



ISSN:2456-9739

BRITISH JOURNAL OF BIO-MEDICAL RESEARCH

Cross Ref DOI: <https://doi.org/10.24942/bjbmr.2021.839> Volume 05, Issue 03, May -June 2021

Research Article

Antihyperlipidemic Effect Of *Leucaena Leucocephala* Linn. Stem Bark In High Fat Diet Induced Hyperlipidemia In Rats

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ARTICLE INFO

ABSTRACT

Article History:

Received on 02th May 2021

Peer Reviewed on 18th May 2021

Revised on 15th June 2021

Published on 28th June 2021

Keywords:

Antihyperlipidemic, LDL, HDL, VLDL

Objective: To evaluate anti-hyperlipidemic effect of *Leucaena leucocephala* Linn. Stem bark in high fat diet induced hyperlipidemia in rats

Method: The bark *Leucaena leucocephala* Collected, size reduced and was subjected to successive Soxhlet extraction. Hyperlipidemia was induced by the administration of cholesterol rich food diet (previously prepared by mixing Cholic acid 1%, cholesterol 2%, coconut oil 10%, sucrose 40%) material till 14 days. Atorvastatin 10 mg/kg and methanolic extract of *L. leucocephala* 250 mg/kg and 500 mg/kg were administered to the respective group for one month on daily basis. Then rats were sacrificed and the blood was collected by cardiac puncture and finally analysed for HDL, TC, TG, LDL, and VLDL. Histopathological analysis was also done with rat's liver tissue.

Result and Discussion: The daily administration of methanolic extract of *L. leucocephala* 250 mg/kg and 500 mg/kg and Atorvastatin 10 mg/kg to the hyperlipidemic rats (previously made hyperlipidemic by administering high cholesterol diet) reduces the undesirable raised level of TC, TG, LDL, and VLDL and improve the HDL level (good cholesterol) as compared to the Diseased rats (untreated group). Histopathological analysis show the regeneration property of Atorvastatin 10 mg/kg and methanolic extract of *L. leucocephala* 250 mg/kg and 500 mg/kg on Degenerated liver cell as compared to the Diseased rats (untreated group).

Conclusion: The above result conclude that The daily administration of methanolic extract of *L. leucocephala* restore the altered lipidemic profile (decreases TC, TG, LDL, and VLDL and increase HDL level. Histopathological analysis also proves the above estimation. Further investigation is needed to isolate single chemical constituent of LL responsible for pharmacological action.

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INTRODUCTION

Hyperlipidemia is characterized as the heterogeneous group of disorder, and is excessive lipidemic level in blood circulation. Various forms of lipid transported in blood stream based on specific density named, LDL, HDL, VLDL, and many more also. Atherosclerotic and other associated condition are major complication are associated with it. Although the complication associated is the result of many more complication. Dislipidemic condition showed major contribution in atherosclerotic risk. Altered lifestyle and diet play major role in developing dyslipidemia [7, 10].

Previous scientific researcher's report 2011 concludes that 4.5 % (2.6 million) people death in all over world was occurred due to hyperlipidemic condition. Global health Organization WHO estimated that cardiovascular disease is more deadly disease. It was estimated that there will be 24 million deaths by 2030^[1].

The disease associated with hyperlipidemia is peripheral vascular disease, coronary heart disease, and ischemic cerebrovascular disease, obesity is also major diseased condition associated with it [9]. Further deposition of fatty material like, cellular waste, cholesterol, calcium and several unwanted things in the innermost layering of an blood artery resulting plaque formation inside the inner arteries, as time passes, it become harden and more thick plaque formation . This plaque formation narrows the arteries, which responsible for macro and micro vascular damage to the blood arteries and vessels [4].

In current ere worldwide interest toward adaptation of herbal medicine is increased and now researcher screening natural remedies and explaining their effectiveness potential. We aimed to evaluate antihyperlipidemic effect of *Leucaena leucocephala* Linn. Stem bark in high fat diet induced hyperlipidemia in rats [3].

MATERIAL AND METHOD

Collection and extraction of plant material

The bark was collected from the plant *Leucaena leucocephala* and it was collected from home town Kunda, Praatapgarh, Uttar Pradesh, India in the month of September. It was reviewed and authorized by Dr. GP Sinha (Senior Head Scientist In Botanical survey (BSI) of India, Prayagraj with Ref. No- BSI/CRC/T/2019-20/523. The isolated bark was shade dried and size reduced to form uniform particle size, Then it was extracted with various extracting solvent in soxhlet apparatus based on the polarity The different solvent extracts were filtered, and solvent were evaporated till moisture content is reduced then it was subjected to freeze drying process and finally the resulting dried extract was stored in suitable environment condition till future use [1].

Preliminary Phytochemical Screening

The resulting extract of *Leucaena leucocephala* was screened for phytochemical contents like alkaloids, coumarin, saponins, cardiac glycosides, tannins glycosides, steroids, flavonoids, and other phyto contents.

Chemicals and Drug

Methanol, cholesterol, coconut oil, sodium cholate, atorvastatin

Animals

Albino wistar rats (170–200 g) were utilized for evaluation of pharmacological activities. Animal were housed under polypropylene cages, with suitable bedding material. The environmental condition such as temperature was maintained at 25±2 °C, and also with RH (relative humidity) between 45-55%. The 12h light and dark cycles was maintained during experimentation duration. Before experimental procedure on animal, they were acclimatized for upto 7 days in to laboratory conditions. Standard pallet diet and water ad libitum was provided to all experimental animals. Before performing experimentation on animal, approval was granted by the

Institutional Animal Ethical Committee (IAEC) under Reg. No. 1632/PO/Re/S/12/CPCSEA/11.

Acute Toxicity Studies

The acute toxicity estimation of the *Leucaena leucocephala* bark extract methanol extract was estimated by introducing wistar albino rat of either sex, which was previously acclimatized under standard temperature and humidity conditions with proper animal pallet diet. The rats were fasted up to 12 hour before experimentation. Fixed dose (OECD. 423) experimental guideline of CPCSEA agency was considered for this evaluation studies. Different Fraction dose was administered to the animal and then the animal were observed for 24 hr. and next 72 hr. any sign of physical behavioral, and mortality was also observed.

High Fat Diet Hyperlipidemia

Experimental design

Wistar rats weighing 150-180 g were divided into 5 groups of 6 animals each.

Group 1: Served as normal control and were given only vehicle (distilled water)

Group 2: Received high fat diet served as hyperlipidemic control (positive control)

Group 3: Received 10 mg/kg/day atorvastatin served as standard

Group 4: Received 250 mg/kg/day plant extract

Group 5: Received 500 mg/kg/day plant extract

The extract and standard drug administered to the animal on daily basis for one month^[1, 12].

Experimental Induction of Hyperlipidemia

(High fat diet method)

Hyperlipidemia was induced by ingesting high cholesterol food content which consists of 2% cholesterol, 2% coconut oil, 1% sodium cholate, and standard animal food pallet. All ingredients were mixed and small pallet was prepared which finally placed in rats' cage for administration for 20 days^[1, 13].

Collection of blood and biochemical estimation

Rat was killed by cervical dislocation under ether anaesthesia. Blood was collected by puncturing heart. The blood was centrifused at 2000 rpm for 10 min. The serum was separated and biochemical parameter like HDL, TC, TG, LDL,

and VLDL was estimated using diagnostic machine kit. VLDL and LDL were estimated statically through Friedward method and, VLDL: TG/5 [1, 14].

Histopathological evaluation

The rats were sacrificed by cervical dislocation under mild ether anaesthesia. Rats aorta were dissected and transferred into 10% formalin solution. Histopathological study was done by pathological laboratory for eosin and hematoxilin staining^[1, 12].

Statistical Analysis

The result of experimental study were expressed as mean \pm SEM. The statistical analysis was done using One-way Analysis of variance (ANOVA) followed by Dunnet multiple comparison test. The value $P < 0.05$ was taken as significant value^[14, 15].

RESULT AND DISCUSSION

Result

Phytochemical screening

Phytochemical screening showed the presence of flavanoid, phenolic compound, glycoside, alkaloid and other phyto compound was also confirmed.

Acute toxicity test

All testing animal were alive at the dose 2000 mg/kg. LL. There was no sign of toxicity in experimental animal.

Anti Hyperlipidemic activity of LL

Table 1 represents the Effect of treatment on biochemical parameter in different group. DC control rat shows significant increase ($p < 0.0001$) in TC, TG, LDL, VLDL level, and decrease in HDL level as comparison to normal rats. SC control rat significantly ($p < 0.0001$) reduces TC, TG, LDL, VLDL level, and increases HDL level as compared to DC rats. The methanolic fraction of LL at dose 250 and 500 mg/kg also significantly ($p < 0.0001$) reduces TC, TG, LDL, VLDL level, and improve HDL level in comparison to DC rats.

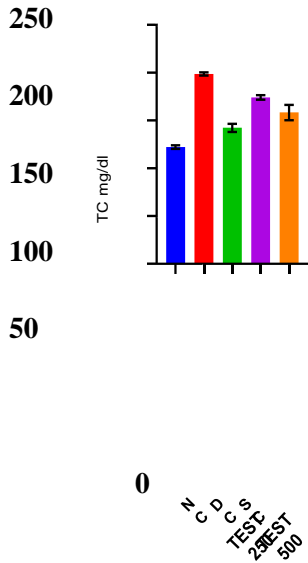
Table 1: Effect of treatment on biochemical parameter in different group

Group	Treatment	Serum level (mg/dl)				VLDL
		TC	TG	LDL	HDL	
1	NORMAL	122.16	141.16	55.33	44.50±1.52*	18.50±2.32
		±1.92****	±3.17	±4.11****		**

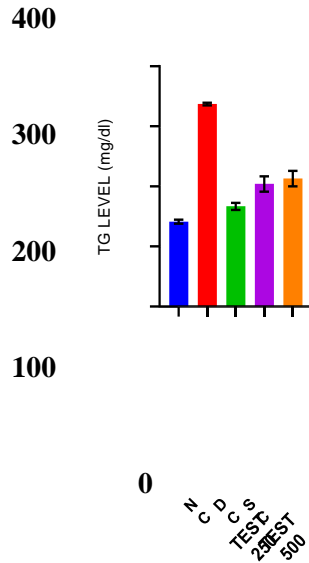
2	DISEASE	198.66	337.00	114.83	24.83 ±1.85	36.33±2.52
	CONTROL	±1.70	±2.36	±7.04		
3	GLIBENCLAMIDE	142.33	166.67	67.66	45.16±2.75****	22.50±2.11
		±4.20*	±5.74**	±24.71		
4	T 250	174.16	204.00	73.83	40.33 ±5.58****	24.00±2.85
		±2.38*	±12.96**	±2.28		
5	T 500	158.33	213.00	79.50	36.50 ±5.46**	27.00±4.59
		±7.99	±12.83**	±0.05		

Each value expressed as Mean±SD, (n=6)

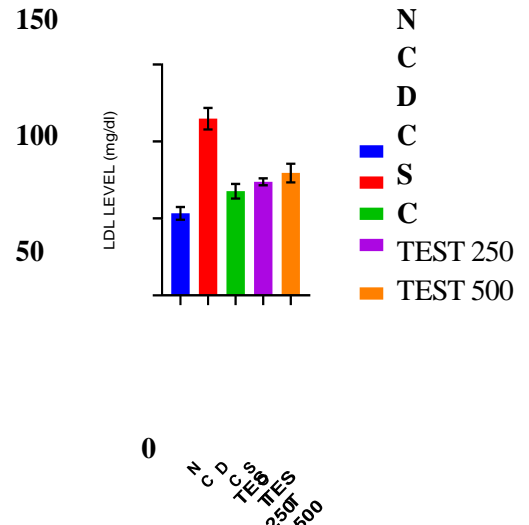
**** $p < 0.0001$ compared with Disease Control



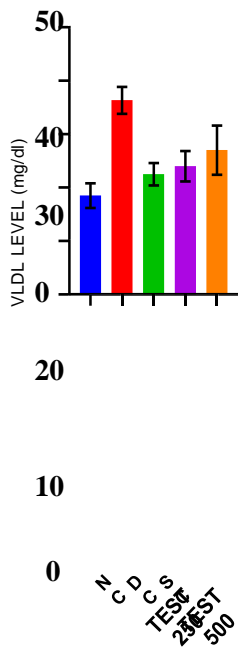
Effect of treatment in different group



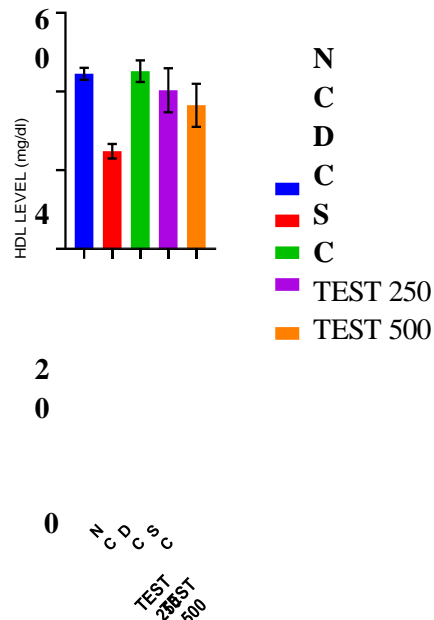
Effect of treatment in different group



Effect of treatment in different group



Effect of treatment in different group



Effect of treatment in different group

Each value = mean \pm SEM, Nc = Normal Control, Dc = Diseased Control, Sc = Standard Control

Fig 3.1 Effect of treatment on various group, NC= Normal control, DC= diseased control, SC=standard control (receive Atorvastatin), TEST 150= *L. leucocephala* 150 mg/kg and TEST 150=300 mg/kg

Histopathological evaluation

Histopathological test of liver is conformational supportive evidence to the testing parameter. Microscopic structure of group 1 rat (normal rat) showed normal cellular area. Necrotic structure was seen in microscopical image of diseased rat (group 2). Microscopic structure of Atorvastatin treated rat (group 3) showed no necrotic area in liver cell, which shows protective ability of atorvastatin against liver cell necrosis. MELL at dose 250 and 500 mg/kg (in group 4 and 5 respectively) showed dose dependent action on liver tissue necrosis. MELL 500 mg/kg showed greater liver cell regeneration activity than MELL 250 mg/kg.



Fig3.2. Microscopic image of Group 1

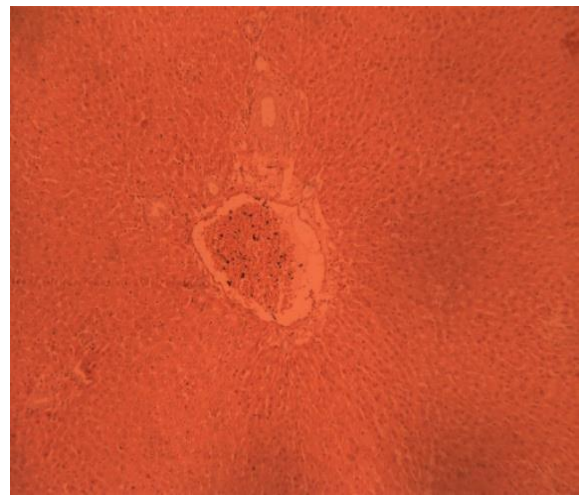


Fig.3.3 . Microscopic image of Group 2

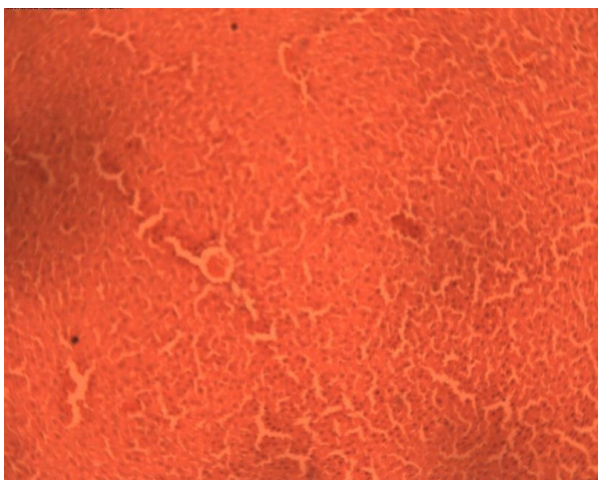


Fig.3.4 Microscopic image of Group 3

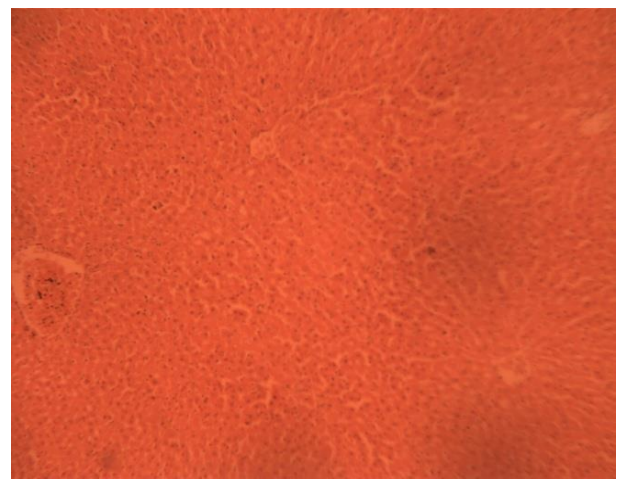


Fig.3.5 . Microscopic image of Group

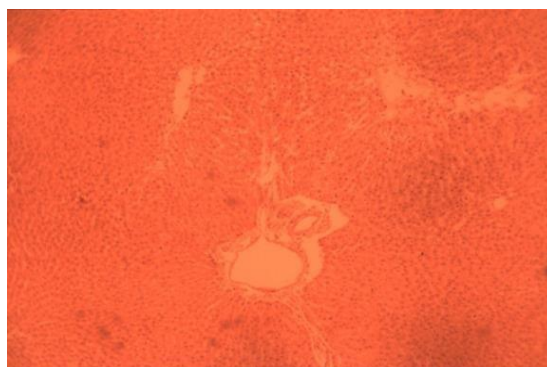


Fig.3.6 Microscopic image of Group 5

DISCUSSION

Distinct increment in serum TC, TG, LDL, VLDL level and reduction in HDL is the major risk to coronary artery disease. HMG CoA is rate limiting enzyme in biosynthesis pathway of cholesterol. By inhibiting above factor help to maintain the lipidemic level. Cholesterol rich diet increases the TC, TG, LDL, VLDL level, and reduces the HDL level in experimental rat. The standard drug atorvastatin in SC group reduces TC, TG, LDL, VLDL level, and increases HDL level in comparison to diseased rat (untreated rats). Methanolic fraction of LL at 250 and 500 mg/kg also reduces the TC, TG, LDL, VLDL level, and improve HDL level in comparison to the diseased control rats. LL extract 250 mg/kg showed more efficient result as compared to the LL extract 500 mg/kg. This results of occurs due to protective effect LL which improve the lipidemic Level of Serum. [12, 13, 14, 15]

CONCLUSION

We conclude that, alteration in lipidemic level invites other complication like diabetes, microvascular, and macrovascular complication. We find that, alteration in lipidemic level due to high cholesterol diet were restored by standard drug Atorvastatin and testing drug MELL. Alteration in histopathological structure was also restored by the standard drug Atorvastatin and teting drug MELL. Thus we conclude that LL can be used as a supportive medication for treatment and

management of hepatic disease [1, 12,14].

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How to cite this article:

Kapil Jaiswal, Sana Raza, Suresh Kumar Chauhan, Chetan Kesarvani *Antihyperlipidemic Effect Of Leucaena Leucocephala Linn. Stem Bark In High Fat Diet Induced Hyperlipidemia In Rats Br J Bio Med Res , Vol.05, Issue 03, Pg.1636 - 1644, May - June 2021. ISSN:2456-9739 Cross Ref DOI : <https://doi.org/10.24942/bjbm.2021.839>*

Source of Support: Nil

Conflict of Interest: None declared.

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