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Hypolipidemic activity of *Ailanthus excelsa* Roxb.

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Abstract

Methanolic extract of *Ailanthus excelsa* leaves and its fractions were investigated for hypolipidemic activity on triton induced hyperlipidemic model. Methanolic extract decreased total Cholesterol level upto 02.22, 04.36, 05.57 and 11.01% in 50, 100, 200 and 300mg/kg body weight dose respectively. On the other hand methanolic extract increased HDL level upto 0.74, 01.33, 02.37 and 03.02% in 50, 100, 200 and 300mg/kg body weight dose respectively. methanolic extract decreased Triglycerides level upto 09.87, 10.72, 12.34 and 13.04% in 50, 100, 200 and 300mg/kg body weight dose respectively. methanolic extract decreased LDL-cholesterol level upto 04.50, 06.46, 06.79 and 09.04% in 50, 100, 200 and 300mg/kg body weight dose respectively. methanolic extract decreased VLDL-cholesterol level upto 09.87, 10.73, 12.35 and 13.05% in 50, 100, 200 and 300mg/kg body weight dose respectively. Initial total cholesterol of isolate CT1 treated group was found to be 134.83±1.99 which was increased upto 161.16±7.02 by triton. After the treatment with isolate CT1 the total cholesterol level was decreased upto 134.16±1.52. In this group the initial HDL was 22.58±1.07 which was increased upto 24.83±2.21 by triton and after the treatment with isolate CT1 it was reached normal level upto 22.33±0.91. The initial level of triglyceride was 76.00±1.29 which was increased upto 90.25±3.62 by triton and after the treatment with isolate CT1 it was decreased upto 78.75±1.58 and the initial LDL-cholesterol level was 97.05±1.97 which was increased upto 119.28±4.45 and after treatment with isolate CT1 it was reduced upto 94.48±2.33.

Key-Words: *Ailanthus excelsa*, Hypolipidemic activity, Triton WR 1339

Introduction

Ailanthus is a genus of tall, lofty trees, distributed in India, China, Japan and Australia[1]. *Ailanthus excelsa* Roxb. (Simaroubaceae) is commonly known as "Mahanimba" due to its resemblance with neem tree (*Azadirachta indica*). Ailanthus is an important ingredient in most of the ayurvedic preparations like, Pusyanuga churna, Brahat Gangadhara churna and Aralu putpaka, used in the management of atisara, krimi, arsa, sannipatajwara, brama, tvakroga, chardi, kustha, pravahika, grahani, prameha, gulma, swasa, musaka and visaja roga[1,2,3]. In Indian traditional medicine, it is highly valued for the treatment of hyperlipidemia[4]. The plant is considered to be the main therapeutic constituents for dysentery (amebic and bacterial) and diarrhoea, intestinal worms, internal parasites, malaria, astringent and externally for wounds, viral infections[5], antifertility[6,7], insect feeding[8], antifungal[9], antimicrobial[10], antibacterial[11], hypoglycemic[12], hepatoprotective[13], antiproliferative[14]. The aim of present work was to further evaluate the hypolipidemic activity of *Ailanthus excelsa* which grows commonly in India.

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Material and Methods

Plant material

The leaves of *Ailanthus excelsa* Roxb. was identified and authenticated by Dr. (Mrs) Tesi Thomas, Assistant Professor, Department of botany, Chandra Shekhar Azad Government Post Graduate College, Sehore (M.P.). The leaves of *Ailanthus excelsa* Roxb. (Mahanimb) was collected from the area adjoining to Bhopal city in the month of October, 2008. The leaves were dried in shade. The dried leaves were crushed to moderately coarse powder

Extraction and fractionation

The drug powder was packed in a soxhlet apparatus and was defatted with petroleum ether for 72 h. The defatted material was completely freed of petroleum ether and the marc was extracted with methanol. The methanolic extract was obtained was freed from solvent under vacuum (yield: 9.13% w/w). The methanolic extract was subjected for bioactivity directed fractionation. The active fraction then subjected for column chromatography with a view to isolate individual active component. Amongst four isolated compounds (CT1, CT2, CT3 and CT4) only CT1 was found to be most active.

Screening for hypolipidemic activity

Screening for hypolipidemic activity was carried out in albino rats (Wistar strain) weighing (100-200 g) of either sex maintained in standard conditions for temperature, relative humidity, and light/day cycles and fed with normal diet and water ad libitum, were used. Triton WR 1339 (Tyloxapol) was purchased from Sigma Chemicals Co., USA.

Preparation of test material

Methanolic extract and its fraction were suspended in distilled water with PVP (2% w/v) Triton (Sigma) was dissolved in normal saline to give a 7% solution.

Measurement of biochemical parameters

The methanolic extract of *Ailanthus excelsa* leaves and its fraction were studied for hypolipidimic activity in albino rats. In this study albino rats were divided in 15 groups of 6 animal each for estimation of cholesterol, triglyceride, HDL, LDL and VLDL. Group-1 normal control treated with normal saline solution and PVP solution (2%), group-2 triton control treated with triton WR 1339 200mg/kg and PVP solution (2%), group-3 standard drug treated with fenofibrate 50mg/kg, group-4, 5, 6 and 7 are treated with triton WR 1339 200mg/kg and methanolic extract dose 50, 100, 200 and 300 mg/kg respectively, group-8, 9, 10 and 11 are treated with triton WR 1339 200mg/kg and aqueous extract dose 50, 100, 200 and 300 mg/kg respectively and group-12, 13, 14 and 15 are treated with triton WR 1339 200mg/kg and petroleum ether extract dose 50, 100, 200 and 300 mg/kg respectively. The blood samples were withdrawn from the eye vein and transferred directly into centrifuge tubes and allowed to clot at room temperature for 20–25 min and centrifuged for 15–20 min at 2000 r.p.m. The supernatant clear serum thus obtained was transferred carefully with the help of micropipette into small test tubes for estimation. The serum concentration of total cholesterol, HDL, and triglyceride were measured by standard procedure using auto-analyzer, while the LDL-cholesterol and VLDL-cholesterol level was calculated with the help of the equation which are LDL-C = TC− (HDL-C-TG/5) and VLDL-C = TG/5 respectively.

Statistical analysis

The blood lipid level data of different dose level was tabulated and analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test for comparisons with control. The data are expressed as mean ± standard error mean. The Dunnet's test was employed to analyze the result and P value less than >0.05 was considered statistical significant[15,16,17].

Results and Discussion

The aim of present study was to asses the hypolipidemic activity of *Ailanthus excelsa* leaves. The yield of methanolic extract was 09.13% w/w of crude drug. The methanolic extract was fractioned and the yield of fractions was found 12.85% w/w of methanolic extract. In methanolic extract treated group the total cholesterol level was decreased upto 02.22, 04.36, 05.57 and 11.01% in 50, 100, 200 and 300mg/kg body weight dose respectively as compared to Triton control group in 48 hours (Table-1). In methanolic extract treated group the Triglycerides level was decreased upto 09.87, 10.72, 12.34 and 13.04% in 50, 100, 200 and 300mg/kg body weight dose respectively as compared to Triton control group in 48 hours(Table-2). In methanolic extract treated group the HDL level was increased upto 0.74, 01.33, 02.37 and 03.02% in 50, 100, 200 and 300mg/kg body weight dose respectively as compared to Triton control group in 48 hours(Table-3). In methanolic extract treated group the LDL-cholesterol level was decreased upto 04.50, 06.46, 06.79 and 09.04% in 50, 100, 200 and 300mg/kg body weight dose respectively as compared to Triton control group in 48 hours(Table-4). In methanolic extract treated group the VLDL-cholesterol level was decreased upto 09.87, 10.73, 12.35 and 13.05% in 50, 100, 200 and 300mg/kg body weight dose respectively as compared to Triton control group in 48 hours(Table-5). In isolated fraction isolate CT1 were more active as compared to other isolates. Initial total cholesterol of isolate CT1 treated group was found to be 134.83±1.99 which was increased upto 161.16±7.02 by triton. After the treatment with isolate CT1 the total cholesterol level was decreased upto 134.16±1.52. In this group the initial HDL was 22.58±1.07 which was increased upto 24.83±2.21 by triton and after the treatment with isolate CT1 it was reached normal level upto 22.33±0.91. The initial level of triglyceride was 76.00±1.29 which was increased upto 90.25±3.62 by triton and after the treatment with isolate CT1 it was decreased upto 78.75±1.58 and the initial LDL-C level was 97.05±1.97 which was increased upto 119.28±4.45 and after treatment with isolate CT1 it was reduced upto 94.48±2.33.

An increased risk of coronary heart disease is associated with a high serum concentration of total cholesterol, low-density lipoprotein (LDL) and triglyceride. On the other hand, low serum concentration of high-density lipoprotein (HDL) is also responsible for coronary heart diseases. Atherosclerosis, congestive heart diseases and some other diseases are strongly associated with disorders of lipid metabolism and plasma lipoproteins. The non-

ionic detergent, Triton WR 1339 acts as a surfactant to block the uptake of lipoprotein from the circulation by extra hepatic tissues resulting in an increase in the level of circulatory lipoproteins in animal models which are often used for a number of objectives, in particular for screening natural or chemical hypolipidemic drugs. With this aim, many medicinal plants, such as *Cassia tora*, *Ocimum basilicum* etc. have been assessed for their hypolipidemic activity in a Triton WR-1339-induced hyperlipidemic model. On parenteral administration of Triton in rats, the maximum blood cholesterol and triglyceride levels were reached at 20 hours, followed by a decline to normal values. This result demonstrates the feasibility of using Triton induced hyperlipidemic rat as an experimental model to investigate the hypolipidemic effect of *Ailanthus excelsa* extracts. This work was aimed at the assessment of the possible hypolipidemic activities of methanolic extracts and its isolated fractions of *Ailanthus excelsa* leaves.

Conclusion

The results revealed that the isolate CT1 is potent hypolipidemic agent as compared to other isolates and methanolic extract which is due to presence of Flavanoids, triterpenoids and sterols. The literature review revealed that flavanoid possess a remarkable spectrum of biochemical and pharmacological activities, which also include hypolipidemic agents. The activity of isolate CT1 is due to its flavanoidal characteristic. Based on the reports published earlier, it may be presumed that hypolipidemic activity of *Ailanthus excelsa* is due to its flavanoids such as Apigenin, Kaempferol and Luteolin etc. In conclusion, the present study justifies the traditional claim for lipid lowering property of *Ailanthus excelsa* leaves.

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Table 1: Effect of different extract on Total Cholesterol level in Triton induced hyperlipidemic Model

Groups/Time Dose in Mg/Kg b.w.	0 hours (Initial)	6 hours	24 hours	48 hours	% Change After 2-days Treatment
Normal Control	222.20±11.64	222.47±11.59	222.13±11.69	222.23±11.69	
Triton Control	223.30±15.28	286.73±13.84	278.77±13.35	274.00±10.08	
Triton+Std.50 (Fenofibrate)	235.36±10.77	294.00±11.29	249.87±10.95*	236.70±11.05	13.61 ↓
Triton+ Me.Ext-50	231.70±11.57	288.27±11.63	271.53±11.06*	267.90±11.13	02.22 ↓
Triton+ Me.Ext-100	233.76±09.35	293.90±08.46	284.90±09.65	262.03±08.69	04.36 ↓
Triton+ Me.Ext-200	225.16±10.62	291.00±06.70	279.83±05.85	258.73±11.14**	05.57 ↓
Triton+ Me.Ext-300	213.10±05.82	275.47±05.85*	268.70±05.96*	243.83±05.81**	11.01 ↓
Triton+ Aq.Ext-50	215.83±08.21	276.03±07.16	271.03±06.96	269.67±06.89	01.58 ↓
Triton+ Aq.Ext-100	221.96±10.02	282.83±10.63	272.17±10.29	266.23±09.85	02.83 ↓
Triton+ Aq.Ext-200	224.13±09.66	279.60±09.89	269.07±10.58	264.50±09.62	03.46 ↓
Triton+ Aq.Ext-300	232.73±08.67	286.73±09.87	268.17±09.99	259.97±05.77**	05.12 ↓
Triton+ Pet.Ext-50	225.33±10.85	288.57±08.59	279.70±09.58	270.37±11.03*	01.32 ↓
Triton+ Pet.Ext-100	221.60±12.73	279.43±11.98	271.77±10.35*	269.17±11.83	01.76 ↓
Triton+ Pet.Ext-200	222.60±07.73	276.80±06.91	268.47±06.79	265.23±07.42	03.20 ↓
Triton+ Pet.Ext-300	223.90±12.60	278.83±10.68	269.77±10.65*	260.10±11.80*	05.07 ↓

Values are expressed as mean ± S.E.M. for six animals in each group.
** = P<0.05, ** = P<0.01, ↓ = Decrease

Table 2: Effect of different extract on Triglycerides level in Triton induced hyperlipidemic Model

Groups/Time Dose in Mg/Kg b.w.	0 hours (Initial)	6 hours	24 hours	48 hours	% Change After 2-days Treatment
Normal Control	61.10±1.14	60.46±1.20	60.05±1.19	60.90±1.80	
Triton Control	59.85±1.30	92.58±1.41	92.76±1.44	92.70±1.44	
Triton+Std.50 (Fenofibrate)	61.75±1.45	82.73±1.47**	72.35±1.27**	66.03±0.88**	28.77 ↓
Triton+ Me.Ext-50	59.30±1.46	85.96±0.58*	85.08±0.47**	83.55±0.75	09.87 ↓
Triton+ Me.Ext-100	59.83±1.30	84.21±1.59**	83.06±0.87**	82.76±1.22*	10.72 ↓
Triton+ Me.Ext-200	61.63±1.40	85.96±0.58**	82.76±1.22**	81.26±1.39*	12.34 ↓
Triton+ Me.Ext-300	59.25±1.42	84.21±1.59**	81.26±1.12**	80.61±1.52**	13.04 ↓
Triton+ Aq.Ext-50	59.76±1.27	88.45±1.03	87.38±0.92*	86.93±1.57	06.22 ↓
Triton+ Aq.Ext-100	61.61±1.40	87.68±1.10	87.10±1.59**	85.83±1.04	07.41 ↓
Triton+ Aq.Ext-200	59.40±1.50	84.21±1.59**	85.08±0.47**	84.66±0.87**	08.67 ↓
Triton+ Aq.Ext-300	59.16±1.27	84.05±1.10**	84.00±1.66**	83.73±0.78**	09.67 ↓
Triton+ Pet.Ext-50	61.71±1.43	88.96±1.23	88.26±1.32	89.41±0.63	03.54 ↓
Triton+ Pet.Ext-100	59.13±1.47	87.38±1.74	89.75±0.73	88.58±0.65	04.44 ↓
Triton+ Pet.Ext-200	59.80±1.28	85.05±1.64**	88.70±0.60	87.73±0.73*	05.36 ↓
Triton+ Pet.Ext-300	59.81±1.29	86.88±0.70*	87.95±0.86*	86.85±0.85**	06.31 ↓

Values are expressed as mean ± S.E.M. for six animals in each group.
** = P<0.05, ** = P<0.01, ↓ = Decrease

Table 3: Effect of different extract on HDL level in Triton induced hyperlipidemic Model

Groups/Time Dose in Mg/Kg b.w.	0 hours (Initial)	6 hours	24 hours	48 hours	% Change After 2-days Treatment
Normal Control	41.16±0.88	42.50±1.51	41.91±0.75	41.31±0.96	
Triton Control	41.43±1.33	62.61±1.60	64.06±1.80	64.45±1.81	
Triton+Std.50 (Fenofibrate)	42.00±1.38	63.75±1.14	65.46±1.33	67.50±0.91	4.73 ↑
Triton+ Me.Ext-50	42.33±1.47	62.81±1.48	64.61±1.02	64.93±1.14	0.74 ↑
Triton+ Me.Ext-100	41.70±1.40	63.00±1.38	64.95±1.14	65.31±1.25	1.33 ↑
Triton+ Me.Ext-200	42.05±1.40	63.11±1.32 *	65.33±1.26	65.98±0.87 *	2.37 ↑
Triton+ Me.Ext-300	42.16±1.40	63.25±1.27 *	66.01±0.88 **	66.40±0.96 **	3.02 ↑
Triton+ Aq.Ext-50	41.85±1.48	62.78±1.50	64.18±0.88	64.48±1.03	0.04 ↑
Triton+ Aq.Ext-100	42.18±1.47	62.96±1.39	64.50±1.03	64.75±1.09	0.46 ↑
Triton+ Aq.Ext-200	42.51±1.56	63.08±1.34	64.78±1.09	64.83±0.95	0.58 ↑
Triton+ Aq.Ext-300	42.63±1.25	63.21±1.28 *	64.85±0.95	65.43±1.32 **	1.52 ↑
Triton+ Pet.Ext-50	42.45±1.36	62.76±1.51	64.20±0.65	64.31±0.72	0.21 ↓
Triton+ Pet.Ext-100	42.46±1.46	62.95±1.40	64.25±0.69	64.43±0.76 *	0.03 ↓
Triton+ Pet.Ext-200	42.26±1.35	63.06±1.35 *	64.36±0.74	64.46±0.94	0.01 ↑
Triton+ Pet.Ext-300	42.03±1.58	63.18±1.30	64.45±0.93 *	64.83±0.95 *	0.58 ↑

Values are expressed as mean ± S.E.M. for six animals in each group.

* = P<0.05, ** = P<0.01,

↓ = Decrease ↑ = Increase

Table 4: Effect of different extract on LDL level in Triton induced hyperlipidemic Model

Groups/Time Dose in Mg/Kg b.w.	0 hours (Initial)	6 hours	24 hours	48 hours	% Change After 2-days Treatment
Normal Control	121.36±2.78	122.00±2.18	122.12±2.24	122.13±2.57	
Triton Control	120.92±3.23	191.95±2.07	192.17±2.03	192.00±1.99	
Triton+Std.50 (Fenofibrate)	122.21±2.82	173.85±2.09**	158.65±2.19*	144.93±2.18**	24.51 ↓
Triton+ Me.Ext-50	120.43±3.33	189.20±2.54	187.55±2.64.	183.35±2.16*	04.50 ↓
Triton+ Me.Ext-100	121.20±3.44	186.95±1.87	183.20±2.33	179.58±1.54*	06.46↓
Triton+ Me.Ext-200	121.36±2.78	185.20±3.22	181.68±2.18*	178.97±1.45**	06.79 ↓
Triton+ Me.Ext-300	120.92±3.23	183.37±1.85	180.57±2.06**	174.63±1.55**	09.04 ↓
Triton+ Aq.Ext-50	122.21±2.82	189.57±1.15	188.17±2.56	184.83±2.72	03.73 ↓
Triton+ Aq.Ext-100	120.43±3.33	187.23±2.20	185.20±3.22	182.52±1.62*	04.94 ↓
Triton+ Aq.Ext-200	121.20±3.44	186.08±1.93	183.20±2.33	180.42±1.34**	06.03 ↓
Triton+ Aq.Ext-300	122.21±2.82	184.72±2.24	180.93±2.03*	178.22±1.35**	07.18 ↓
Triton+ Pet.Ext-50	120.43±3.33	190.68±1.65	187.90±2.60	185.15±3.20	03.56 ↓
Triton+ Pet.Ext-100	121.20±3.44	190.18±1.73	185.43±1.55	182.60±1.97*	04.89 ↓
Triton+ Pet.Ext-200	121.36±2.78	189.77±1.90	183.20±2.33	181.68±2.18*	05.37 ↓
Triton+ Pet.Ext-300	120.92±3.23	188.17±2.81	182.60±1.97*	179.00±1.70**	06.77 ↓

Values are expressed as mean ± S.E.M. for six animals in each group.
** = P<0.05, ** = P<0.01, ↓ = Decrease

Table 5: Effect of different extract on VLDL level in Triton induced hyperlipidemic Model

Groups/Time Dose in Mg/Kg b.w.	0 hours (Initial)	6 hours	24 hours	48 hours	% Change After 2-days Treatment
Normal Control	12.22±0.32	12.09±0.24	12.01±0.43	12.18±0.21	
Triton Control	11.97±0.26	18.51±0.28	18.88±0.35	18.54±0.28	
Triton+Std.50 (Fenofibrate)	12.35±0.29	16.54±0.29**	14.47±0.25**	13.20±0.17**	28.80 ↓
Triton+ Me.Ext-50	11.86±0.29	17.19±0.11	17.01±0.09*	16.71±0.15*	09.87 ↓
Triton+ Me.Ext-100	11.96±0.26	16.84±0.31*	16.61±0.17**	16.55±0.24**	10.73 ↓
Triton+ Me.Ext-200	12.32±0.28	17.19±0.11**	16.55±0.24*	16.25±0.27**	12.35 ↓
Triton+ Me.Ext-300	11.85±0.28	16.84±0.31**	16.25±0.22**	16.12±0.30**	13.05 ↓
Triton+ Aq.Ext-50	11.95±0.25	17.69±0.20	17.47±0.18**	17.38±0.31**	06.25 ↓
Triton+ Aq.Ext-100	12.32±0.28	17.53±0.22	17.42±0.31*	17.16±0.20**	07.44 ↓
Triton+ Aq.Ext-200	11.88±0.30	16.84±0.31	17.01±0.09**	16.93±0.17**	08.68 ↓
Triton+ Aq.Ext-300	11.95±0.25	16.81±0.22*	16.80±0.09**	16.74±0.15**	09.70 ↓
Triton+ Pet.Ext-50	12.34±0.28	17.79±0.24	17.65±0.26**	17.88±0.12	03.55 ↓
Triton+ Pet.Ext-100	11.82±0.29	17.47±0.34	17.95±0.14	17.71±0.13	04.47 ↓
Triton+ Pet.Ext-200	11.96±0.25	17.01±0.32	17.74±0.12**	17.54±0.14*	05.39 ↓
Triton+ Pet.Ext-300	11.96±0.25	17.37±0.14*	17.59±0.17**	17.37±0.17**	06.31 ↓

Values are expressed as mean ± S.E.M. for six animals in each group.
** = P<0.05, ** = P<0.01, ↓ = Decrease