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**Research Article**

# CNS depressant and analgesic effects of *Withania coagulans* (Stocks) dunal fruits collected from Khuzdar district of Balochistan, Pakistan

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**Abstract**

*Withania coagulans* (Stocks) Dunal (Solanaceae) have been reported with various uses such as emetic, sedative, diuretic, alterative in chronic liver disorders, dyspepsia, intestinal infections and colic pain. This study aims to investigate the central nervous system (CNS) depressant, anxiolytic and analgesic effects of methanolic extract of *W. coagulans*. CNS depressant activity of crude methanolic extract of *W. coagulans* fruits was evaluated using standard animal behavioural models such as open field, cage crossing, rearing and traction test. Anxiolytic activity was evaluated through forced swimming test whereas analgesic activity was carried out via acetic acid induced writhing and formalin test in mice. The extract (100 and 250mg/kg) showed a dose-dependent suppression of motor and exploratory (Neuropharmacological) activity for mice in open field, cage crossing, rearing and traction test. Significant writhing effect i.e. decrease in number of writhes as well as analgesic effects were observed ($P$<0.05).The findings of current research work supports the CNS sedative and analgesic potential of *W. coagulans*.

**Keywords:** Analgesic; Writhing test; Balochistan; Khuzdar; *Withania coagulans*

## Introduction

The family Solanaceae is famous for plants with potent medicinal properties **[1]**. *Withania coagulans* (Stocks) Dunal, a shrub from Solanacea family, locally known as Paneer bandh, is distributed mostly in the Mediterranean region (east), ranging to South Asia **[2-4]**. In Pakistan, it is found in Khuzdar region of Balochistan where it is used as ethno-pharmacologically by the

local inhabitants [5, 6]. The berries from this plant due to the presence of enzyme in husk and pulp are used as milk coagulating agent. The phytochemical investigation revealed the presence of free amino acids, esterases, essential oils, fatty oils and withanolides in the berries. Main skeleton of Withanolides is ergostane with a steroidal lactones [7, 8]. In addition; the fruit of plant is emetic, sedative, diuretic and used as alterative in chronic liver disorders as well as employed for gastrointestinal problems such as flatulence, dyspepsia, intestinal infections and colic pain. The famous local use found for this plant is; chewing the small branches for dental cleaning whereas smoking the branches for toothache problem [7, 9-11]. The plant also have an eminent place in Unani and Ayurvedic medicine. Furthermore, *W. coagulans* have been reported as a significant source of constituents with steroidal lactones having biological activity [5, 11]. Numerous studies have revealed the pharmacological uses of crude plant, its extracts, fractions, and isolated withanolides in various ailments such as leishmaniasis, tumor, addiction, diabetes mellitus, wounds, epilepsy, hyperlipidemia and many other diseases [11-17].

Furthermore; many literature have reported the fruit of the plant as sedative and analgesic drug, thus current study aims to evaluate the Central Nervous system (CNS) depressant and analgesic activities of its methanolic extract.

## Material and methods

### Plant material

The Fruits of *W. coagulans* were collected from Khuzdar district of Balochistan in the month of June. Plant was identified by Mrs Bushra aziz khan Department of Pharmacognosy, Faculty of Pharmacy and Health Sciences, University of Balochistan Quetta, Pakistan. Voucher specimen number NA-216 was deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy and Health sciences, University Balochistan Quetta, Pakistan.

### Experimental animals

Mice (either sex; weigh, 25 to 28 gram) were collected from Animal House of Dow University of Health Sciences (DUHS), Karachi, Pakistan, placed in plastic cages with free access to water and food. The animals were kept in a controlled area (30 ± 1°C 12/12 h light/dark cycle) in order to familiarize them with standard environmental conditions (at least 07) prior to any testing or extract/drug administration.

### Forced swim test (FST)

Despair of behavior is suggested as a classical model for testing antidepressant activity [18]. Animal were divided in 4 groups i.e. Control (Non treated), 250 and 500mg/kg and standard drug (diazepam) treated groups and forced to swim individually in an open cylindrical apparatus (diameter 10 cm, height 25 cm), containing 19 cm of water (depth) at 25 ± 1°C. The total duration of time (minutes) for every mice, persisted immobile for a duration of 6 minutes, was noted as immobility time and determined as mentioned [19]. The immobility refers to mice when they remain immobile and stops struggling in stationary water, doing solitary those actions required to retain their head above the water. An enhancement in immobility time is revealing antidepressant like effect [18].

### Novelty induced rearing (NIR)

NIR is considered as a central excitatory behavior [20]. The observation are made i.e. counting the number of times a mice makes upright position using hind limb whereas the forelimbs remains against the walls of observation beaker or in free air [21]. The number of rearing activities were counted for 10 minutes [22].

### Open field activity (OFT)

The exploratory and loco motor activities were determined in an OFT. The apparatus

was made of plastic cube, walled (30 cm in height) and the floor was divided into twenty five (25) squares i.e. length and width 10 cm). The mice, after thirty minutes (30 min) following oral administration of the vehicle, plant extract (100 and 250 mg/kg) and diazepam, were evaluated individually at the center of OFT apparatus (10 min) in order to record locomotor activity (expressed by squares crossing with the all four paws) [23].

**Cage crossing test (CCT)**

For CCT, animal were placed in a plastic cage with crossing i.e. 26x 26 x26, and the number of movements to touch the cage edges (10 min) were recorded [24].

**Traction test (TT)**

For traction test an iron rod (one meter) is used while the animals were made pre-skilled to walk this rod. For TT observation, the mice time (seconds) in order to travel the rod is recorded. Any enhancement or reduction in time consumed by the extract treated mice were compared with control (non-treated) which shows stimulant or sedative activity of extract, respectively [25].

**Analgesic activity**

**Acetic acid writhing reflex method**

This activity was performed according to the modified method Danbisya *et al.* [26] as adopted from Koster *et al.* [27]. Twenty (20) mice (of both sexes) were arbitrarily divided in four (4) groups of five mice. Group I (Control group) mice were administered distilled water (10 ml/kg), group II mice were administered Diclofenic potassium (as standard drug) 50mg/kg whereas group III and IV received 250 and 500 mg/kg of *W. coagulans* extract respectively. Following the administration of *W. coagulans* extract and drug (after thirty (30) minutes), glacial acetic acid (0.7%; 10 ml/kg; i.p.) was introduced to all mice in order to induce pain as characterized by writhes or abdominal constrictions. Number of writhes experiential in every mice was calculated for thirty (30) minutes whereas the %age protection compared to abdominal writhing was utilized as a tool to measure the degree of analgesia using the formula [28].

$$\frac{\text{Mean control} - \text{Mean treated group} \times 100}{\text{Mean of control group}}$$

**Formalin test**

For formalin test; mice were divided in four (Control i.e. distilled water, 250&500mg/kg and aspirin treated) groups with 5 mice in individual group. Formalin solution (20 µl; 2%) was injected subcutaneously (s.c.) in right hind paw dorsal surface of mouse, using a 26-gauge micro syringe. After formalin injection, mice were put back in the observation chamber. Early phase (1$^{st}$ phase) was started instantly and continued for five minutes. The late phase response (2$^{nd}$ phase) was started after 15 minutes of formalin injection and continued (15 minutes). In the both phases licking or biting of injected site was referred as a nociceptive response, and the overall time of the response was determined [29].

**Statistical analysis**

The data was observed by ANOVA (analysis of variance) and Student's t test when the analysis was restricted to 2 means. Statistical significance was accepted at the 5% level (P -C 0.05). Results are given as Mean $\pm$ S.E.M [28].

**Results**

**Forced swimming test**

In FST the mean mobility and immobilty times were as; control (non-treated) group (180.01$\pm$0.01 and 179.09$\pm$0.03 seconds), for 100mg/kg *W. coagulans* treated group (141$\pm$0.02 and 159$\pm$0.04 seconds), for 250mg/kg *W. coagulans* treated group (225$\pm$0.02 and 135$\pm$0.04 seconds) whereas for diazepam treated group as (95$\pm$0.01 and 265$\pm$0.04 seconds). The results showed that 250 mg/kg dose of *W. coagulans* produced anxiolytic effect (Table 1).

**Table 1. Effect of *W. coagulans* on swimming test of mice**

| S. No | Treatment | Mean mobility time (seconds)±SEM | Mean immobility time (seconds)±SEM |
|---|---|---|---|
| 1 | Control | 180.01±0.01 | 179.09±0.03 |
| 2 | *W. coagulans* crude extract 100mg /kg | 141±0.02 | 159±0.04 |
| 3 | *W. coagulans* crude extract 250mg/kg | 225±0.02 ** | 135±0.04 ** |
| 4 | Diazepam 2mg/kg | 95±0.01 ** | 265±0.04 ** |

All values are mean ± SEM; n=5; * = Significant results ($P$<0.05), ** = highly significant results ($P$<0.01)

**Novelty induced rearing (NIR)**

The mean number of rearing activity observed were as; 39.5 ± 0.92 (control group), 35 ± 1.86 (100 mg/kg treated group), 20± 10.4 (250mg/kg treated group) and 13.4± 0.75 (diazepam treated group). These results shows a significant decrease in rearing activities (p<0.05), thus supporting the plant extract with sedative effects (Table 2).

**Table 2. Effect of *W. coagulans* on rearing activities of mice**

| S. No | Treatment | Rearing activities (Mean±SEM |
|---|---|---|
| 1 | Control | 39.5 ± 0.92 |
| 2 | *W. coagulans* crude extract 100mg/kg | 35 ± 1.86* |
| 3 | *W. coagulans* crude extract 250mg/kg | 20± 10.4 * |
| 4 | Diazepam 2mg/kg | 13.4± 0.75 ** |

All values are mean ± SEM; n=5; * = Significant results ($P$<0.05), ** = highly significant results ($P$<0.01).

**Open field activity**

The mean squares travelled by mice in open field test were as; control group (141.32 ± 2.18), 100 mg/kg treated group (62.2 ± 2.49), 250mg/kg treated group (88.2± 1.5) and for diazepam (40.8± 2.31). The results shows a significant decrease in open field activity (p<0.05) at 100mg/kg oral dose (Table 3).

**Table 3. Effect of *W. coagulans* on open field activity of mice**

| S. No | Treatment | Open field activities (Mean±SEM) |
|---|---|---|
| 1 | Control | 141.32 ± 2.18 |
| 2 | *W. coagulans* crude extract 100mg/kg | 62.2 ± 2.49 * |
| 3 | *W. coagulans* crude extract 250mg/kg | 88.2± 1.5 |
| 4 | Diazepam 2mg/kg | 40.8± 2.31** |

All values are mean ± SEM; n=5; * = Significant results ($P$<0.05), ** = highly significant results ($P$<0.01)

**Cage crossing activity**

The number of cage crossing were observed as; 43.8 ±1 for control group, 32.2 ± 1.1 and 24.2± 1.5 for 100 & 250mg/kg oral dose of mice treated with *W. coagulans* crude extract, as well as 20.8± 0.86 for diazepam treated group. Results reveal that cage crossing activity was significantly decreased (Table 4).

**Table 4. Effect of *W. coagulans* on cage crossing test of mice**

| S. No | Treatment | Cage crossing activities (Mean±SEM) |
|---|---|---|
| 1 | Control | 43.8 ±1 |
| 2 | *W. coagulans* crude extract 100mg/kg | 32.2 ± 1.1* |
| 3 | *W. coagulans* crude extract 250mg/kg | 44.2± 1.5 * |
| 4 | Diazepam 2mg/kg | 20.8± 0.86** |

All values are mean ± SEM; n=5; * = Significant results ($P$<0.05), ** = highly significant results ($P$<0.01)

**Traction time test**

The iron travel as recorded during traction test were; 9.2 ± 0.07 for control group, 25 ± 1.71 for 100 mg/kg and 34.2± 1.99 for 250 mg/kg of mice treated with *W. coagulans* crude extract respectively whereas for diazepam it was 52.8± 0.93. Results show that crude extract of *W. coagulans* showed significant ($p$<0.05) decrease in activity (Table5).

**Table 5. Effect of *W. coagulans* on traction time of mice**

| S. No | Treatment | Mean traction time (Seconds)±SEM |
|---|---|---|
| 1 | Control | 9.2 ± 0.07 |
| 2 | *W. coagulans* crude extract 100mg/kg | 25 ± 1.71 * |
| 3 | *W. coagulans* crude extract 250mg/kg | 34.2± 1.99 * |
| 4 | Diazepam 2mg/kg | 52.8± 0.93** |

All values are mean ± SEM; n=5; * = Significant results ($P$<0.05), ** = highly significant results ($P$<0.01)

**Analgesic activity**

**Writhing test**

The mean number of writhing observed were as; control group (44.5 ± 1.6), 100 mg/kg treated group (42.2± 3.2), 250mg/kg treated group (24.2± 0.6) whereas for diclofenac potassium treated group it was 17.6± 0.68. Results reveal that the *W. coagulans* at 250mg/kg showed significant analgesic activity (Table 6).

**Table 6. Effect of *W. coagulans* on acetic acid induced writhing test of mice**

| S. No | Treatment | Writhing activities ( Mean±SEM) |
|---|---|---|
| 1 | Control | 44.5 ± 1.6 |
| 2 | *W. coagulans* crude extract 100mg/kg | 42.2± 3.2 * |
| 3 | *W. coagulans* crude extract 250mg/kg | 24.2± 0.6* |
| 4 | Diclofenic potassium 50mg/kg | 17.6± 0.68** |

All values are mean ± SEM; n=5; * = Significant results ($P$<0.05), ** = highly significant results ($P$<0.01)

**Formalin test**

The results of formalin test (Table 7) reveals that *W. coagulans* at 100 & 250mg/kg showed significant analgesic activity in both 1st and 2nd phases of formalin induced inflammatory pain.

**Table 7. Effect of Crude extract of *W. coagulans* in formalin induced inflammatory pain on mice**

| Treatment | Dose mg/kg orally | First phase Mean No. of observations ±S.E.M | | Second phase Mean No. of observations ±S.E.M | |
|---|---|---|---|---|---|
| | | Number of licking and biting | Time spent (Seconds) | Number of licking and biting | Time spent (Seconds) |
| Control | 0.5ml Distil water | 72.6±0.51 | 84.6±0.9 | 83.2±1.24 | 94.8±1.7 |
| Crude extract of *W. coagulans* | 100 mg/kg | 51.6 ±2.66* | 64.4±1.33* | 61.8±0.60* | 54.6±1.2* |
| | 250mg/kg | 36.6±2.40* | 29.6±3.42* | 57.2±2.60* | 49.8±0.18* |
| Diclofenic potassium | 50mg/kg | 30±1.418** | 21.4±3.77** | 27.6±2.21** | 22.6±3.01** |

## Discussion

During last few decades, the role of herbal medicines for treatment of numerous psychological conditions has extended and developed effectively. Numerous herbal products are utilized for the treatment of anxiety, insomnia and cognitive abnormalities and these herbal preparations contain active constituents that effect the ionotropic receptors function for major (inhibitory) neurotransmitters, GABA and serotonin in the brain [30]. Depressive and anxiety disorders are among the most recurrent psychiatric disorders faced today. It has been reported that 20% of adult people suffer from such conditions at certain stage throughout their life. Thus a CNS depressant agent from natural source with less or no toxicity is very essential in these conditions [31]. Significant decrease in the open field, rearing, cage crossing and traction test shows that *W. coagulans* crude extract may act on central nervous system. Since it is recognized that rearing is the function of level of excitability of CNS [32]. Flavonoids are known to possess CNS depressant effect [33]. The CNS depressant activity produced by *W. coagulans* extract may be due to flavonoids.

For screening of the potential antidepressant drug, FST is considered among the most communal animal model [34, 35] In this test a state of the immobility is induced in animals facing an unavoidable state (in a swim tank). This immobility behaviour is considered to reflect behavioural despair, which is a reflection of disorders of depression in humans. Hence, decrease in immobility time shows the antidepressant like activity of drug as shown by animals acquiesced to swimming. This change in behavioural is subtle to the major antidepressant classes, including tricyclic's, monoamine oxidase (MOA) inhibitors, inhibitors of selective 5-HT reuptake [36]. The, results of current study suggest that antidepressant like activity of *W. coagulans* was comparable to standard antidepressant drug.

Result of current study reveals that the crude methanolic extract of berries of *W. coagulans* showed an antinociceptive activity when evaluated by chemical models of nociception i.e acetic acid-induced writhing test and formalin test. In writhing test, antinociceptive activity was dependent on dose of the extract. It is hypothesized that, acetic acid indirectly acts by prompting the release of the endogenous mediators which stimulate the nociceptive neurons, sensitive to the opioids and NSAIDs (non-steroidal anti-inflammatory drugs) [37]. Flavonoids are naturally occurring polyphenolic compounds in plants which have anti-inflammatory and analgesic properties. Flavonoids have ability to cross blood-brain and regulate pain by numerous mechanisms like effect on opioid, GABA-A,

adrenergic receptors and inhibits the enzymes participated in inflammation in brain. GABA-A, adrenergic and opioid receptors are reported to present in various areas of nervous system together with in rostral ventrolateral medulla in which paragigantocellular nucleus is present. Stimulation of these receptors responsible for relief of pain. Flavonoids inhibit the cyclooxigenase present in tissues, which inhibit the formation of prostaglandins. Prostaglandins stimulate the receptors of pain **[38]**. *W. coagulans* has contain flavonoids **[39]**, flavonoids are reported to exert analgesic effects **[40]**. In present study the analgesic activity may be due to flavonoids.

## Conclusion

The results of present study reveal that the administration of methanolic crude extract of *W. coagulans* exhibited significant decrease in neuropharmacological activities, forced swimming test, number of writhes in acetic acid writhing test alongwith the number and time spent on licking and biting. Thus it is evident from our results; crude methanolic extract of *W. coagulans* contains pharmacological active constituents with CNS depressant and analgesic effect. Hence, further pharmacological and biochemical studies are required to postulate the exact mechanism and chemical compound responsible for pharmacological effects.

## Authors' contributions

Conceived and designed the experiments: S Muhammad, K Fatima & Rizwan Ahmad, Performed the experiments: N Ahmad, Analyzed the data: Mehjabeen & Marvi, Contributed reagents/ materials/ analysis tools: NA Shahwani, Wrote the paper: N Ahmad.

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