxicity, such as coma, drooling, diarrhea, trembling, convulsions, or abnormal sleep, were carefully noted and recorded.

Anti-hyperlipidemic Activity of Extracts HEC and HEE

Preparation of extracts

About 5% gum acacia suspension (GAS) was prepared by dissolving acacia in warm distilled water, followed by filtration, and used as the medium for drug administration. Separately, weighed amounts of HEC and HEE were suspended in the acacia suspension, resulting in 30% suspensions of the respective extracts. Atorvastatin drug was dissolved in the acacia suspension, creating a 2% solution. All solutions were then filtered and set aside for subsequent use in the study.

Drug (triton) administration for induction of hyperlipidemia

A total of seven groups of overnight fasted rats were randomly assigned, each with six animals. First, a negative control group (NCG) received normal water orally as a control. The second group, serving as the hyperlipidemic control group (HCG), and all other groups from 3 to 7 were administered with a single dose of triton intraperitoneally (100 mg/kg B.W) through saline and distilled water was given *via* oral gavage.[8,9] Following this, after 72 hours, the third and fourth groups received the HEC extracts (100 and 200 mg per kg) via the oral route, and they served as test groups 1 and 2. The fifth and sixth groups received the HEE extracts at a dose of 100 and 200 mg per kg body weight via the oral route, and they served as test groups 3 and 4. The seventh group was treated as the standard group and received atorvastatin (10 mg per kg). Each rat's initial body weight was recorded before the experiment began. In order to determine whether any changes or effects have occurred as a result of treatment administered after seven days, each group's final body weights were recorded.[10,11]

Estimation of lipid indices and parameters

Using anesthesia and, blood was withdrawn from the retro-orbital plexus to separate serum to estimate final lipid parameters. The naso-anal length was noted for each rat for the determination of fat indices like the Lee index and BMI.[12,13]

$$\text{Lee index} = (\sqrt[3]{\text{bodyweight}}/\text{naso-anal length}) \times 100$$

$$\text{BMI} = \text{Body weight}/\text{body length}^2$$

The isolated serum samples were analyzed for the lipid parameters like total cholesterol, triglycerides (TG), low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). The biochemical parameters were analyzed using the commercially available diagnostic kits and as per the standard procedures. The atherogenicity index was also calculated from the available data of lipids using the standard formula.[14]

The separated serum was used to perform the biochemical estimation of the enzymes like superoxide dismutase (SOD), catalase (CAT), lipid peroxidase (LPx), glutathione reductase (GR) and glutathione peroxidases (GPx). The estimations were done as per the standard procedures following in the reference through the commercially available kits in the market.[15,16]

Histopathological studies

The liver tissues of the rats were kept in 10% formalin solution for histological analysis after the study. A standard histological procedure involves washing, sectioning, and staining with hematoxylin and eosin (H and E) and examining microscopically for morphological changes.

Statistical Analysis

ANOVA and Dunnett's multiple comparison tests were utilized with GraphPad Prism version 5.0 to analyze the data. Results are expressed as mean ± standard error of the mean. The significance level was determined by $p < 0.001$.

RESULTS

Acute Toxicity Studies

The extracts of *R. hypocrateriformis* (Desr). leaves (HEC and HEE) were investigated for acute oral toxicity at various doses ranging from 2000 to 100 mg/kg body weight following the OECD 423 guidelines. Observations show that both extracts showed no changes in any of those behaviours or features. Both extracts showed no sign of toxicity through changes in any of the above-stated features. Interestingly, rats administered with HEC showed excessive sleeping compared to HEE which was not significant but noted. The symptoms indicating the changes in motor activity were also normal. Overall, it can be assumed that both extracts showed no toxicity in any of the rats at 2000 mg/kg. The rats showed no mortality or any sign of disease or abnormality in behavior. Thus, the effective dose of the extracts was determined as 5%, the lowest dose, and 10%, the highest dose.

Effect of extracts of R. hypocrateriformis (Desr). leaves on body weights of rats in triton-induced hyperlipidemia

Triton was administered to the rats at 100 mg/kg body weight to induce acute hyperlipidemia in Sprague Dawley rats on the starting day. Extracts were administered to the rats at 100 and 200 mg/kg and compared with the standard drug atorvastatin at 10 mg/kg. Initial body weights of the rats were noted before exposing them to the drug and after the experiment was over that is 7 days, the body weight of the rats was noted and compared for changes and induction of hyperlipidemia due to triton. The values were tabulated in Table 1.

Figure 1 illustrates the comparison between the HEE and HEC for the effect of controlling the rise in body weight induced by triton. The rats treated with the inducing agent showed a significant increase in body weight in 7 days and the final body weight was about 336.83, which is almost 50% increase in the weight. This might be due to the increase in appetite of the rats and also the induction of fat gain due to adipocyte deposits into the liver. Although the normal group of rats showed increased weight, it was non-significant compared to the induction group. The extract groups in both doses showed significant control of the increase in weights due to triton. The suppression of rise in body weight was comparatively similar to atorvastatin standard and HEE and HEC at 200 mg/kg showed better activity than standard. In comparison with the extracts, HEE at 200 mg/kg showed a better control of raise of body weight.

Effect of extracts of R. hypocrateriformis (Desr). leaves on lipid indices of rats in triton-induced hyperlipidemia

Table 1 and Figure 2 also show rats' lipid indices, liver weight, Lee Index, and body mass index in different groups. The control group's liver weight, Lee index and BMI were 6.95 gm, 43.27 and 1.17, respectively. The liver weight of the triton-induced

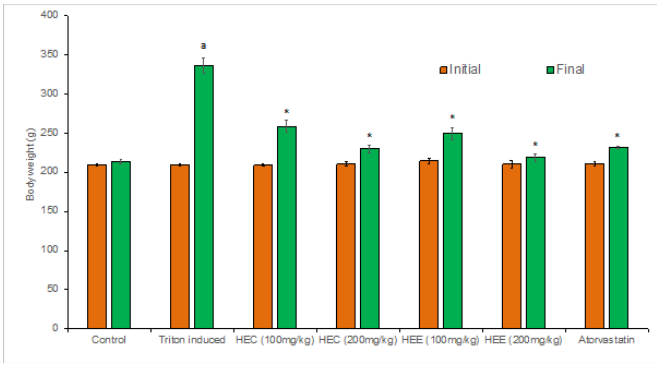

Figure 1: Effect of HEC and HEE on the body weights of triton induced hyperlipidemia

Table 1: HEC and HEE effects on the body weights of Triton induced hyperlipidemia

Group	*Body weight of rats (g)*		*Obesity indices of rats*		
	Initial	*Final*	*Lee Index*	*BMI*	*Liver weight (g)*
Control	209.5 ± 1.22	213.5 ± 2.58	43.27 ± 1.74	1.17 ± 0.09	6.95 ± 0.94
Triton induced	209.66 ± 1.5[a]	336.83 ± 9.66[a]	49.73 ± 3.76[a]	1.73 ± 0.27[a]	10.71 ± 0.61[a]
HEC 100 mg/kg	209.16 ± 1.72*	258.66 ± 7.71*	43.69 ± 2.06*	1.21 ± 0.12*	7.31 ± 0.55*
HEC 200 mg/kg	210.23 ± 2.93*	229.92 ± 4.99*	42.92 ± 2.88*	1.2 ± 0.11*	6.93 ± 0.52*
HEE 100 mg/kg	214.3 ± 3.03*	249.33 ± 7.58*	44.72 ± 3.28*	1.26 ± 0.12*	6.88 ± 0.43*
HEE 200 mg/kg	210.08 ± 4.87*	218.71 ± 4.33*	42.78 ± 2.84*	1.19 ± 0.13*	5.46 ± 0.45*
Atorvastatin 10 mg/kg	211.1 ± 2.89*	231.44 ± 1.72*	45.12 ± 2.01*	1.28 ± 0.11*	7.27 ± 0.48*

The values were represented as Mean ± SEM (n = 6); *$p < 0.001$ compared to the induction group; [a]$p < 0.001$ compared to the normal control group

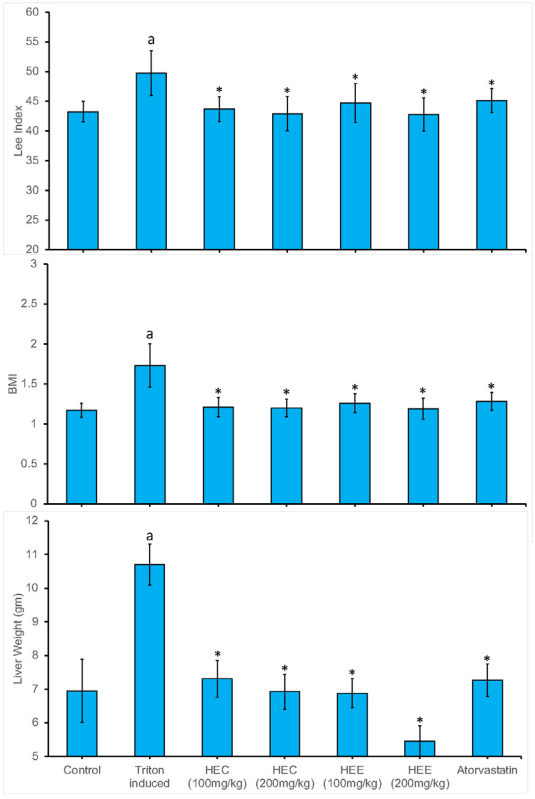

Figure 2: Effect of extracts of *R. hypocrateriformis* (Desr). on the obesity indices

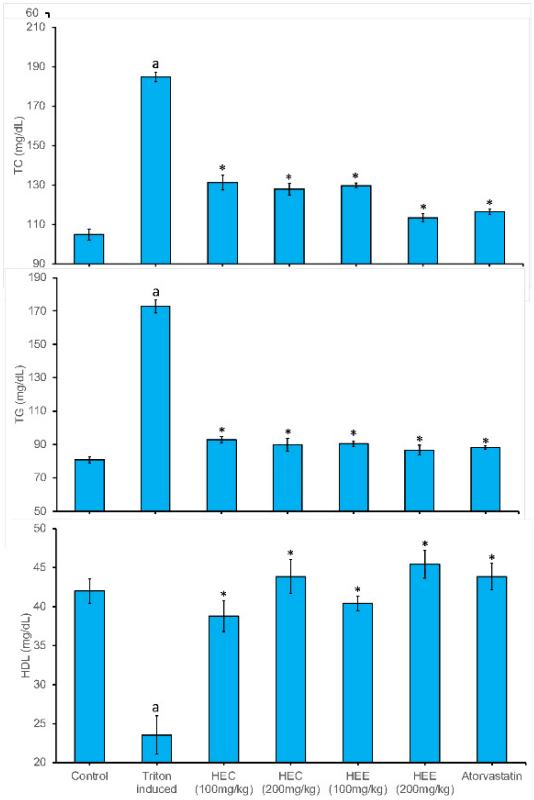

Figure 3: Extract's effect on the Lipid levels of triton induced hyperlipidemia

group was raised to 10.71, whereas the Lee index was 49.73, and the BMI was 1.73, which showed a significant change from the control group. The values of the liver weight, Lee index and BMI of the extract-treated groups suggest a significant activity of the extracts compared with the standard drug. Also, the HEE 200 mg/kg showed much better activity in controlling liver weight compared to the standard drug. HEE and HEC at 200 mg/kg showed better activity than the standard drug and those at lower dose, suggesting the extracts showed a dose-based activity in controlling lipid accumulation and hyperlipidemia.

Table 2: HEC and HEE effects on lipid levels in triton induced hyperlipidaemia

Group	*Lipid levels of rats (mg/dL)*		
	TC	*TG*	*HDL*
Control	104.87 ± 2.79	80.86 ± 1.82	42.01 ± 1.54
Triton induced group	184.78 ± 2.37[a]	172.94 ± 3.96[a]	23.55 ± 2.44[a]
HEC 100 mg/kg	131.22 ± 3.64*	92.94 ± 1.94*	38.8 ± 2*
HEC 200 mg/kg	127.94 ± 2.98*	89.88 ± 3.87*	43.87 ± 2.18*
HEE 100 mg/kg	129.76 ± 1.3*	90.46 ± 1.62*	40.42 ± 0.91*
HEE 200 mg/kg	113.47 ± 1.99*	86.81 ± 2.89*	45.44 ± 1.78*
Atorvastatin 10 mg/kg	116.56 ± 1.39*	88.32 ± 0.86*	43.87 ± 1.68*

The values were represented as Mean ± SEM (n = 6); *$p < 0.001$ compared to the induction group; [a]$p < 0.001$ compared to the normal control group

Effect of extracts of R. hypocrateriformis (Desr). leaves on TC, TG and HDL of rats in triton induced hyperlipidaemia

The rats were sacrificed in the last hour and blood was withdrawn to separate the serum. Serum was collected and analyzed for the lipid levels. Table 2 and Figure 3 shows the lipid levels of rats in different groups, including total cholesterol, triglycerides, and high-density lipoprotein. Overall, the lipid levels, including TC, TG, and HDL, were altered by the different treatments as shown in Table 2, indicating the potential therapeutic effect of HEC and HEE at higher doses significant to the standard on lipid metabolism in rats. The triton-induced group showed significant differences from the control group in all three indices, indicating that triton caused significant metabolic changes in the rats. Also, similar to the above, extracts showed a dose-based activity where HEE at 200 mg/kg outperformed standard drugs in elevating HDL and controlling total cholesterol and triglycerides.

Effect of extracts of R. hypocrateriformis (Desr). Leaves on LDL, proteins and atheronogenic index of rats in triton-induced hyperlipidemia

Table 3 and Figure 4 shows the lipid parameters such as LDL, VLDL, total protein, and atherogenicity index of rats in different experimental groups. The results show that the triton-induced hyperlipidemia group had significantly higher levels of LDL and VLDL and a significantly lower total protein level than the normal control group. The atherogenicity index was also significantly higher in the triton-induced group compared

Table 3: HEC and HEE effects on lipid parameters in triton induced hyperlipidemia

Group	*Lipid parameters of rats*			
	LDL (mg/dL)	*VLDL (mg/dL)*	*Total Protein (mg/dL)*	*Atherogenicity index (%)*
Control	73.26 ± 2.66	32.7 ± 0.6	8.34 ± 0.61	2.49 ± 0.09
Triton induced group	149.13 ± 4.03[a]	72.82 ± 1.96[a]	2.84 ± 0.19[a]	7.92 ± 0.94[a]
HEC 100 mg/kg	93.88 ± 2.22*	51.82 ± 2.08*	3.87 ± 0.46*	5.71 ± 0.17*
HEC 200 mg/kg	79.04 ± 3.75*	43.89 ± 1.88*	5.86 ± 0.46*	3.38 ± 0.14*
HEE 100 mg/kg	86.99 ± 2.34*	46.78 ± 2.19*	4.56 ± 0.58*	4.66 ± 0.05*
HEE 200 mg/kg	73.52 ± 3.27*	31.32 ± 2.16*	8.1 ± 0.54*	2.49 ± 0.12*
Atorvastatin 10 mg/kg	74.09 ± 2.49*	32.02 ± 2.06*	6.96 ± 0.5*	2.66 ± 0.12*

The values were represented as Mean ± SEM (n = 6); *p <0.001 compared to the induction group; [a]p <0.001 compared to the normal control group

Table 4: HEC and HEE effects on antioxidant levels in triton-induced hyperlipidaemia

Group	*SOD (U/mg protein)*	*CAT (μM H_2O_2/mg protein)*	*GPx (μg/mg protein)*	*GSH (μg/mg protein)*	*MDA (nM/mg protein)*
Control	9.33 ± 0.5	95.18 ± 1.55	10.01 ± 0.59	55.44 ± 1.36	6.69 ± 0.49
Triton induced group	4.63 ± 0.35[a]	42.33 ± 2.7[a]	5.38 ± 0.33[a]	22.11 ± 1.76[a]	12.38 ± 0.27[a]
HEC 100 mg/kg	5.34 ± 0.49*	63.88 ± 2.04*	6.24 ± 0.35*	32.89 ± 1.85*	6.32 ± 0.32*
HEC 200 mg/kg	7.24 ± 0.33*	77.91 ± 2.01*	7.32 ± 0.43*	42.98 ± 1.76*	4.69 ± 0.44*
HEE 100 mg/kg	6.87 ± 0.26*	69.78 ± 1.66*	6.92 ± 0.43*	38.44 ± 1.28*	5.49 ± 0.36*
HEE 200 mg/kg	8.58 ± 0.54*	83.04 ± 1.89*	9.4 ± 0.43*	52.37 ± 1.34*	2.26 ± 0.42*
Atorvastatin 10 mg/kg	7.71 ± 0.43*	55.87 ± 1.6*	7.57 ± 0.48*	40.97 ± 1.93*	4.77 ± 0.33*

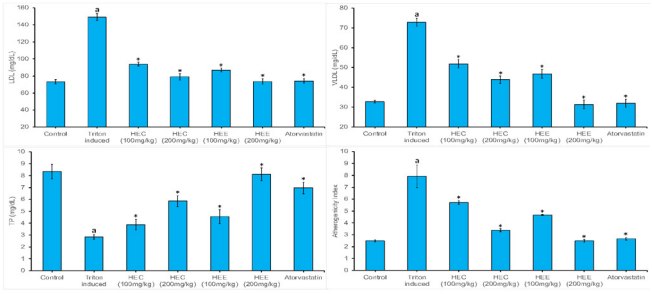

Figure 4: Effect of HEC and HEE on the lipid parameters in triton-induced hyperlipidemia

to the normal control group. The treatment with HEC, HEE, and atorvastatin significantly improved the lipid parameters of the rats with triton-induced hyperlipidemia. Specifically, these treatments significantly decreased LDL and VLDL levels and increased total protein levels. These changes resulted in a significantly lower atherogenicity index compared to the triton-induced group. In summary, the results suggest that the treatments with HEC 200 mg/kg and HEE 200 mg/kg are effective in improving the lipid profile of rats with triton-induced hyperlipidemia compared with the standard.

Effect of extracts of R. hypocrateriformis (Desr). leaves on antioxidant enzymes of rats in Triton-induced hyperlipidemia

Table 4 and Figure 5 shows the levels of oxidative stress markers like SOD, CAT, GPx, GSH, and MDA activity in different groups of rats. While the MDA levels in the control group were lower than in the triton-induced group, the SOD, CAT, GPx, and GSH levels were higher in the control group. This suggests that Triton induction resulted in increased oxidative stress and lipid peroxidation in the rats. The groups treated with HEC 100 and 200 mg/kg, HEE 100 and 200 mg/kg, and atorvastatin 10 mg/kg had higher levels of SOD, CAT, GPx, and GSH compared to the triton-induced group, indicating that these treatments were able to mitigate oxidative stress. These groups also had lower levels of MDA compared to the Triton-induced group, suggesting that they were able to reduce lipid peroxidation. MDA levels were significantly decreased in the HEE 200 mg/kg treated group compared to others in the triton-induced group, indicating that HEE has a protective effect against lipid peroxidation. These results suggest the order of activity as HEC 100 mg/kg <HEE 100 mg/kg <HEC 200 mg/kg <standard < HEE 200 mg/kg, suggesting the highest activity in HEE at the highest dose, which have significant clinical importance as potential antioxidants in treating hyperlipidemia-induced oxidative stress.

Histopathology of liver tissue treated with extracts of R. hypocrateriformis (Desr). leaves in triton-induced hyperlipidemia

In Figure 6, illustrations corresponding to the histopathology of the liver tissue in various groups were made. Liver tissue of the normal group showed normal hepatocyte cells with scattered nuclei and no fat deposits between the cells. Cellular size was also normal, and there were no structural disturbances or inflammatory evidence like cell membrane disruption and bulging of cells. In the triton group, clear enlargement of liver cells was observed with marked infiltration of lymphocytes

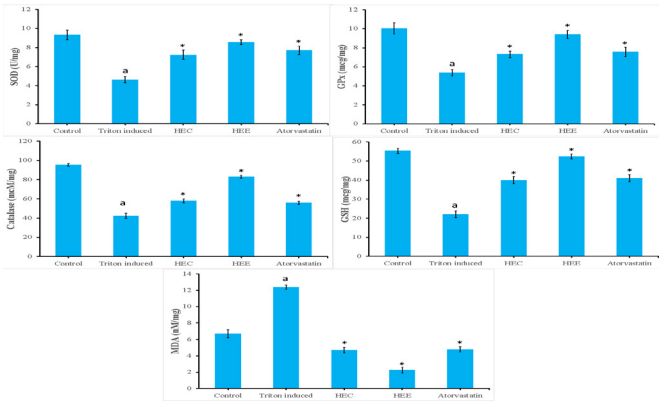

Figure 5: Effect of HEC and HEE on the antioxidant activity in triton-induced hyperlipidemia

and monocytes, indicating inflammation. The cell membrane disruption was also evident with an accumulation of fat deposits. Clearly, this was lowered by the treatment with extracts that had the highest activity, as the cellular integrity was restored and the cell size was significantly lowered. Thus, it can be assumed that the extracts showed enhanced activity compared to atorvastatin.

Loss of hepatic tissue integrity and blood accumulation (Blue box), aggregation of lymphocytes and monocytes (Green box), fatty infiltration in liver tissue (Yellow box).

DISCUSSION

Diabetes and heart disease are linked to hyperlipidemia or elevated blood lipid levels. While statins and fibrates have been effective in managing hyperlipidemia, their side effects have spurred interest in discovering new hypolipidemic drugs with fewer adverse effects.[17] Natural alternatives for hyperlipidemia management have gained attention to address concerns of over-prescription and potential adverse reactions.[18] In this context, medicinal plant extracts have shown promise in reducing atherosclerotic lesions and attenuating atherosclerosis development in animal models, leading to decreased plasma lipid concentrations and atherogenic indexes.[19] Among these plants, *R. hypocrateriformis (Desr)* has been of particular interest for its potential therapeutic effects against hyperlipidemia. In this study, chloroform (HEC) and ethanol (HEE) extracts of *R. hypocrateriformis* (Desr) were investigated for their anti-hyperlipidemic activity in a triton-induced hyperlipidemic rat model. The extracts were evaluated for their effects on lipid profiles and atherogenic indexes in the rat model, providing valuable insights into their potential as natural therapeutic agents for managing hyperlipidemia.

Using triton, peripheral tissues are blocked from absorbing triacylglycerol-rich lipoproteins from plasma in animal models. This method is commonly employed for various objectives, particularly in screening natural or chemical hypolipidemic drugs.[20] As a result of triton's inhibition of lipoprotein lipase activity, triglyceride-rich lipoproteins are less likely to be cleared from the circulation, leading to hyperlipidemia.[21] By interfering with the clearance of triglyceride-rich

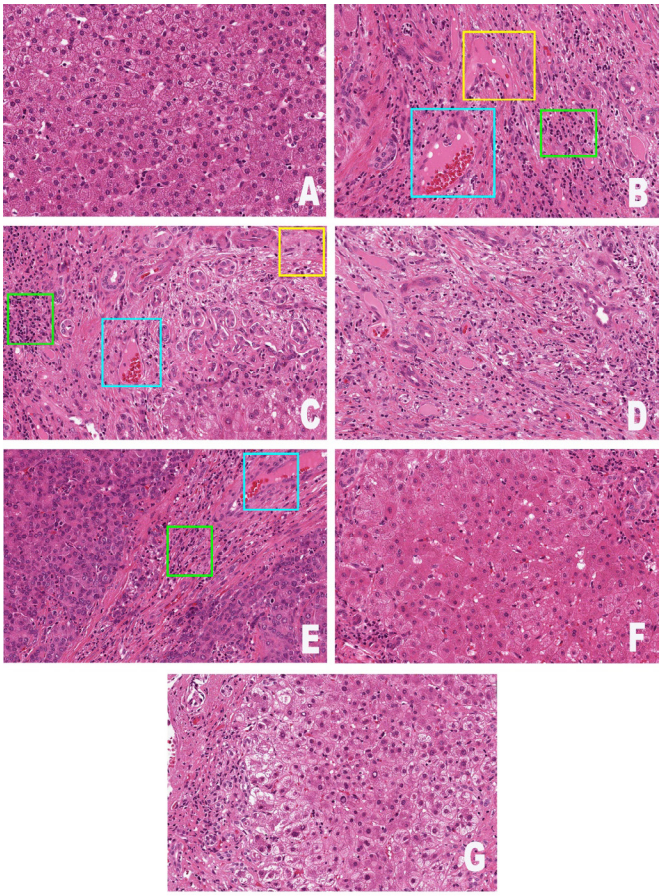

Figure 6: Effect of extracts on the histopathology of adipose tissue
A. Control; B. Triton group; C. HEC 100 mg/kg; D. HEC 200 mg/kg; E. HEE 100 mg/kg; F. HEE 200 mg/kg; G. Standard

lipoproteins, triton causes an increase in plasma triglyceride and cholesterol levels. This occurs due to enhanced very low-density lipoprotein (VLDL) secretion by the liver and reduced catabolism of VLDL and low-density lipoprotein (LDL).[22,23] Acute hyperlipidemia may develop as a result of triton WR-1339 altering VLDL structure, increasing their resistance to lipolytic enzymes in the body.[24]

In this study, we observed that the triton-induced group exhibited a substantial increase in body weight and obesity indices compared to the control group, confirming the success of the model in inducing hyperlipidemia. Treatment with both HEC and HEE extracts at 100 and 200 mg/kg doses resulted in a significant reduction in final body weight compared to the triton-induced group. This reduction in body weight suggests that the extracts may have a role in controlling lipid metabolism. Moreover, we evaluated the effects of HEC and HEE extracts on lipid levels in the rats. The triton-induced group had an adverse lipid profile, indicating elevated TC and TG levels and reduced HDL levels. However, treatment with both HEC and HEE extracts and the standard drug atorvastatin resulted in a significant reduction in TC and TG levels and an increase in HDL levels. These observations suggest that the extracts may play a role in modulating lipid metabolism, leading to improved lipid profiles.

Further, LDL and VLDL lipid parameters were significantly elevated in triton-induced mice, indicating a greater level of atherogenic lipoproteins. Treatment with HEC and HEE extracts, similar to atorvastatin, reduced LDL and VLDL levels, potentially mitigating the risk of atherosclerosis. Atherogenicity index (AI), which is a measure of the extent of the atherosclerotic lesion, was significantly reduced by the oral treatment of alendronate and atorvastatin, thus confirming previous findings.[25] Our study observed that the triton-induced group exhibited a significantly elevated atherogenicity index compared to the control group. This finding suggests that the triton-induced hyperlipidemia model successfully induced an adverse lipid profile characterized by higher LDL-C and/or lower HDL-C levels, which predisposes the rats to atherosclerosis. However, treatment with both HEC and HEE extracts, as well as the standard drug atorvastatin, reduced the atherogenicity index. The decrease in the atherogenicity index indicates an improvement in the lipid profile, with a potential reduction in LDL-C and/or an increase in HDL-C levels. This is a positive outcome, as a lower atherogenicity index is associated with a reduced risk of atherosclerosis and related cardiovascular complications.

An important factor contributing to hyperlipidemia was oxidative stress. As a result of metabolic and signal-transduction processes in cells, reactive oxygen species (ROS) are produced. These radicals include oxygen-free radicals. Biological membranes and lipoproteins are broken down by free radical oxidation.[26] Studies have shown that hypercholesterolemia is associated with higher levels of ROS compared to the normal state.[27] Supporting this, research conducted on mice revealed that 18 hours after triton administration, plasma thiobarbituric acid reactive substances (TBARS) increased after indicating oxidative damage. Catalase and glutathione peroxidase (GPx), two hepatic detoxicating enzymes, were also decreased as compared to control, further confirming oxidative stress.[28] The evidence suggests that oxidative stress is a key contributor to hyperlipidemia-related complications, and measures to reduce ROS levels and enhance antioxidant defenses may hold potential in managing hyperlipidemia and its associated health risks.

Therefore, we investigated the effects of HEC and HEE extracts on antioxidant enzymes in the triton-induced hyperlipidemia model. The triton-induced group showed decreased levels of SOD, CAT, GPx, and GSH, while elevated levels of MDA indicated oxidative stress. Treatment with both HEC and HEE extracts, similar to atorvastatin, restored antioxidant enzyme levels and reduced MDA levels, suggesting enhanced antioxidant defenses against oxidative stress. Overall, the results also suggest that the extracts showed a dose-dependent activity in controlling the hyperlipidemia induced due to the exposure to triton and HEE 200mg/kg being more potent than standard drug. These findings collectively demonstrate the potential of *R. hypocrateriformis (Desr)* extracts to exert anti-hyperlipidemia effects in the triton-induced hyperlipidemia rat model. The observed reductions in body weight and improved lipid profiles, along with the restoration of antioxidant enzyme levels, suggest that the extracts may act through multiple mechanisms like countering the inflammatory processes, restoration of the normal metabolism of fats *via* repairing the liver tissue to alleviate hyperlipidemia which is evident in the histopathology studies.

CONCLUSION

In conclusion, the study demonstrates the promising anti-hyperlipidemic and antioxidant properties of *R. hypocrateriformis* (Desr) extracts. The extracts offer a natural and multifaceted approach to address hyperlipidemia by modulating lipid metabolism and enhancing antioxidant defenses. Future research opportunities lie in identifying specific bioactive compounds, elucidating underlying mechanisms, and conducting clinical trials for human validation. *R. hypocrateriformis* (Desr) presents a compelling subject for drug development and nutraceutical research, potentially improving hyperlipidemia management and cardiovascular health.

REFERENCES

1. Ramkumar S, Raghunath A, Raghunath S. Statin Therapy: Review of Safety and Potential Side Effects. Acta Cardiol Sin. 2016; 32(6):631-639. doi: 10.6515/acs20160611a. PMID: 27899849; PMCID: PMC5126440.
2. Mukim M, Kabra A, Hano C, Drouet S, Tungmunnithum D, Chaturvedi M, Patel R, Ayaz M, Shadrack DM. Rivea hypocrateriformis (desr.) choisy: an overview of its ethnomedicinal uses, phytochemistry, and biological activities and prospective research directions. Journal of Chemistry. 2022 Jun 22;2022:1-1.
3. Borkar SD, Naik R, Shukla VJ, Acharya R. Evaluation of phytochemical content, nutritional value and antioxidant activity of Phanji-Rivea hypocrateriformis (Desr.) Choisy leaf. AYU (An international quarterly journal of research in Ayurveda). 2015 Jul 1;36(3):298-302.
4. Godipurge SS, Biradar JS, Biradar NJ, Shivarudrappa HP, Yallappa S, Hungund BS, Sreedhara Ranganath Pai K. Structural elucidation of alkaloids from aerial parts of rivea hypocrateriformis and their antihemolytic and cytotoxic activity. Chemistry of natural compounds. 2018 Jan;54:142-146.
5. Patel VB, Patel DG, Makwana AG, Patel JM, Brahmbhatt MR. Comparative study of Rivea hypocraterformis, Cynodon dactylone and Balanite aegypticae using antilithiatic activity in vitro. Journal of Pharmaceutical Sciences and Research. 2010;1(12):85-87.
6. Pariyani R, Safinar Ismail I, Azam AA, Abas F, Shaari K, Sulaiman MR. Phytochemical screening and acute oral toxicity study of Java tea leaf extracts. BioMed research international. 2015 Dec 27;2015. Available from: http://dx.doi.org/10.1155/2015/742420
7. Olaniyan JM, Muhammad HL, Makun HA, Busari MB, Abdullah AS. Acute and sub-acute toxicity studies of aqueous and methanol extracts of Nelsonia campestris in rats. Journal of Acute Disease. 2016; 5:62–70. Available from: http://dx.doi.org/10.1016/j.jo ad.2015.08.0 06
8. Abdelgadir AA, Hassan HM, Eltaher AM, Mohammed KG, Mohammed LA, Hago TB, Aboalbashar TH, Aalim TH, Ahmed AM, Mohamed AK. Hypolipidemic effect of cinnamon (Cinnamomum zeylanicum) bark ethanolic extract on triton

X-100 induced hyperlipidemia in albino rats.
9. Sudha S, Karthic R, Naveen JR. Anti hyperlipidemic activity of Spirulina platensis in Triton X-100 induced hyperlipidemic rats. Hygeia J D Med. 2011; 3: 32-37.
10. Parwin A, Najmi AK, Ismail MV, Kaundal M, Akhtar M. Protective effects of alendronate in Triton X-100-induced hyperlipidemia in rats. Turk J Gastroenterol. 2019; 30(6):557-564. doi: 10.5152/tjg.2019.18076. PMID: 31144662; PMCID: PMC6565349.
11. Saleem U, Riaz S, Ahmad B, Saleem M. Pharmacological Screening of Trachyspermum ammi for Anti-hyperlipidemic Activity in Triton X-100 Induced Hyperlipidemia Rat Model. Pharmacognosy Res. 2017; 9: 34-40.
12. Li H, Matheny M, Tumer N and Scarpace PJ. Aging and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression. American Journal of Physiology, Endocrinology and Metabolism. 1998; 275: 405–411
13. Lee MR, Kim JE, Choi JY, Park JJ, Kim HR, Song BR, Choi YW, Kim KM, Song H, Hwang DY. Anti-obesity effect in high-fat-diet-induced obese C57BL/6 mice: Study of a novel extract from mulberry (Morus alba) leaves fermented with Cordyceps militaris. Exp Ther Med. 2019;17(3):2185-2193. doi: 10.3892/etm.2019.7191. Epub 2019 Jan 21. PMID: 30867704; PMCID: PMC6395968.
14. Vijaya C, Ramanathan M, Suresh B. Lipid lowering activity of ethanolic extract of leaves of Aegle marmelos (Linn.) in hyperlipidaemic models of Wistar albino rats. Indian J Experimental Biol. 2009; 47:182–5.
15. Maehly AC, Chance B. In: methods of biochemical analysis, Vol. I, Glick D, editors, New York, Interscience. 1954; 357.
16. Ramesh, K. G., Mahesh, D., Burande. Elements of Clinical Pharmacy, 5th edition, 2007; 341-353.
17. Sultan S., D'Souza A., Zabetakis I., *et al.* The Impact of Nutrition and Statins on Cardiovascular Diseases. Amsterdam, Netherlands: Elsevier; 2019. Statins: rationale, mode of action, and side effects; pp. 171–200.
18. Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med. 2013; 369 (5):448–457. doi: 10.1056/NEJMra1201534.
19. Sedighi M., Bahmani M., Asgary S., Beyranvand F., Rafieian-Kopaei M. A review of plant-based compounds and medicinal plants effective on atherosclerosis. Journal of Research in Medical Sciences. 2017; 22(1) doi: 10.4103/1735-1995.202151.
20. Harnafi H., Caid H.S., Bouanani N. el H., Aziz M., Amrani S. Hypolipemic activity of polyphenol-rich extracts from Ocimum basilicum in Triton WR-1339-induced hyperlipidemic mice. Food Chem. 2008; 108:205–212.
21. Schurr P. E., Schultz J. R., Parkinson T. M. Triton-induced hyperlipidemia in rats as an animal model for screening hypolipidemic drugs. Lipids. 1972;7(1):68–74. doi: 10.1007/bf02531272
22. Otway S, Robinson DS. The effect of a non-ionic detergent (Triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rat. J Physiology. 1967; 190:309–19. doi: 10.1113/jphysiol.1967.sp008210.
23. Sikarwar M.S., Patil M.B. Anti-hyperlipidemic activity of *Salacia chinensis* root extracts in Triton-induced and atherogenic diet-induced hyperlipidemic rats. Indian J. Pharmacol. 2012; 44 (1):88–92.
24. Friedman M., Byers S.O. The mechanism responsible for the hypercholesteremia induced by Triton WR-1339. J. Exp. Med. 1953; 97(1):117–130.
25. Véniant MM, Sullivan MA, Kim SK, et al. Defining the atherogenicity of large and small lipoproteins containing apolipoprotein B100. J Clin Inves. 2000; 106:1501–10. doi: 10.1172/JCI10695.
26. Morgan MJ, Kim YS, Liu Z. Lipid rafts and oxidative stress–induced cell death. Antioxidants & redox signaling. 2007 Sep 1;9(9):1471-1484.
27. Stokes KY, Cooper D, Tailor A, Granger DN. Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. Free Radical Biology and Medicine. 2002 Oct 15;33(8):1026-1036.
28. Oh PS, Lee SJ, Lim KT. Hypolipidemic and antioxidative effects of the plant glycoprotein (36 kDa) from Rhus verniciflua stokes fruit in Triton WR-1339-induced hyperlipidemic mice. Bioscience, biotechnology, and biochemistry. 2006;70(2):447-456.