ANTI-INFLAMMATORY, ANTIHYPERLIPIDEMIC ACTIVITY OF VARIOUS EXTRACT OF FRUITS OF *Withania coagulance*

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Summary

In the present investigations, attempts were made to study detail phytochemical parameters and pharmacological activities, particularly antinflammatory, antihyperlipidemic activity of fruits of *Withania coagulance dunal* belonging to family Solanaceae. The hydroalcholic extract of *Withania coagulance* showed significant inhibition of rat paw volume i.e. 80 % at 1st hr and 90.73 % 3rd h whereas constituent No. 1 showed significant inhibition of rat paw volume i.e. 84.6 % at 1st hr. and 92.65 % 3rd hr. The hydroalcholic extract of *Withania coagulance* showed significant decrease in total cholesterol i.e. 88.87 % at 24th hr. and 91.05 % 48th hr wheras hydroalcholic extract of *Withania coagulance* showed significant decrease in total triglycerides i.e. 96.93 % at 24th hr. and 91.55 % 48th hr. when compared to standard. The decrease in triglycerides value by other extracts was not significant when compared to standard.

Keywords: *Withania coagulance,* Anti-inflammatory, Antihyperlipidemic,Carrageenan induced paw edema, triton x –100.

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Introduction

Withania coagulance belongs to the solanaceae and cultivated throughout central Asia. This family is well known for its medicinal properties like immunomodulatary, mental disability and depression disorders[1]. In the literature survey, it was found that *withania cogulance* has been found to be relatively rich in withonolides. Which have attracted considerable interest due to their biological activity and their chemical and physical properties have been investigated extensively[1-8]. The selected plant *Withania coagulance* have multiple therapeutic properties like of antifungal, larvicidal, emetic, antibacterial, sedative, antiasthematic, antinflammatory, cardiovascular, diuretic, and hepatoprotective action but its antinflammatory, antioxidant, antihyperlipidemic and antimicrobial activity of active constituent. Plant from other species of the same family i.e. *Withania somnifera* is reported to possess all these activities. The berries of selected plant *Withania coagulance* in folkloric is used for similar purpose but the scientific data of *Withania coagulance* targeting these activities till date is not reported.

Materials And Methods

Drugs and chemicals

All the solvents used for the extraction process are of Laboratory grade. triton x –100 (SMAR Chemicals, Nagpur), Carrageenan (SD Fine chemicals. Mumbai).

Plant Extract

The powdered material of fruits of *Withania coagulance* was extracted in a Soxhlet apparatus by charging (500g each batch) and successive hot continuous extraction was carried out using petroleum ether (60-80°C), benzene, chloroform, acetone, rectified spirit and hydroalcohol (50:50). The percentage extractive value of the hydroalcoholic extract was found to be highest i.e. 3%w/w.

Animals

All the extracts were subjected to evaluation of anti-inflammatory, antihyperlipidemic. Albino rats, weighing (200-250 g) of either sex were used for the investigation. The were housed in standard laboratory conditions and feed on commercial rat cubes (standard pellets chow, Lipton, India) and allowed free access to fresh water in bottles *ad libitum*. All experimental protocols were in compliance with Nagpur University, animal ethical committee as well as international accepted principle for laboratory animal use and care.

Preliminary chemical tests

Preliminary phytochemical screening of the extracts was studied, which showed presence of Sterols, Alkaloids, Flavonoids, Saponins, Sugars, Coumarins and Aminoacids

Carrageenan induced paw edema

Carrageenan induced rat paw edema method was used to carry out anti-inflammatory activity. Indomethacin was used as a standard. The test preparation was administered orally at a dose of 500mg/kg body weight. After an hour 0.1 ml of 1 % carrageenan was injected into the plantar tissue of right hind paw and immediately paw volume was measured.

Antihyperlipidemic activity

The determination of antihyperlipidemic activity was done using enzymatic kits of Medsource Ozone Biomedical pvt. Ltd. Pravastatin was used as a standard. Hyperlipidemia was induced by single subcutaneous injection of triton x –100 (200 mg/kg body wt.). The test preparations were administered orally at a dose of 400mg/kg body weight immediately after triton administration. Blood was withdrawn after 24 hr. & 48 hr. for investigating lipid profile **(Cholesterol and Triglycerides).**

Results

Column chromatography of hydroalcoholic extract

The Column chromatography was carried out using gradient elution technique to isolate the number of chemical constituents present in the hydroalcoholic extract. The elution of the column yielded 3 compounds. The results are given in table 1.

Table 1. Column chromatography of hydroalcoholic extract

Solvent	Fractions (5 ml)	Constituents	Detection
Petroleum ether	1-12	Solvent front	Detection on preparative TLC Methanol: Ethylacetate: Toluene (2 : 2 : 5)
Petroleum ether: Benzene			
4 : 1	13-24	-	
3 :2	25-36	-	
2 :3	37-48	-	
1 : 4	49-60	-	
Benzene	61-72	-	
Benzene: Acetone			
4 : 1	73-84	-	
3 :2	85-96	-	
2 :3	97-108	-	
1 : 4	109-120	-	
Acetone	121-132	-	

Acetone: Methanol			
4 : 1	133-144	-	
3 :2	145-156	III	
2 :3	157-168	III	
1 : 4	169-180	III, II	
Methanol	181-192	II	
Methanol : Water			
4 : 1	193-204	II, I	
3 :2	205-216	I	
2 :3	217-228	I	
1 : 4	229-230	-	

All three separated constituents of hydroalcoholic extract were subjected to evaluation of anti-inflammatory activity, antihyperlipidemic activity. Purification of the constituents obtained was done by preparativeTLC.

Anti-inflammatory Activity

The paw volume was again recorded after one and three hours for extracts and isolated constituents. The results are shown in table 2 and 3 respectively.

Table 2. Anti-inflammatory activity of different extracts

Test Group (N= 6)	0 hour	1 hour	3 hour	Percent inhibition% 1 hour	3 hour
Control	1.69±0.01	2.15±0.1	2.55±0.1	-	-
Standard (Indomethacin)	1.63±0.03	1.83±0.05	1.95±0.07	56.52	62.79
Petroleum ether extract	1.63±0.04	2.10±0.32	2.53±0.04	2.17	4.65
Benzene extract	1.68±0.02	2.12±0.02	2.60±0.01	4.34	6.97
Chloroform extract	1.68±0.05	2.17±0.03	2.48±0.04	6.52	6.97
Acetone extract	1.64±0.02	2.12±0.02	2.46±0.03	4.30	4.65
Rectified spirit extract	1.69±0.03	2.06±0.06	2.35±0.08	19.56	23.25
Hydroalcholic extract	1.66±0.03	1.91±0.06	2.03±0.08	45.65	56.97

Table 3. Anti-inflammatory activity of isolated constituents

Test Group (N= 6)	Dose in mg/kg	0 hour	1 hour	3 hour	Percent inhibition 1 hour	3 hour
Control	-	1.69±0.01	2.15±0.1	2.55±0.1	-	-
Standard-Indomethacin	10	1.63±0.03	1.83±0.05	1.95±0.07	56.52	62.79
Constituent no 1	200	1.65±0.04	1.89±0.36	2.01±0.23	47.82	58.18
Constituent no 2	200	1.67±0.12	2.09±0.05	2.72±0.03	8.69	22.09
Constituent no 3	200	1.70±0.21	2.23±0.09	2.87±0.18	15.21	24.41

Antihyperlipidemic activity

The total cholesterol and total triglycerides values are recoded after 24h and 48h for fruit extracts (table 4 and 5) and isolated constituents (6 and 7).

Table 4. Effect of fruit extracts on total cholesterol

Sr. No.	Group	After 24 hr.	After 48 hr.
1.	**Control**	407.6 ± 15.55	179.9 ± 28.25
2.	**Standard**	150.5 ± 21.11	113.3 ± 16.72
3.	**Petroleum ether extract**	295.3 ± 22.15	192.9 ± 27.90
4.	**Benzene extract**	300.4 ± 31.70	250.1 ± 16.65
5.	**Chloroform extract**	196.1 ± 28.90	180.0 ± 15.65
6.	**Acetone extract**	190.7 ± 28.90	175.5 ± 15.63
7.	**Rectified spirit extract**	185.8 ± 13.74 a	163.7 ± 9.98
8.	**Hydroalcholic extract**	167.6 ± 20.59 a	123.45 ± 6.650

N = 6, values are mean ± S.E.M., (a) $P < 0.01$ – Significant compared to triton

Table 5. Effect of fruit extracts on total triglyceride

Sr. No.	Group	After 24 hr.	After 48 hr.
1.	**Control**	174.48 ± 7.100	144.95 ± 18.35
2.	**Standard**	120.1 ± 3.713	103.72 ± 3.705
3.	**Petroleum ether extract**	156.93 ± 13.95	145.20 ± 6.960
4.	**Benzene extract**	165.12 ± 12.25	154.00 ± 1.110
5.	**Chloroform extract**	157.81 ± 17.45	142.15 ± 4.816
6.	**Acetone extract**	169.29 ± 0.835	156.08 ± 5.315
7.	**Rectified spirit extract**	149.66 ± 4.165	133.55 ± 2.750
8.	**Hydroalcholic extract**	123.79 ± 3.31 a	112.49 ± 4.980

N = 6, values are mean ± S.E.M., (a) $P < 0.01$ – Significant compared to triton

Table 6. Effect of isolated constituents on total cholesterol

Sr. no.	Group	Dose – 200 mg/kg body wt.	
		After 24 hr.	After 48 hr.
1.	**Control**	407.6 ± 15.55	179.9 ± 28.25
2.	**Standard**	150.5 ± 21.11	113.3 ± 16.72
3.	**Constituent no 1**	350.2 ± 11.16	292.9 ± 27.40
4.	**Constituent no 2**	325.5 ± 15.26	255.1 ± 15.45
5.	**Constituent no 3**	295.10 ± 18.90	247.3 ± 16.35

N = 6, values are mean ± S.E.M., (a) $P < 0.01$ – Significant compared to triton

Table 7. Effect of isolated constituents on total triglyceride

Sr. no.	Group	Dose – 200 mg/kg body wt.	
		After 24 hr.	After 48 hr.
1.	**Control**	174.48 ± 7.100	144.95 ± 18.35
2.	**Standard**	120.1 ± 3.713	103.72 ± 3.705
3.	**Constituent no 1**	171.36 ± 14.36	165.3 ± 7.532
4.	**Constituent no 2**	163.35 ± 13.2	157.2 ± 2.34
5.	**Constituent no 3**	156.29 ± 16.26	150.02 ± 3.86

N = 6,values are mean ± S.E.M., (a) $P < 0.01$ – Significant compared to triton

Conclusions

The hydroalcholic extract of *Withania coagulance* showed significant inhibition of rat paw volume i.e. 80 % at 1st hr and 90.73 % 3rd h when compared to standard (Table 3). The inhibition of paw volume by other extracts was not significant when compared to standard.
The constituent No. 1 showed significant inhibition of rat paw volume i.e. 84.6 % at 1st hr. and 92.65 % 3rd hr. When compared to standard. The inhibition of paw volume by other constituent was not significant when compared to standard.
The hydroalcholic extract of *Withania coagulance* showed significant decrease in total cholesterol i.e. 88.87 % at 24th hr. and 91.05 % 48th hr. when compared to standard. The decrease in cholesterol value by other extracts was not significant when compared to standard.
The hydroalcholic extract of *Withania coagulance* showed significant decrease in total triglycerides i.e. 96.93 % at 24th hr. and 91.55 % 48th hr. when compared to standard. The decrease in triglycerides value by other extracts was not significant when compared to standard.
The decrease in cholesterol and triglycerides value by all the three separated constituents was not significant when compared to standard.

References

1 Choudhary MI, Rahman AU, Antifungal steroidal Lactones from withania coagulancs, Phytochem 1995; 40: 1243-46.
2 Andallu B, Radhika B, Hypoglycemic, Diuretic and Hyper chlolesterolemic Effect of withania cherry root, Ind J Exp Biol 2000; 38: 607-9.
3 Archna R, Namasivayam A, Antistressar effect of withania somnifera, J. Ethanopharmacol 1999; 64: 91-93.
4 Choudhary RR, Vohra SB, Plant with possible hypoglycemic activity in advance in Research in Indian Medicine, Banars Hindu University, Varanasi, 1970; 57.
5 Hemlatha S, Wahi AK, Singh PN at al, Hypoglycemic activity of withania cogulance Dunal in streptozotocin induced Diabetic Rats, J Ethanopharmacol 2004; 93: 261-64.
6 Mohmud TH, Anti-inflammatory activity of ashwagandha, Hamdard Medicus 2000; XLIII: 77.
7 Prasad S, Luthura SP, Pharmacogonastic studies on withania coagulance Dunal 1959; 21: 189-94.
8 Kharc CP, Rational western Therapy, Ayurvedic and other Traditional usages, Springer verlog Berlin Heidelberg, Encylopedia of Indian Medicinal Plants, 2004; 479-83.