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Research Article

ANALGESIC ACTIVITY OF WITHANIA COAGULANS DUNAL FRUIT EXTRACTS IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Objective: *Withania coagulans* (WC) dunal fruits has shown anti-inflammatory, cardio tonic activities, hepatoprotective, anti-fungal, hypoglycemic, free radical scavenging activity, hypolipidemic and wound healing activity. The present study was undertaken to assess the central analgesic activity of *Withania Coagulans* Dunal fruit extract in Swiss albino mice.

Methods: Hot plate method and tail immersion method is generally used *in vivo* model for estimation of central analgesic activity. Methanolic and hydro-alcoholic extract of WC was used. In both cases Diclofenac Sodium was used as standard drug.

Results: Both the extracts at doses of 100 mg/kg and 200 mg/kg have elicited significant analgesic activity in dose dependant manner by using Hot plate method and Tail immersion method. But hydro-alcoholic extract of WC at 200 mg/kg showed more significant activity than other extracts.

Conclusion: Hence both methanolic and hydro-alcoholic extract of WC fruits have potent analgesic activity against different stimuli due to significant increase in reaction time.

Keywords: *Withania coagulans* dunal, central analgesic activity, Hot plate method, Tail immersion method, Diclofenac sodium

INTRODUCTION

Withania coagulans Dunal, Family- Solanacea is distributed in the east of the Mediterranean region and extends to South Asia, [1] common in drier parts of Punjab, Gujarat, Simla and Kumaon in India, Baluchistan in Iran, Pakistan and Afghanistan and East India. The main component of berries are esterase, fatty oil, essential oils, lignan amino acids such as proline, hydroxyproline, valine, tyrosine, aspartic acid, glycines, aspargines, cysteine and glutamic acid and alkaloids are the phytoconstituents. Most of the activities of the plants are due to the presence of an active component as, Withanolide. Withanolides are steroidal lactones with an ergostane skeleton. It is also used in folk medicine.

Fruits of the plant have a milk-coagulating characteristic. WC Dunal is commonly known as 'Indian cheese maker' or 'vegetable rennet' because fruits and leaves of this plant are used as a coagulant. The milk coagulating property of the fruits is attributed to the pulp and husk berries which contain an enzyme called Withanin, having milk-coagulating activity. One ounce of the fruits of WC when mixed with one quart of boiling water makes a decoction, one table spoonful of which is capable to coagulate a gallon of milk in just an hour. In Pakistan, the berries of WC are commonly used to clot milk that is called, 'paneer'.

The milk of buffalo or sheep is boiled to 100^0C with crushed berries of the plant, tied in a cloth. This causes the milk to curdle within 30-40 minutes. [2]

Fig. 1: It shows *Withania Coagulans* Flower and Leaves

Fig. 2: It shows *Withania Coagulans* Fruits

It has shown to have anti-inflammatory, cardio tonic activities, hepatoprotective, anti-fungal, hypoglycemic, free radical scavenging activity, hypolipidemic and wound healing activity. Recent investigation has shown that withanolides isolated from the aqueous extract of fruits possessed a good anti-hyperglycemic and anti-dyslipidemic activity. [3, 4]

The present study is to determine the analgesic activity of WC fruits in experimental animal model.

MATERIALS AND METHODS

Plant material

The fruits of WC belong to the family Solanacea is distributed throughout India. For the present study fruits are collected in the month of August from Mumbai (Maharashtra). The dried fruits was identified, confirmed and authenticated by A.S.Upadhye, Scientist, Plant Drug Authentication Service, Botany group, Plant Sciences Division, Agharkar Research Institute, Pune (V.No. F-180). The dried fruit material was then pulverized by a mechanical grinder. The resulting coarse powder was then used for extraction.

Preparation of extracts

Dried fruits of *Withania Coagulans* Dunal were coarse powdered and packed into Soxhlet column and extracted with methanol for methanolic extract [5] and water: methanol (40:60) for hydro alcoholic extract. [6] The extract was concentrated under reduced pressure. The dried extract was stored in airtight container in

refrigerator below 10 ^{0}c. To convert hydro alcoholic extract into powder, distillation process have been followed to recover solvent and then dried.

Experimental animal

Swiss albino mice of either sex weighing between 30-40 gm were selected for the analgesic activity was housed under the uniform laboratory condition fed with commercial diet and provided with water ad-libitum, during the experiment. The animals were procured from Dr. L. H. Hiranandani College of pharmacy and permitted for the study under the Institutional Animal Ethical Committee (IAEC). All protocols of the study were approved by the Institutional Animal Ethical Committee with reference number IAEC/PCOL-08/2013. The IAEC is approved by committee for the purpose of control and supervision of experiments on animals (CPCSEA) with registration number 879/ac/05/CPCSEA.

Chemicals

For the phytochemical study chemicals procured from college chemical store supplied by Molychem and SD fine chemical Ltd.

Drugs and dosage

The formulation was administered orally at doses of 100 mg/kg and 200 mg/kg in the form of suspension prepared in doubled distilled water containing carboxy methyl cellulose (1%, w/v, CMC). Diclofenac Sodium, brand name Voveran (Mfgd, by NOVARTIS).

Preliminary Phytochemical studies [7]

The preliminary phytochemical analysis was carried out on methanolic extract of *Withania coagulans* dunal fruits. It is subjected to the phytochemical tests in ordered to identify the presence of various phytoconstituents.

Phytochemical studies of methanolic extract and hydro alcoholic extract of fruits part of *Withania coagulans* Dunal was done.

Preliminary Toxicological Evaluation:

Determination of acute oral toxicity study (LD_{50}) [8]

The acute oral toxicity study of methanolic and hydro alcoholic extract of *Withania Coagulans* Dunal fruits were determined in female Sprague Dawley rats in accordance with OECD (Organization for Economic Co-operation and Development) guideline (425) to evaluate safety profile of these plant extract.

Experimental design

Animals were divided in 6 groups of 6 mice each.

Group I: Control received normal saline.

Group II: Standard received Diclofenac sodium (9mg/kg) orally.

Group III and IV: Test groups administered orally with methanolic extract of WC (WCME) fruits at dose of 100 mg/kg and 200 mg/kg respectively.

Group V and VI: Test group administered orally with hydro-alcoholic extract of WC (WCHAE) fruits at dose of 100 mg/kg and 200 mg/kg respectively.

Assessment of Analgesic Activity

1. Hot Plate Method:

Mice were placed on the hot plate maintained at 55±0.5^{0}C before the treatment and its reaction time is noted. After noting initial reaction time, the treatment should be given to each mouse. The response time was noted at the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. [9-12] Mice with baseline latencies of <58 or >30s were eliminated from the study. The response latency was measured at 0,1,2,3,4,5,6 h.

2. Tail Immersion Method

The tip of tail was dipped up to 5 cm in hot water maintained at 58 ^{0}C. The response time was noted as a sudden withdrawal of the tail from the hot water. Cut off time of 20 sec was maintained to avoid damage to the tail. Time required for withdrawal of the tail was measured at 0,1,2,3,4,5,6 h. [11-13]

Statistical Analysis

All values are shown as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test *p<0.05, **p<0.01 was considered statistically significant.

RESULTS

Preliminary Phytochemical Evaluation

Both methanolic and hydro alcoholic extract of WC are positive for presence of flavonoids, alkaloids, steroids, saponin, fixed oil, glycosides, and carbohydrates.

Table 1: It shows preliminary phytochemical evaluation of methanolic and hydro alcoholic extract of WC

S. No.	Test / Reagent	Positive (+ve) or Negative (-ve)	Inference
1.	Carbohydrates-Molish's test	+ve	Carbohydrates present
	- Fehling's test	+ve	
2.	Saponin glycosides- Foam test	+ve	Saponin glycoside present
3.	Flavonoids -Lead acetate test	+ve	Flavonoids present
4.	Alkaloids -Dragendorff's test	+ve	Alkaloids present
	- Hager's test	+ve	
	- Wagner's test	+ve	
	- Mayer's test	+ve	
5.	Glycoside – Legal's test	+ve	Glycosides present
6.	Steroidal Compounds- Liebermann burchard's test	+ve	Steroidal compounds present
7.	Test for fixed oil- Spot test	+ve	Fixed oils presents

Acute Toxicity of WCME and WCHAE

Acute toxicity of WCME and WCHAE did not show any deleterious or toxic effects up to 2000 mg/kg oral dose indicating low toxicity of WCME and WCHAE at high doses. Rats were administered up to maximal possible dose.

Hot plate method in mice

In analgesic studies, the extract showed significant analgesic activity at all tested dose levels. In Hot plate method TA2 (200 mg/kg) showed significant activity 9.168±1.141** (p<0.01) after 6 hr.

TB1 (200 mg/kg) showed significant activity 8.7±0.8692 after 5 hrs and TB2 (200 mg/kg) 6.06±0.4509** showed after 3 hr as shown in Table 2 and Fig.3.

Table 2: It shows effect of WC Fruits extracts on Hot plate reaction in mice

Experimental Group	Reaction Time (sec) ± SEM						
	0 min	**60 min**	**120 min**	**180 min**	**240 min**	**300 min**	**360 min**
Control	2.143± 0.289	2.356± 0.150	2.933± 0.392	3.538± 0.230	3.615± 0.195	3.998± 0.418	4.161± 0.232
STD	2.476± 0.160	3.956± 0.505**	4.981± 0.360**	6.468± 0.444**	9.266± 0.923**	9.468± 0.961**	10.671± 0.982**
TA 1	2.725± 0.238	2.503± 0.188	3.455± 0.305	4.402± 0.331	6.648± 1.179	7.345± 0.968*	8.583± 1.087*
TA 2	2.983± 0.144	2.771± 0.242	3.763± 0.341	4.98± 0.675	7.516± 0.993*	8.048± 0.848*	9.168± 1.141**
TB 1	2.885± 0.200	2.725± 0.143	4.07± 1.014	5.521± 0.563*	7.648± 1.039*	8.7± 0.869**	9.361± 1.155**
TB 2	2.183 ± 0.268	3.316± 0.264	4.566± 0.339*	6.06± 0.450**	8.168± 0.831**	9.098± 0.955**	10.186± 1.071**

Values are expressed in mean ± S.E.M. (n=6); *p<0.05, **p<0.01 vs Control group, Data analyzed by One-way ANOVA test followed by Dunnett's multiple test for comparison. Where STD:Diclofenac Sodium-9 mg/kg, TA1:WCME-100 mg/kg, TA2:WCME-200 mg/kg, TB1:WCHAE-100 mg/kg, TB2:WCHAE-200 mg/kg.

Table 3: Effect of WC Fruits extracts on Tail immersion test in mice

Experimental Group	Reaction Time (sec) ± SEM						
	0 min	**60 min**	**120 min**	**180 min**	**240 min**	**300 min**	**360 min**
Control	2.293± 0.128	2.185± 0.279	2.483± 0.285	2.386± 0.220	2.161± 0.056	2.588± 0.234	2.758± 0.196
STD	5.56± 0.836	5.954± 0.783**	6.906± 1.013**	9.793± 1.107**	10.763± 0.727**	11.011± 0.911**	12.558± 0.687**
TA 1	3.22± 0.288	3.53± 0.261	4.218± 0.578	4.083± 0.412	7.13± 0.411**	7.403± 0.437**	8.116± 0.094**
TA 2	3.575± 0.421	4.77± 0.614	4.903± 0.576	5.398± 0.781*	7.27± 0.341**	7.808± 0.817**	8.685± 0.566**
TB 1	3.606± 0.360	4.796± 0.709	5.918± 0.686**	5.746± 0.160*	7.958± 0.538**	8.658± 0.994**	9.608± 0.318**
TB 2	3.64± 0.360	4.883± 1.197*	6.378± 0.885**	6.433± 1.152**	8.513± 0.684**	8.793± 1.131**	10.018± 1.070**

Values are expressed in mean ± S.E.M. (n=6); *p<0.05, **p<0.01 vs Control group, Data analyzed by One-way ANOVA test followed by Dunnett's multiple test for comparison. Where STD:Diclofenac Sodium-9 mg/kg, TA1:WCME-100 mg/kg, TA2:WCME-200 mg/kg, TB1:WCHAE-100 mg/kg, TB2:WCHAE-200 mg/kg

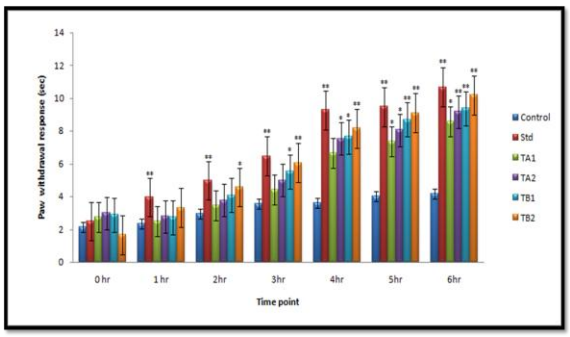

Fig. 3: Graphical representation of Analgesic effect of methanolic and hyroalcoholic extract of WC fruits in pain using Hot plate method

STD: Diclofenac Sodium-9 mg/kg, TA1:WCME-100 mg/kg, TA2:WCME-200 mg/kg, TB1:WCHAE-100 mg/kg, TB2:WCHAE 200 mg/kg

Tail immersion method in mice

In analgesic studies, the extract showed significant analgesic activity at all tested dose levels. In Tail immersion method TA1 (100mg/kg), TA2 (200 mg/kg), TB1 (100 mg/kg) have showed significant activity 7.13±0.4115**, 7.27±0.3417**, 7.958±0.5378** (p<0.01) after 4 hr respectively. TB2 (200 mg/kg) have showed significant activity 6.3783±0.8853** (p<0.01) after 2 hrs as shown in Table 3 and Fig.4.

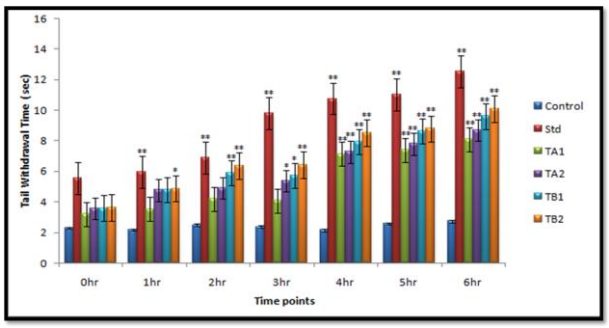

Fig. 4: Graphical representation of Analgesic effect of methanolic and hyroalcoholic extract of WC fruits in pain using Tail immersion method

STD: Diclofenac Sodium-9 mg/kg, TA1:WCME-100 mg/kg, TA2:WCME-200 mg/kg), TB1:WCHAE-100 mg/kg, TB2:WCHAE 200 mg/kg

DISCUSSION

Hot plate method and tail immersion method in mice were employed to assess the central mechanism of compound in producing analgesia. These two methods were differing from each other in their tendency to respond to nociceptive stimuli conducted through the neuronal pathway. Hot plate method involves higher brain functions and is considered supraspinally organized response whereas tail immersion method mediates spinal reflex to painful stimuli. μ, k3 and d2 are the opioid receptor sub-type primarily responsible for supraspinally

mediated analgesic action i.e., Hot plate method whereas spinal analgesia appears to be mediated through μ2, k1 and d2 receptor. [14] The hot-plate method is useful in elucidating centrally mediated anti-nociceptive responses, which focuses mainly on changes above the spinal cord level. The significant increase in pain threshold produced by WCME and WCHAE suggests involvement of central pain pathways. Number of complex processes including opiate, dopaminergic, descending nor-adrenergic and serotonergic systems are involved in centrally modulated pain. Central mechanisms involving these receptor systems or peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in inflammation and pain were responsible for analgesic effect of the extracts. [15, 16] But WCHAE at 200 mg/kg showed more significant activity than other extracts.

The preliminary phytochemical screening of WCE showed the presence of alkaloids, saponin, steroids, flavonoids, carbohydrates and glycosides in our laboratory. [17] These compounds have well-known anti-inflammatory effects. The effects observed with WCE could possibly be due to the synergistic actions of these compounds. In the present study, WCE demonstrated a significant ($P < 0.05$) analgesic activity at different dose levels in various animal models of pain. The hot plate method and tail immersion method has been found to be suitable for the evaluation of centrally but not peripherally acting analgesics.[17] The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model. In our study, WCE (100 mg/ kg and 200 mg/ kg) exhibited a significant analgesic effect in all above models of pain.

CONCLUSION

From the above investigation, it is quite apparent that methanolic and hydro alcoholic extracts of *Withania Coagulans* Dunal fruits possesses potent analgesic effect against different stimuli. This is evidenced by significant increase in the reaction time by stimuli in different experimental models.

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