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Anti-Hyper Lipidemic Activity of Desmostachya Bipinnata Extracts in Triton X-100 Induced Hyper Lipidemic Rats

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1. INTRODUCTION

Hyperlipidemia, a condition characterized by increased levels of triglycerides, cholesterol, cholesterol esters, and phospholipids in the plasma, has been studied extensively by researchers over the years (J.L. Goldstein, 1973; D. Harrison, 2003). This elevation in plasma lipids can be attributed to either primary factors, such as genetic defects, or secondary factors like diet, drugs, or diseases. It is important to note that while there are variations in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic rat models have been developed for research purposes. These rat models are established through various methods, including dietary supplementation with lipids or sucrose over a period of 2 to 3 months, depending on the study's requirements. Additionally, some models involve the administration of progesterone (SK Asmath Begum, 2019) or the oral usage of nonionic surfactants like methionine, poloxamer-407, triton X-100, and WR-1339 (R.H. Raasch, 1988; Vasu K. & Srinivas P., 2009; Madariaga YG, 2015). Among these, Triton X-100 solution has proven to be a successful inducer of hyperlipidemia in rats, as shown in various research studies (DS. Mohale, 2008). Due to its convenience, reproducibility, and availability, Triton X-100 was chosen as the hyperlipidemic model for the current study. However, the use of synthetic drugs can lead to adverse effects, including nausea, diarrhea, gastric irritation, and hyperuricemia. As a result, researchers are increasingly focused on exploring plant-derived extracts and phytoconstituents with hypolipidemic properties due to their minimal or non-existent side effects (Pahan K, 2006).

Studies have demonstrated the potential of various plant extracts in this regard. For instance, the methanolic extract and juice of *Lagenaria siceraria* (Family: Cucurbitaceae) fruits (Agarwal S.S., 2008 & Nainwal P, 2011), the ethyl acetate extract of *Commiphora wightii* (Family: Burseraceae) (Sahni CA, 2005 & Hasani RS, 2010), the ethanolic (95%) extract of *Glycyrrhiza glabra* (Family: Fabaceae) (Sitohy MZ, 1991 & Maurya SK, 2009), and the methanolic extract of *Desmostachya bipinnata* (Family: Poaceae) (Yaso D, 2016) have all demonstrated anti-obesity potential. These natural alternatives offer promising benefits for addressing lipid-related issues.

The present study was carried out to examine anti-hyperlipidemic activity in aerial parts of the plant. Extracts of aerial parts of *Desmostachya bipinnata* were prepared and their anti-hyperlipidemic activity was investigated using triton- X100 induced hyperlipidemia in rats.

2. METHODOLOGY

Plant material: collection, authentication

Whole plants of *Desmostachya bipinnata* (L.) Stapf were collected in September 2013 from Chirawa, district Jhunjhunu, Rajasthan, India. The collected plant was authenticated by Dr. R. P. Pandey from Botanical Survey of India, Jodhpur, India. A voucher specimen, JNU/PH/2010/D_b D_2, was deposited in the herbarium of Jodhpur National University, Jodhpur, India.

3. EXTRACTION

The plant material *i.e*; aerial parts of *Desmostachya bipinnata* (Extractive yield in Table 7.4.1) was grinded using electric mixer-grinder and screened using BSS standard sieve. Powder which passed through seive no. 22 of average aperture size 710 µm and retained on sieve no. 44 of average aperture size 355 µm was selected and used for extraction. The powdered drug was packed in a paper cylinder made from a filter paper and placed in the body of soxhlet extractor. The solvent poured (n-Hexane, Chloroform and Alcohol) in soxhlet extractor and allowed to run for 3-4 cycles. After that the apparatus was fitted in appropriate manner and drug was extracted. The obtained extracts were filtered through Whatman filter paper and concentrated and dried by evaporating the solvent on water bath. The residual moisture in the extract was removed by drying in an oven followed by keeping the extract in desiccator.

4. CLOUD POINT EXTRACTION

Cloud point extraction (Ultrasonic cloud point extraction) was performed as shown in Figure 1. Three factors were studied *i.e;* temperature, time and surfactant concentration and at two levels as given in Table 1 and total 8 extractions were performed (Paleologos EX, 2005)

Table 1: Factors used in cloud point extraction

Name	Factor	Lowest level	Highest Level
A	Temp	40°c	60°c
B	Time	1 hr	2 hr
C	Surfactant	2% w/v	5% w/v

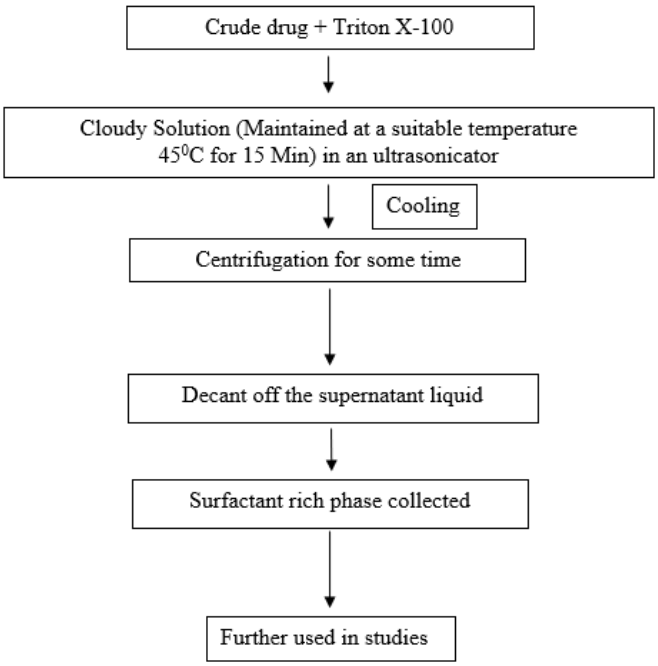

Figure 1: Steps followed in cloud point extraction

The extracts of aerial parts of *Desmostachya* were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, fixed oils and fats, proteins, amino acids, flavonoids, saponins, etc. using reported methods. (Mukherjee, 2002)

5. TRITON X-100 INDUCED HYPERLIPIDEMIC ACTIVITY

Hyperlipidemic activity of chloroform, alcohol and cloud point extract 8 extracts of *Desmotachya bipinnata* (Aerial parts) were evaluated using Triton X-100 induced Anti- hyperlipidemic in albino rats (n=5) (Vasu K. & Srinivas P., 2009)

Animals

Wistar albino rats of either sex weighing between 150-250 gm were used for the study. They were obtained from (CPCSEA) government of India and Institutional animal ethics committiee. They were kept in the departmental animal house at 25 ± 2°C and relative humidity 45-51.5% on a 12 hours light/dark cycle.

Grouping and Treatment Protocol

Group I: Control group

Normal saline 0.9% NaCl

Group II: Standard group

Atorvastatin (10 mg/kg)

Group III: Positive control

Triton X-100 (5.3 mg/kg)

Group IV: Test group I

Alcoholic extract (500 mg/kg)

Group IV: Test group II

Rats were treated with chloroform extract (500 mg/kg)

Group VI: Test group III

Rats were treated with cloud point extract 8 (500 mg/kg)

6. BIOCHEMICAL ESTIMATION

Biochemical estimation was carried out 24 h after last dosing of 24 h at the end of experimental protocol, animals were fasted over-night and blood was taken from retro-orbital plexus under mild chloroform anesthesia, serum was separated by centrifugation (Remi motor LTD. Mumbai) at 3000 rpm for 10 minutes and resultant supernatant phase was used for biochemical estimation.

After, that serum parameters like serum cholesterol, serum LDH (Lactate dehydrogenase) and serum triglycerides. CHO (Cholesterol), HDL (High density lipoprotein), LDL (Low density lipoprotein), VLDL (Very low-density lipoprotein). Serum parameters were analyzed by the biochemical kits (ERBA).

7. HISTOPATHOLOGY OF LIVER

Harvested liver tissue was fixed in formalin (4% v/v) and sectioned perpendicularly (Kumar V & Singh P, 2009) Tissue sections were stained with hematoxylin-eosin using standard histopathology protocol.

Statistical analysis

All the data were represented as Mean ± S.E.M., n=5, and analyzed by One-way ANOVA followed by Dunnett's t-test for the possible significant interrelation between the various groups.

8. RESULTS

Extraction of aerial parts *Desmostachya bipinnata* was performed using soxhlation method and cloud point extraction method. In soxhlation method, three solvents were used *i.e;* n-hexane, chloroform and alcohol and in cloud point extraction triton X-100 is used as surfactant. Maximum extractive yield was found in Alcohol 20.00 ± 0.68%w/w than in chloroform 4.44 ± 0.18 % w/w and n-hexane 1.90 ± 0.57% w/w respectively. Amount of phenolics and flavonoids present in alcoholic extract was 10.92%w/w and 20.81 %w/w respectively. Phenolics and flavonoids present in chloroform extract are 7.76 % and 8.59 %w/w. In cloud point extraction, cloud point extract 8 was found best among all eight extracts of cloud point extraction (Temperature 60^{o}c, time 2 hours and triton x-100 concentration 5%) having maximum extraction yield 11.2 ± 01.26 % w/w, flavonoid 13.44%w/w and phenolics 43.63%w/w. In phytochemical screening of extracts phenolics,

flavonoids, alkaloids and steroids were present in different extracts of *Desmostachya bipinnata.*

9. HYPERLIPIDAEMIC ACTIVITY

Increased triglycerides, cholesterol, cholesterol esters and phospholipids (Plasma lipids) results in hyperlipidemia (J.L. Goldstein; 1973, D. Harrison; 2003). Increase in plasma lipids may be due to primary (genetic defect) or secondary (diet, drugs or diseases) factors. Although there are differences in lipoprotein distribution and metabolism in humans and rats. Hyperlipidemic rat models are based on dietary supplementation with lipid or sucrose or both for 2 to 3 months depending upon requirements; based on administration of progesterone (SK Asmath Begum, 2019) and based on the oral usage of nonionic surfactant such as methionine, poloxamer-407, triton X-100 and WR-1339 are extensively used in lipid research (R.H. Raasch; 1988, Vasu K. & Srinivas P., 2009, Madariaga YG; 2015). Triton X-100 solution has successfully been used to induce hyperlipidemia in rats in various reported research studies (DS. Mohale; 2008). Because of convenience, reproducibility and availability of the Triton X-100, it was chosen as the hyperlipidemic model for the current study.

Serum parameters (TRG, CHO, HDL, LDL, VLDL) were recorded. All serum parameters were analyzed by the biochemical kits. Blood was collected from retro-orbital of rats, then blood was centrifuged and Serum was separated.

Table 2: Serum parameters after 24 hours of treatment

Groups	**Serum biochemical parameters (mg/dl)**				
	TRG	**CHO**	**HDL**	**LDL**	**VLDL**
Control	151.00 ± 2.82	114.6 ± 1.58	36.76 ± 0.77	47.00 ± 2.09	30.28 ± 0.57
Positive control	320.10 ± 1.68*	171.40 ± 0.69*	51.81± 0.48 *	89.62 ± 0.36 *	63.96 ± 0.33*
Standard	183.40 ±0.58 ***	131.90 ± 1.37***	37.76 ± 0.43***	58.35 ± 1.60***	36.80 ±0.22***
Test 1 (Alcohol)	304.00 ± 0.35*	148.6 ± 0.25***	49.24 ± 0.62*	78.78 ± 0.71*	60.96 ± 0.29*
Test 2 (Chloroform)	198.70 ± 0.59**	163.5 ± 0.86*	44.89 ± 0.67**	75.78 ± 0.93*	39.58 ± 0.25***
Test 3 (C.P.E 8)	310.40 ± 0.71*	159.6 ± 0.38**	47.98 ± 0.96*	71.32 ± 0.84**	61.70 ± 0.38*
TRG (Triglycerides), CHO (Cholesterol), HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein), VLDL (Very Low-Density Lipoprotein).					

All serum parameters were increased in positive control. In the treatment groups such as standard marketed drug, alcoholic, chloroform and cloud point extract 8.

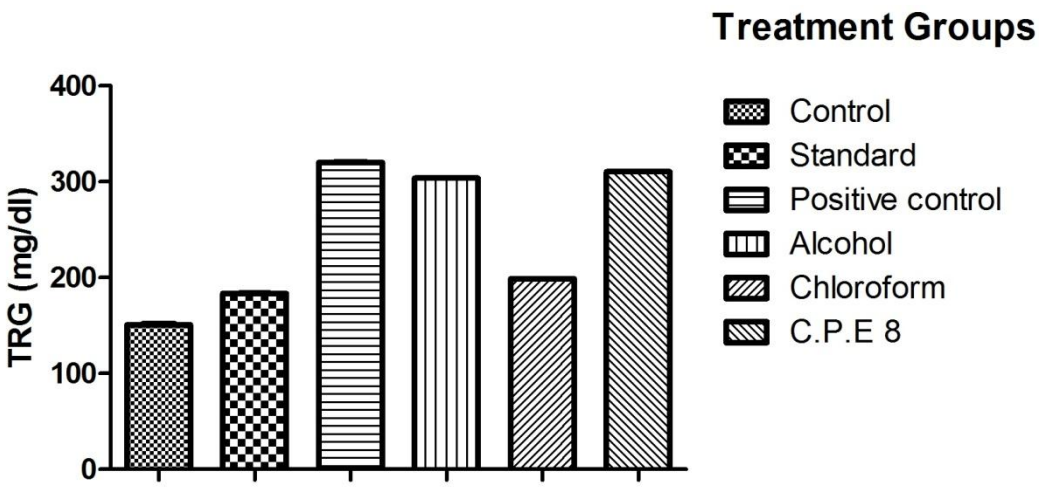

Figure 2: Effect of *Desmotachya bipinnata* (aerial parts) extracts on release of triglycerides (TRG)

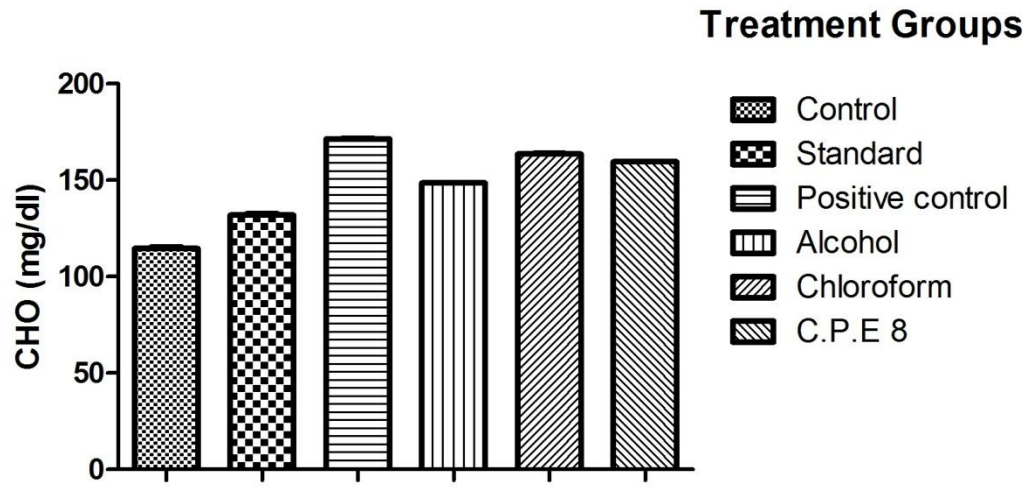

Figure 3: Effect of *Desmotachya bipinnata* (aerial parts) extracts on release of cholesterol (CHO)

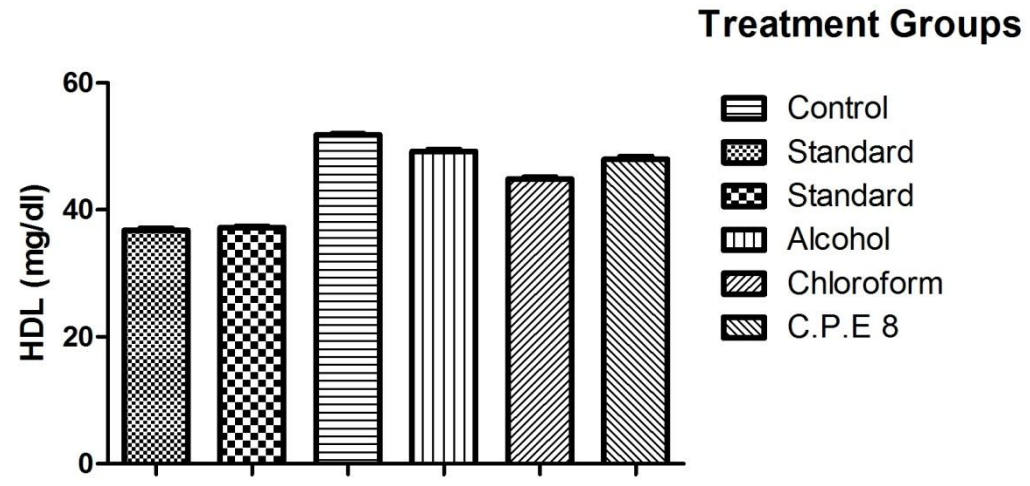

Figure 4: Effect of *Desmotachya bipinnata* (aerial parts) extracts on release of high-density lipoprotein (HDL)

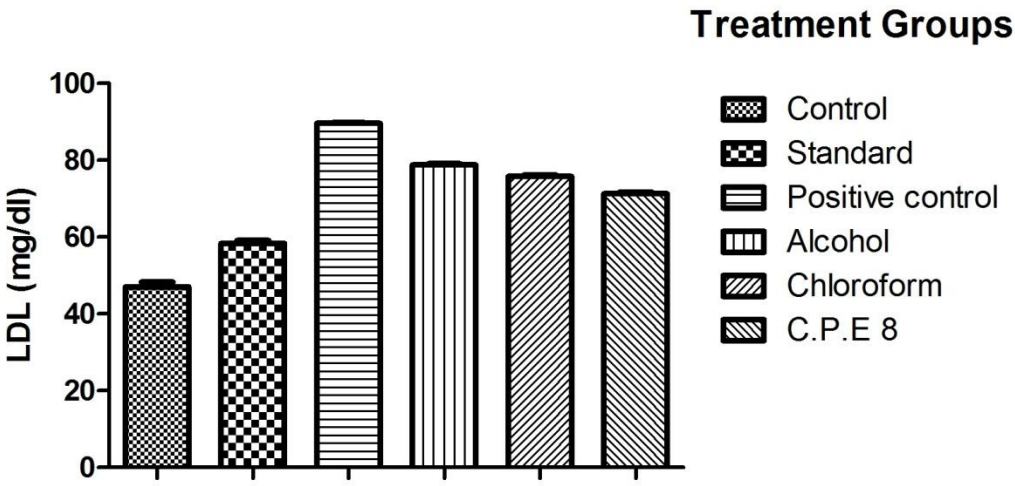

Figure 5: Effect of *Desmotachya bipinnata* (Aerial parts) extracts on release of low-density lipoprotein (LDL)

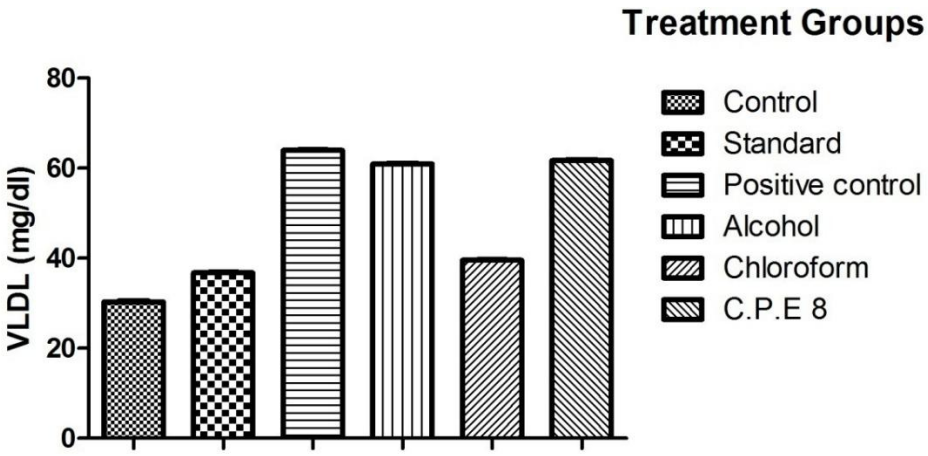

Figure 6: Effect of *Desmotachya bipinnata* (Aerial parts) extracts on release of very low-density lipoprotein (VLDL)

Table 3: Organs (Liver, heart and kidney) weight after 24 hours of treatment.

Groups	Organ's weight (g/kg)			
	Liver	Heart	Kidney	
			Left	Right
Control	4.61 ± 0.09	0.52 ± 0.04	0.86 ± 0.04	0.74 ± 0.03
Positive control	6.24 ± 0.42	0.91 ± 0.04	0.86 ± 0.03	0.83 ± 0.04
Standard	5.45 ± 0.18	0.68 ± 0.03	0.77 ± 0.04	0.74 ± 0.02
Test 1 (Alcohol)	6.02 ± 0.12	0.75 ± 0.04	0.81 ± 0.03	0.75 ± 0.02
Test 2 (Chloroform)	6.06 ± 0.20	0.80 ± 0.04	0.84 ± 0.04	0.65 ± 0.03
Test 3 (C.P.E 8)	5.04 ± 0.13	0.62 ± 0.02	0.77 ± 0.02	0.75 ± 0.02

During the anti-obesity study, changes in organ weights (Liver, Heart, and Kidney) were carefully observed. In the positive control group, the weights of these organs were higher, whereas in the various treatment groups of *Desmostachya bipinnata* extracts, the weights of these organs were notably reduced. Remarkably, the weights of the different organs in the C.P.E.8 extract showed a significant reduction, comparable to the standard group. This indicates the potential efficacy of the C.P.E.8 extract in managing organ weight and suggests its promising role in addressing obesity-related issues.

These observations shed light on the influence of *Desmostachya bipinnata* extracts on organ weights and contribute to the understanding of their anti-obesity properties. The findings pave the way for further research and the exploration of potential therapeutic applications.

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Effect of treatment groups on histopathology of rats' liver

The results obtained from the histopathological studies provided valuable supportive evidence for the biochemical analysis. The histology of the normal control group displayed reparative changes in a few hepatocytes, with no indication of cirrhosis or toxicity. Similar results were observed in the treatment groups, except for the C.P.E group, where glycogen degeneration was absent, accompanied by the presence of a cyst and mild inflammation.

In terms of tissue and serum analysis for the treatment of hyperlipidemia, the order of hyperlipidemic effect was found to be atorvastatin > chloroform extract > alcohol extract > C.P.E 8. The variation in the activity of these extracts in reducing hyperlipidemia can be attributed to the active constituents present in each of them. Notably, the standard drug demonstrated the highest effectiveness, while the C.P.E 8 extract exhibited comparatively lesser effectiveness among all.

To assess the statistical significance of the differences between means, one way Analysis of Variance (ANOVA) was initially conducted, followed by Dunnett's test for multiple comparisons, and t-test followed by an unpaired test. The values are expressed as Mean ± SEM, with statistical significance denoted by *P<0.05, **P<0.01, ***P<0.001, and a sample size of n=5 in each group.

These findings shed light on the impact of the treatment groups on liver histopathology and provide insights into their efficacy in managing hyperlipidemia. The combined histopathological and biochemical analyses contribute to a comprehensive understanding of the effects of the tested extracts, aiding further research and potential therapeutic applications.

Many phytoconstituents have been identified from *D. bipinnata* and reported in literature like coumarins (scopoletine and umbelliferone), carbohydrates, sugars, proteins, amino acids, alkaloids, tannins, phenolics, xanthenes (2,6-dihydroxy-7Zmethoxy-3H-xanthen-3- one) (Sabina S., 2011), steroids (stigmasterol, βsitosterol, daucosterol, etc) (Shrestha S., 2011), flavonoids (kaempferol, quercetin, quercetin-3- glucoside, trycin and trycin-7-glucoside) (Awaad AS.,2008), triterpenoids and glycosides (Golla U., 2014, Ashok KBS., 2010, Hifnawy MS.,1999, Kumar V., 2010). Essential oils obtained from the aerial parts of *D. bipinnata* consists of camphene, isobornyl acetate, tricyclene, caryophyllene diepoxide, eudesmol, eseroline and calarene as the main components oil (Kumar Ak., 2011).

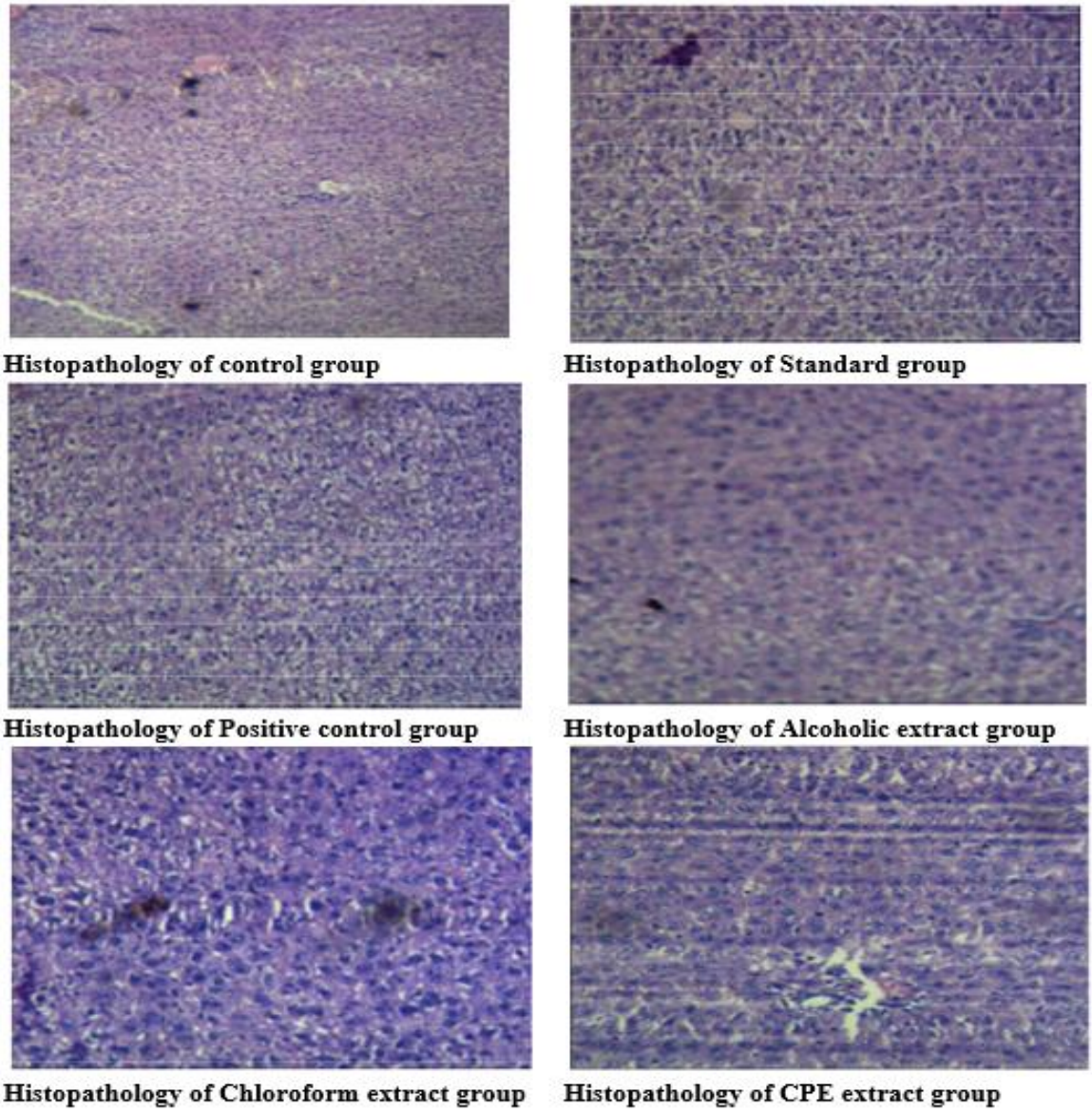

Figure 7: Histopsthology of rat's liver in different groups of anti-hyper lipidemic activity.

10. DISCUSSION

In the preliminary phytochemical screening of extracts, phenolics, flavonoids, alkaloids, and steroids were found in different extracts of *D. bipinnata*. In Anti-hyper lipidemic activity, CPE 8 was found to be more effective among all treatment groups in tissue, serum, and histopathological studies. The results of our studies validate the traditional use of the plant as a Anti-hyper lipidemic agent. Detailed studies, including phytochemical analysis, isolation of phytoconstituents, mechanisms, formulation, etc., are warranted for exploitation of this plant for developing herbal medicinal product.

11. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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