CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.2205207

Available online at: http://www.iajps.com Research Article

A STUDY ON ANALGESICS AND ANTIBACTERIAL ACTIVITIES OF WITHANIA COAGULANS FRUITS COLLECTED FROM DISTRICT LORALAI, BALOCHISTAN

Saima Ghazal[1*], Amanullah Khan[2], Muhammad Kamran Taj[3], Muhammad Shoaib[1]

[1]Department of Pharmacology, Faculty of Pharmacy & Health Sciences, University of Balochistan, Quetta, Pakistan.

[2]Drug Testing Laboratory, Government of Balochistan, Quetta.

[3] Centre for Advanced Studies in Vaccinology And Biotechnology, Quetta.

Abstact:

The herb Withania coagulans which belongs to the family Solanaceae is mostly found in the wild desert areas of Pakistan and India and is commonly called as a cheese maker due to its ability to convert the milk into cheese. Traditionally, it is most commonly used as medicinal plant by local herbal practitioners for hyperglycemia. It is also used for curing a number of other medicinal purposes including wound healing, inflammation, hepatic and cardiovascular diseases. The present study aims to assess the analgesic and anti-microbial effects (against Escherichia coli and Clostridium perfringens) of Crude Methanoic Extract (CME) of Withania coagulans. The analgesic activity was performed on albino mice by two tests, i.e. (i) Acetic Acid induced writhing test and (ii) Formalin induced licking and biting test and the antimicrobial activity was also assessed by performing Well Diffusion and Disc Diffusion methods. However, CME exhibited significant antimicrobial effects in higher concentrations, whereas it did not show any remarkable analgesic effect as compared to control group.

Key words: *Withania coagulans, Antibacterial and Analgesic activity.*

***Corresponding author:**

Saima Ghazal,

Faculty of Pharmacy & Health Sciences,
University of Balochistan,
Quetta, Pakistan
Email: saimahghazal@gmail.com

QR code

Please cite this article in press Saima Ghazal et al., ***A Study On Analgesics And Antibacterial Activities Of Withania Coagulans Fruits Collected From District Loralai, Balochistan.,*** *Indo Am. J. P. Sci, 2018; 05(10).*

INTRODUCTION:

Plants are known to be the earliest source of traditional medication to fight against different types of aliments. The plants having medicinal properties comprise a great part of medicines used in this era. The medicinal plants create a basis for new medication. The bioactive extract ought to be standardized on the basis of active compounds, (Mathur and Agrawal 2011). According to the report by WHO, nearly 3500 million of world population is dependent on medicinal plants in developing countries for treatment of various diseased condition, (Balick and Cox 1996). Now on a daily basis there's a growing specialization in the importance of medicative plants within the ancient health care system in world, due to varied blessings over the artificial medication. The plants square are utilized in drugs since antiquity with success to treat totally different ailments. The present organic process setup mainly concentrates on the synthetic approach of medicines. A minute alteration in the functional groups leads to a big change in the properties of the medicine. However, still most of the efforts are employed is on the natural source of medicaments, (Rates 2001).

In developed countries of the world like United States of America , the plants put up a part of about 25% of all of the medicines prepared and used, whereas in undeveloped countries such as Pakistan and India, the contribution of medicinal plants is about 80%, (Joy and Thomas). The Himalayan herbs have been identified for this purpiose since ancient civilization. The first use of plants as medicine was noticed 600 B.C in Vedas, hence possesses the old information which includes 67 species, (Sheng-Ji 2001).

The nature has gifted Pakistan with every season and has privileged with seas, grounds, deserts and also has ranges of high altitude mountains. Therefore, Pakistani land represents a very fertile area for a vast variety of medicinal plants with about 6000 species, (Ali and Qaiser 1986). One among these natural resources of medicines that is employed to treat varied diseases in Pakistan is the plant of Withania coagulans, (Pandey & Nama, 2015).

Plant information:

The plant is locally known as Paneer band, cheese maker or vegetable rennet. (Ali and Qaiser 1986) , This traditional name of the plants occurs due to its property of coagulating milk into cheese (Gupta 2012). Withania coagulans is distributed in the drier parts of subcontinent (Kirtikar and Basu 1918). Its plant is an Angiosperm belonging to family Solanaceae and is a hard, grey coloured shrub which has a height of about 60 to 120 centimetres. The flowering time of Withania coagulans starts from November and extends till April and their fruit matures during January to May. The natural revival occurs by the scattered seed from the dried ripened fruit (Hemalatha, Kumar et al. 2008)

Pharmacological activities of Withania coagulans:

Withania coagulans is one of the most prominent crude drugs of the old Indian medical system. It has a wide range of uses in Ayurvedic and Unani forms of medications. The fruit of this very plant is sweetened in nature and possess significant pharmacological activities, Such as sedatives, emetics and diuretics. These are also reported as blood purifier and are useful in dyspepsia. The ancient people have used this plant for asthma and biliousness too (Maurya, Akanksha et al. 2010). The plant extract of Withania coagulans (berries) has also been reported for the coagulation of milk (Chadha 1976). However, present study aimed to analyze analgesic activities of the plant's fruit collected from Loralai, District Zhob of Balochistan, Pakistan. This, to the best of our knowledge was not evaluated earlier. Moreover, the antibacterial activity on Clostridium Perfringens and E.Coli was evaluated first time in Balochistan. Furthermore, ethanol, for the first time in Balochistan was used for the extraction of the crude drug from the fruits of Wathania coagulans.

MATERIAL AND METHOD:

Collection and identification of Plant:

The fully ripened fruit with stalk of the plant Withania coagulans were collected from Loralai, District Zhob of Balochistan, Pakistan. The plant was recognized by Department of Pharmacognocy, Faculty of Pharmacy, University of Balochistan, Quetta.

Methanoic extract preparation:

After identification, the plant parts were kept safe in Drug Testing Laboratory and were shade dried for about two weeks at room temperature to prevent any loss of medicinally important constituents. The fully dried fruit of the plant (Withania coagulans) was converted into fine powder with the help of Grinder and was sieved using a strainer commonly used in houses. The hard seeds left behind in sieve were again grinded and sieved again. 50g of fine powder of the plant part was mixed and immersed in 150ml Methanol in airtight amber colored glass bottle for about 7 to 10 days. Later on, the solvent was filtered and evaporated by using Rotary Evaporator. Dark brown semi-solid extract was

obtained which was stored in the refrigerator for further use whenever needed.

Experimental Animals:
25-30 grams of Albino mice of either sex were used in this study for the assessment of analgesic activity of Withania coagulans. We get these mice from CASVAB (i.e. a research center of University of Balochistan).

Analgesic activity:-
Acetic acid induced writhing test:
To determine the analgesic activity of the plants, the mice were treated with acetic acid for the induction of pain (Apu, A. S., et al 2012).10 ml per body weight of 0.6% acetic acid solution in Normal Saline (v/v) was administered intra-peritoneal to the mice (Parimaladevi, Boominathan et al. 2003) After 30 minutes of the administration of the saline treated (control group), Wathania coagulans 250 & 500 mg/kg treated group and Aspirin 150mg/kg treated group. Meanwhile the writhings were counted for 30 minutes. Decrease in the writhings is deemed to have analgesic activity of the plant in contrast to control (Hunskaar, S., et al 1986). However, the plant did not show any significant decrease in the writhingsas compared to standard.

Formalin test :
Mice were treated with formalin for the sake of pain induction. 20 µl of 5% formalin in Normal Saline(V/V) was subcutaneously injected to the paw (dorsal hind) of the mice with a micro syringe (26-gauge needle), after 30 minutes of administration of saline (control group), Wathania coagulans 250 & 500 mg/kg oral dose and Aspirin 150mg/kg orally (Sayah, Chemlal et al. 2017) After injection the mouse was put into a chamber where the licking and biting of the injected paw were counted. The time of licking and biting was divided in two phases, the first phase was from 0-15 and the second was taken from 26-40 minute (Ahmad, 2014)

Bacteria for test:
The pure cultures of bacteria which are used in the test were collected from CASVAB (Centre for Advanced Studies in Vaccinology and Biotechnology) are:
1. Gram positive anaerobic bacteria: Clostridium perfringens
2. Gram negative facultative anaerobic bacteria: Escherichia coli (E.Coli)
And these microbes were cultured on RCM and Macconkey media respectively.

Antibacterial assay:-
Antibacterial activity was determined by:
Disc Diffusion Method. (Nostro, 2000). and
Well Diffusion Method. (Basu, 2005).

Preparation of Stock Solution:
Stock solution (S.S.) of 500 mg/ml concentration of the extract was prepared in DMSO. Three two-fold serial dilutions were made.

Preparation of Reinforced Clostridial Media:
RCM was prepared by adding 19 grams of RCM and 6 grams of agar in in 500ml water followed by autoclaving this mixture for 15 minutes at 121°C. Then the warm media was transferred to petri plates and refrigerated for further use.

Preparation of Macconkey Media:
Macconkey Media was prepared by mixing 24.76g of dehydrated MacConkey medium in 500ml water followed by autoclaving this mixture for 15 minutes at 121°C. Then the warm media was transferred to petri plates and refrigerated for further use.

Preparation of 0.5 McFarland standard:
The required standard was prepared by adding 0.5 ml. of 0.048 M BaCl2(1.17% w/v BaCl2·2H2O) to 99.5 ml. of 0.18 M H2SO4(1% w/v) while stirring constantly (Andrews, 2004).

Preparation of Bacterial inoculum:
The bacterial inoculums of C. perfringens and E. coli were prepared by mixing the pure bacterial colonies in normal saline, until the turbidities of each bacterial inoculum matches the turbidity of 0.5 McFarland standard.

Disc diffusion method:
In 5ml of the normal saline the inoculum of each bacteria was prepared . then such suspension was compared with 0.5 mcfarland standard.the plates of hard RCM and Macconkey agar were inoculated thoroughly with sterile swabs of cotton. In DMSO (Di Methyl Sulfoxide) solutions of CME(500mg/ml) were prepared. While as Steptomycin sulphate 10µg/ml for positive control was prepared in DMSO. For negative control simple pure DMSO was used. 5mm diameter paper discs of Whatsman filter paper grade 5 were dipped in the solution of CME and were positioned on the inoculated media plates. Streptomycin was used as positive control while DMSO was used as negative control. The plates were incubated at 37°C for 24-48 hours. Each test was repeated three times (Mahesh and Satish 2008).

MIC determination by well diffusion method:
25ml of media was poured in petri dishes and were let to solidify. The wells of 10mm diameter were made in the solidified media. 1-2 µl of bacterial suspension (compared to 0.5 McFarland standard) was deposited on the solidified media with the help of sterile cotton swab. The plates were incubated for 24-48 hrs at 37℃.

RESULTS:

Analgesic Activity:

Table1: Writhing test activity

Mice No.	Placebo Control	Positive Control (Aspirin 150mg/kg)	Test Group 1 (W. coagulans sol.250mg/ml)	Test Group 2 (W. coagulans sol. 500mg/ml)
	Number of Writhings after Intra Peritoneal injection of Acetic Acid Solution			
1	99	47	93	89
2	103	51	97	92
3	150	52	99	93
4	101	49	96	92
5	120	49	97	94
6	109	54	104	98
7	105	50	101	95
Mean	103.428 ± 1.231	50.2857 ± 0.865	98.1428 ± 1.352	93.2857 ± 1.352

The table portrays precisely the analgesic effects of concentrations of CME of Withania coagulans on mice. At the dose of 500mg/kg the average writhings experienced by mice were 98.1428 with ± 1.3527 SEM. And at the dose of 500mg/kg, average number of writhings counted was 93.2857 along with ±1.0626 of SEM. In contrast to Withania coagulans, the standard drug used i.e. Aspirin at a dose of 150mg/kg gave an average of 50.2857 ± 0.865 SEM. The number of writhings experienced by placebo group was 103.428 ± 1.2316 SEM. So we conclude that the CME of Withania coagulans has no significant analgesic activity.

Formalin Test:-

1st phase:
Results show that in saline treated (control group) the number of licking was 55.5714±1.3248 and time spent on licking was 95.285 ±1.8088 seconds. While in 250mg/kg Withania coagulans, the crude extract treated group numbers licking was 51.142±1.2616 and time spent on this was 87.285±1.8608 seconds. In 500mg/kg of crude extract numbers of licking was 43.285±0.8921 and time spent was 79.857±0.7918 seconds and with standard drug (Aspirin) treated group the number of licking was 21.714±0.6060 and time spent was 35.428±1.2316 seconds (Table 2).

2nd phase:
Results have shown that in saline treated (control group) numbers of the licking were 10.714±0.6801 and time spent on this was 18.285±1.2093 seconds. While in 250mg/kg of CME Withania coagulans treated group numbers, the average no.of licking and biting was 9.571±0.6494 and time spent on this was 16.857±0.9110 seconds. In 500mg/kg of crude extract numbers the activity were 14.428±0.8959 and time spent was 23.714±1.0848 second sand with standard drug (Aspirin) treated group the activity was 4.571±0.3688 and time spent for licking and biting was 7.142±0.5084 (table 2).

Formalin induced analgesic activity (table2)

Mice No	Placebo Control Group				Positive Control Group				Test Group 1				Test Group 2			
	Phase 1 (1-15 min)		Phase 2 (26-40 min)		Phase 1 (1-15 min)		Phase 2 (26-40 min)		Phase 1 (1-15 min)		Phase2(26-40min)		Phase 1 (1-15 min)		Phase 2 (26-40 min)	
	No.	Time	No.	Time	No.	Time	No.	Time	No.	Time	No.	Time	No.	Time	No.	Time
1	51	89	8	14	20	32	3	5	46	79	7	13	39	77	11	20
2	55	95	11	18	21	34	5	8	51	87	10	17	42	80	14	22
3	52	90	10	17	20	34	4	7	48	85	9	15	43	71	13	21
4	58	97	12	20	23	37	5	7	53	89	10	18	45	82	16	25
5	59	98	12	22	23	39	4	6	53	90	11	19	45	84	16	26
6	60	103	13	22	24	40	6	9	56	95	12	20	46	85	18	28
7	54	95	9	15	21	32	5	8	51	86	8	16	43	80	13	24
Mean	55.5714	95.285	10.714	18.285	21.714	35.428	4.571	7.142	51.142	87.285	9.571	16.857	43.285	79.857	14.428	23.714
SEM	±1.3248	±1.8088	±0.6801	±1.2093	±0.6060	±1.2316	±0.3688	±0.5084	±1.2616	±1.8608	±0.6494	±0.9110	±0.8921	±0.7918	±0.8959	±1.0848

Antibacterial assay:
Antibacterial activity was determined by Disc Diffusion Method (Nostro et al., 2000). And Well Diffusion Method (Basu et al.,2005).

Activity of Withania coagulans against Clostridium perfringens:
Table 3 elaborates that stock solution of Withania coagulans showed an average inhibition zone of 24.667 mm while as dilution1 showed 21.667mm, dilution2 showed 18.333mm and dilution3 showed 14.00mm zone. Which declares the presence of antibacterial activity of Withania coagulans against Clostridium perfringens (an anaerobic Bacteria).

Table 3: Activity of Withania coagulans on Clostridium perferingens (500mg/ml) by Well Diffusion Method

S. No.	Stock Solution	Dilution #1	Dilution #2	Dilution #3
1	25	21	18	13
2	23	22	18	14
3	26	22	19	15
Mean	24.667	21.667	18.333	14.000

Table 4 explains that with 5mm of disc the stock solution gave a zone of 13.66mm however, dilution1 showed 9.33mm and dilution 2 showed 7.333mm zone of inhibition but dilution 3 did not show any zone of inhibition but the bacterial growth was not on the disc, so we consider the inhibition zone of 3rd 2-fold dilution equivalent to the size of disc (i.e. 5 millimetres). This disc diffusion method declares the antibacterial activity of Withania coagulans against Clostridium perferingens in stock solution of 500mg/ml(w/v) in DMSO in its two-fold dilutions.

Table 4: Activity of Withania coagulans on Clostridium perferingens (500mg/ml) by "Disc Diffusion Method"

S. No.	Stock Solution	Dilution #1	Dilution #2	Dilution #3
1	16	10	7	5
2	17	10	8	5
3	16	9	7	5
Mean	13.667 mm	9.333 mm	7.333 mm	5 mm

Activity of Withania coagulans against Escherichia coli:
The antibacterial activity of Withania coagulans against E.Coli in well diffusion method is being explained in the table 5. The maximum zone of inhibition is 21..667mm which is given by stock solution. Whereas the minimum activity is shown by dilution3 which produced a zone of inhibiton 12.667mm wide. However, Dilution1 of of the CME of Withania coagulans gave a zone of 18.333mm and dilution 2 showed 15.667mm zone of inhibition. Thus , it is concluded that the Withania coagulans has a significant antibacterial activity against E.Coli.

Table 5: Antimicrobial activity of Withania coagulans on Escherichia Coli (500mg/ml) Well diffusion method

S. No.	Stock Solution	Dilution #1	Dilution #2	Dilution #3
1	21	18	15	12
2	20	18	15	11
3	21	19	14	12
Mean	21.667 mm	18.333 mm	15.667 mm	12.667 mm

Disc diffusion method of antibacterial activity of Withania coagulans against E.Coli is well elaborated in table 6. Stock solution gives 15mm zone and dilution1 gave a zone of 11mm while as dilution2 gave a zone of 7.33mm which is the least activity of Withania coagulans as dilution 3 didn't make any zone of inhibition. Hence stock solution and its first two-fold dilutions gave a significant result which shows the positive activity of Withania cogulans against E.Coli.

Table 6: Disc diffusion method of antibacterial activity of Withania coagulans against E.Coli

S. No.	Stock Solution	Dilution #1	Dilution #2	Dilution #3
1	13	10	7	5
2	14	11	7	5
3	13	9	6	5
Mean	13.333	10.00	6.666	5.00

DISCUSSION:

There are more than four lakh medicinal plants where only a few are evaluated for medicinal uses (Sharma, Verma et al. 2009). These plants are utilized by local people to combat about every type of illness from headache to cut and wound (Bhardwaj and Gakhar 2005). Withania coagulans is one of these plants used by local people for a number of purposes is an Angiosperm which belonging to family Solanaceae has a wide range of uses in Ayurvedic and Unani forms of medications. The fruit of this very plant is sweetened in nature and possess significant pharmacological activities, Such as Hepatoprotective, Anti-inflammatory, Anti-hyperglycaemic, Hypolipidemic, Cardiovascular, Antitumour, Wound healing properties and also used as sedatives, emetics and diuretics. These are also reported as blood purifier and are useful in dyspepsia. The ancient people have used this plant for asthma and biliousness too. (Maurya, Akanksha et al. 2010). Wihania coagulans has reported to possess a very significant activity on many microorganisms including Staphylococcus aureus (Khan, Ashraf et al. 1993). The present study has shown antibacterial activity of the plant collected from Dostrict Loralai of the province Balochistan, Pakistan. The has shown significant antibacterial activity against Clostridium Perferingens with 24.667mm of inhibition zone at the dose of 500mg/kg in well diffusion method (Basu et al.,2005). While as in disc diffusion method the zone of inhibition given was 13.667mm. The zone of inhibiton given by Withania coagulans against Escherichia Coli in well diffusion method was 21.667mm while as the zone given by disc diffusion method was 13.333mm. In this study, It is found that Withania coagulans does not possess any significant analgesic activity as compared to Aspirin.

CONCLUSION:

The present study was conducted to evaluate the analgesic and antibacterial activity of Withania coagulans. On the basis of the experiments carried out this could be concluded that it has a very significant effects on E. coli and C. perferingens. However, it did not show any analgesic activity on albino mice.

ACKNOWLEDGEMENT:

The authors wishes to gratitude Mr. Tahriq Mehmood, whose help make this research possible. We thank the staff of CASVAB Mr. Aurangzaib and Mr. Abdullah who helped us in the research. The authers are also greatful to Mr. Abdul Aleem and Mr. Arshad Fayaz from Drug Testing Laboratory Quetta; for their precious time to help us in this research. I also thank all the teachers of the Department of Phamacology, UOB, Quetta, specially Mrs. Bushra Aziz; who help in collection and identification of test plant, and Mr. Shoaib Kakar, Mr. Nouman ul Haq, and Miss Faria; who were always there whenever needed.

REFERENCES:

1. Ali, S. I. and M. Qaiser (1986). "A phytogeographical analysis of the phanerogams of Pakistan and Kashmir." Proceedings of the Royal Society of Edinburgh, Section B: Biological Sciences **89**: 89-101.
2. Ahmad, M., Muhammed, S., Jahan, N., Jan, S. U., & Qureshi, Z. U. R. Anti-dermatitis, anxiolytic and analgesic effects of Rhazya stricta from Balochistan. Pakistan Journal of Pharmaceutical sciences, 2014;27(3): 30.
3. Balick, M. J. and P. A. Cox (1996). Plants, people, and culture: the science of ethnobotany, Scientific American Library.
4. Basu, S., et al. (2005). "Evaluation of the antibacterial activity of Ventilago madraspatana Gaertn., Rubia cordifolia Linn. and Lantana camara Linn.: isolation of emodin and physcion as active antibacterial agents." Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives **19**(10): 888-894.
5. Budhiraja, R., et al. (1984). "Antiinflammatory activity of 3 β-Hydroxy-2, 3-dihydro-withanolide F." Planta medica **50**(02): 134-136.
6. Chadha, Y. (1976). "The Wealth of India, Raw Materials, Vol. 10 (Sp-W), CSIR India." New Delhi.
7. Gupta, P. C. (2012). "Withania coagulans Dunal-an overview." International Journal of Pharmaceutical Sciences Review and Research

12(2): 68-71.
8. Hemalatha, S., et al. (2008). "Withania coagulans Dunal: A review." Pharmacognosy reviews **2**(4): 351.
9. Hunskaar, S., et al. (1986). "Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test." Pain 25(1): 125-132.
10. Joy, P. and J. M. Thomas "S. and Skaria, BP (1998)." Medicinal Plants. Kerala Agricultural University, Kerala, India: 3-8.
11. Khan, M., et al. (1993). "Antibacterial activity of Withania coagulants." FITOTERAPIA-MILANO- **64**: 367-367.
12. Kirtikar, K. R. and B. D. Basu (1918). Indian Medicinal Plants Vol-3, Bishen Singh Mahendra Pal Singh And Periodical Experts.
13. Mahesh, B. and S. Satish (2008). "Antimicrobial activity of some important medicinal plant against plant and human pathogens." World journal of agricultural sciences **4**(5): 839-843.
14. Mathur, D. and R. Agrawal (2011). "Withania coagulans: a review on the morphological and pharmacological properties of the shrub." World journal of science and technology **1**(10): 30-37.
15. Maurya, R., et al. (2010). "Chemistry and pharmacology of Withania coagulans: an Ayurvedic remedy." Journal of pharmacy and pharmacology **62**(2): 153-160.
16. Nostro, A., et al. (2000). "Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity." Letters in applied microbiology **30**(5): 379-384.
17. Pandey, I., & Nama, K. S. (2015). Withania Coagulans (Stocks) Dunal – A Rare Ethnomedicinal Plant of the Western Rajasthan Desert, 2(2), 34–40.
18. Parimaladevi, B., et al. (2003). "Studies on analgesic activity of Cleome viscosa in mice." Fitoterapia **74**(3): 262-266.
19. Rates, S. M. K. (2001). "Plants as source of drugs." Toxicon **39**(5): 603-613.
20. Sayah, K., et al. (2017). "In vivo anti-inflammatory and analgesic activities of Cistus salviifolius (L.) and Cistus monspeliensis (L.) aqueous extracts." South African journal of botany **113**: 160-163.
21. Sheng-Ji, P. (2001). "Ethnobotanical approaches of traditional medicine studies: some experiences from Asia." Pharmaceutical biology **39**(sup1): 74-79.
22. Sharma, A., et al. (2009). "Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens." World Applied Sciences Journal **7**(3): 332-339.