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Analgesic, Anti Inflammatory and Antipyretic Potential of Fruit Extract of *Withania Coagulans* in Experimental Animals.

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***Abstract*-- The objectiveof the present work is to evaluate analgesic, anti-inflammatory and antipyretic activity of fruit extracts of *Withania coagulans* (W.C.) extracts using different animal models. Methods - Analgesia was induced by intraperitoneal injection of 0.6% v/v of acetic acid in mice to access peripheral analgesic action of *WC* whereas central analgesic activity was evaluated by eddy's hot plate method in mice to evaluate central analgesic action of *W.C.* Carragenan o.1 ml of (1%), was administered in sub-plantar region of rat paw and inflammation was induced and the anti-inflammatory activity was evaluated using Plethysmometer. Antipyretic activity was evaluated using cow's milk induced pyrexia model in rats. Results- In acetic acid induced writhing model, administration of *W.C* extract i.e. *WCAQ, WCET,WCEA* (200 mg/kg, p.o) showed significant (P<0.001) inhibition in acetic acid induced writhing as compared to control animals. In hot plate analgesia, *WCET* showed significant (P<0.001) increase in reaction time as compared to control animals from 1h to 3h, whereas *WCAQ* and *WCEA*showed significant increase in reaction time at 4h only in carragenan induced inflammation, administration of *WC* extract i.e. *WCAQ, WCET, WCEA* (200 mg/kg, p.o) as well as diclofenac (25 mg/kg, p.o) reduced the paw volume significantly at 1h, 2h, and 3h. In milk induced pyrexia model, no significant variation was observed in any test rats.**

***Conclusion*-- Based on the results on various animal models, it can be concluded that various extracts of *WC* could be a patent analgesic, moderate anti-inflammatory and passes no anti-pyretic action.**

I. INTRODUCTION

Pain is unpleasant sensation occurs in varying degrees of severity as a consequence of injury, disease, or emotional disorder. Inflammation is protective attempt by organism to remove the injurious stimuli as well as initiates the healing process for tissues (Denko,1992). The process of inflammation is necessary in healing of wounds. Inflammation leads to onset of various diseases like vasomotor rhinnorhoea, rheumatoid arthritis & atherosclerosis (Gabor; 1979). Non-steroidal anti-inflammatory (NSAIDs) are used primarily in the treatment of pain and inflammation. Pyrexia is the increase of body temperature than normal due to metabolic disturbances.

Antipyretic drugs are widely used in treatment of fever and pain. But the greatest disadvantage of these anti-pyretic and (NSAIDs) is gastro-intestinal irritation, due to the inhibition of the protective cyclo-oxygenase enzyme in gastric mucosa (Rang et.al 2008).Various fruit derived extracts are used against diseases in various systems of medicine such as Ayurveda, Unani, and Siddha. Only a few of them have been scientifically explored. Fruits of *W.C* derived natural products such as alkaloids, carbohydrates, protein, amino acids and tannins have received considerable attention in recent years due to their diverse pharmacological properties of analgesic, anti-inflammatory and anti-pyretic activites.

Withania is a small genus of shrubs, belonging to solanaceae family. It is popularly known as "Indian cheese maker". In Punjab, the fruit of *W.coagulans* are used as the source of coagulating enzyme for clotting the milk which is called 'Paneer'. The main components of barriers are esterase, fatty oils, amino acid such as proline, valine, tyrosine, aspartic acid, glycines, asparagines, cysteine and glutamic acid and alkaloids phytoconstituent are present in withania coagulans. The most of the activities reported is due to the presence of an active constituent as, With anolides. (Mar yam Khodaei et al., 2012). Berries of *WC* are used as sources of enzymes which clot the milk to form "Paneer". (Jaiswal et al., 2009). A compound isolated from aqueous extract of WC has shown to exert hepatoprotective activity. (Budhiraja et al., 1986) The dry fruit of WC are used in the treatment of diabetic patient. (Jaiswal et al., 2009). *Withania Coagulans* is used in chronic complaints of liver (Mathur et al., 2011). They are used in dyspepsia, flatulent colic and other intestinal infections. (Mathur et al., 2011). Ethnobacterially *WC* used to decreases the inflammation. (Kirtikar Basu, 1935)

II. MATERIAL AND METHODS

2.1 Plant material

Dried fruits of *Withania coagulans* (family: Solanaceae) was collected from local market of Pune and authenticated by Botanical Survey of India, Pune (Voucher No. of the specimen: BSI/WRC/TECH/2013).

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The fruits samples are stored in the Department of Pharmacognosy of Sinhagad Institute of Pharmacy, Narhe, Pune. (Voucher No: SIOP/12/17)

2.2 Preparation of extract

The fruit of W.C was shade dried and grinded to fine powder. Then 250 g of WC powder was added in 650 ml of distilled water and kept at room temperature for 48 hr with occasional shaking to get aqueous extract (WCAQ). 500g of WC powder was extracted by Soxhletion with different solvents like petroleum ether, (WCPE) for defatting. Ethanol and ethyl acetate (WCET) and (WCEA) respectively.

2.3. *Phytochemical screening*

The extract so obtained was subjected for phytochemical investigation for various chemical constituents in the extract. The crude extracts were screened for the presence of secondary metabolites such as carbohydrates, proteins, amino acids, saponin and tannins.

2.4. Animals

Male swiss albino mice (30-40 g) and wistar rats (190-240 g) were procured from National Toxicological Centre, Punewere used in the present study after acclimatization period of one week.Animals were maintained at 24±1^{o}C, with relative humidity of 45–55% and 12:12 h dark/light cycle. Animals had free access to food (Standard chaw pellet, Chakan oil mills, Sangli) and drinking water *ad libitum*. All experiments were carried between 10.00 and 17.00 h. The experimental protocol was approved from Institutional Animal Ethical Committee (IAEC) of Sinhgad Institute of Pharmacy, Narhe, Pune constituted as per the committee for purpose of supervision and control on the experimental animal [CPCSEA reg. no 1139/a/07]. Protocol approval no. SIOP/IAEC/2012/23.

2.5. Chemicals and drugs

Aspirin, Paracetamol, Diclofenac, Petroleum ether, Ethyl acetate, Ethanol (Research Lab, Mumbai), Carragenan (Hi-Media) and Pentazocine (Ranbaxy, Mumbai).

2.6 *Acute oral toxicity*

Male Wistar rats (190-250g) and Male albino mice (30-40 g) were used to assess the acute toxicity as per OECD guideline No. 425. The aqueous extract of *WC* at dose of 2000 mg/kg was administered.

2.7. Analgesic activity

The analgesic activity of WCAQ, WCEA, WCET extracts were investigated using the following models.

2.7.*1 Acetic acid induced writhing in mice*

The analgesic effect of fruit extract of *W.C* against acetic acid induced writhing in mice was carried out according to the method suggested by (Ezeja et al., 2011). Male albino mice (30-40 g) were divided for the study. The animals were divided into five groups (n=6 in each groups). Group I served as a control, Group II treated with (Aspirin 100 mg/kg, p.o).Whereas Groups III, IV, V were orally administered with WCAQ, WCEA, WCET extracts at dose of 200 mg/kg, p.o each. Thirty minutes after treatment, mice were injected with 0.1 ml of 0.6 % acetic acid solution to induce the characteristic writhing. After 5 min, the mice were placed in an observation box and the number of writhes in a 20 m period was observed.

2.7.2 Eddy's hot plate test

The analgesic effect of *Withania coagulans* was studied in mice using hot plate method described by (Nikajoo, 2009). Male albino mice (30-40 g) and divided into five groups (n=6 in each groups). Group I served as a control. Group II (Pentazocine 10 mg/kg, s.c) served as standard drug and groups III, IV, V were treated with extracts of WCAQ, WCEA, WCET at dose of (200 mg/kg, p.o). Reaction time was noted down in hot plate maintained at 55±2^{0}C at 0, 60, 120, m after treatment. The basal reaction time was taken by observing hind paw licking or jumping response while placed on hot plate. The reaction time was observed with a maximum cut off time of 10 s to avoid damage to the paws.

2.8 Carrageenan induced paw edema

Anti-inflammatory effect of *WC* was studied in wistar rats using carragenan induced paw edema method by (Singh et al., 2008). Animals were fasted overnight prior to start of the experiment and water was allowed *ad libitum.* A mark was made on hind paw, then every time rats paw was inserted in the column up to fixed mark to ensure constant paw volume. The Wistar rats of either sex weighing (200-250 g) were divided into five groups (n=6 in each groups). Acute inflammation was produced by the sub plantar administration of 0.1 ml of 1% carragenan in the left hind paw of the rats. The Group I served as a control, Group II served as standard drug (Diclofenac 25 mg/ kg, p.o). Groups III, IV, V were treated with WCAQ, WCEA, WCET and WCPE extracts at dose of (200 mg /kg, p.o) respectively. The paw volume was noted at 0h, 1h, 2h, 3h, and 4h after carragenan injection. Variation in paw volume was noted at different time interval as mentioned earlier.

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2.9. Anti-pyretic activity

The anti- pyretic effects of extract of *WC* was studied in wistar rats using induction of pyrexia method by (Adesokan et al., 2008). The Wistar rats of either sex weighing (200-250 g) and divided into five groups (n=6 in each groups). The Group I served as a control, Groups II served as standard drug (Paracetamol 10 mg/ kg, p.o). Groups III, IV, V WCAQ, WCEA, WCET extracts at dose of (200 mg /kg, p.o) respectively.Cow's milk was purchased from local market and boiled and cooled to equilibrate room temperature and then administered at a dose of (2 ml/kg, i.p). After 30 minutes rectal temperature of rats was recorded by inserting a bulb of digital tele thermometer in the rectum.

2.10. Statistical analysis

All the results were expressed as mean ± standard error of means (S.E.M.).

The data was statistically analyzed by using two-way analysis of variance followed by Bonferonni's posthoc-test and by using one-way analysis of variance followed by Dunnett's test using Graph Pad Prism Version-5.0 software.

III. Result

3.1 Acute oral toxicity test:-

Oral administrations of extracts of WC (2000 mg/kg) are not associated with any toxic symptoms with 48 hrs. No animal death was observed after treatment. This indicates safety of extracts up to 2000 mg/kg.

3.2 Analgesic activity

3.2.1. Acetic acid induced writhing test

After administration of acetic acid (10 ml/kg) animals in control groups shows 65 writhing which was reduced significantly (P < 0.001) with all treatments.

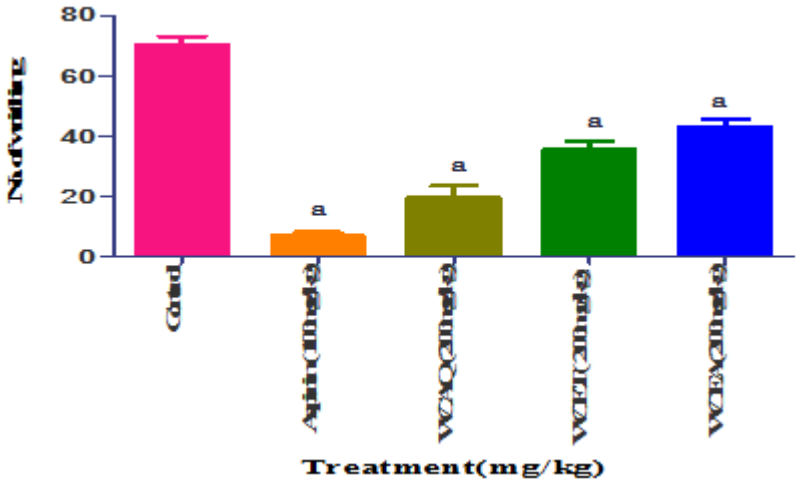

Fig 1: Effect on acetic acid induced writhing in mice. Data expressed as Mean ± S.E.M one way ANOVA followed by Dunnett test a: P<0.001 as compared to control. Values in parentheses indicated the dose in mg/kg (n=6).

3.2.2. Eddy's hot plate method

The analgesic effect of *W*C fruit was done using hot plate test in mice. Oral administration of either standard Pentazocine (10 mg/kg s.c) of *W.C* extract WCAQ, WCET, (200 mg/kg, p.o) significantly attenuated the hot plate thermal stimulation from 30 m onwards till 120 m. (P<0.001) then compared to control at different time intervals.

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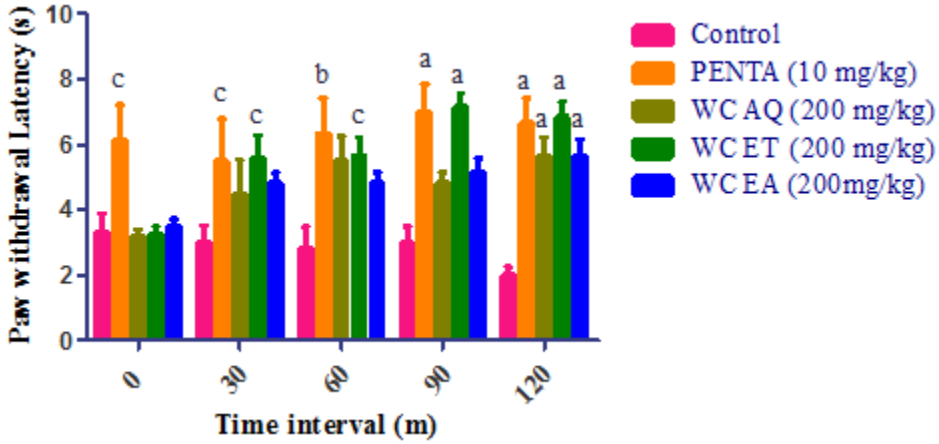

Fig 2: Effect of fruit extracts of *WC* on analgesia using Eddy's hot plate. Data expressed as Mean ± S.E.M Two way ANOVA followed by Bonferroni post test. a: P< 0.001, b: P<0.01 and c: P<0.05. Values in parentheses indicate the dose in mg/kg. (n=6).

3.3 Anti-inflammatory activity

3.3.1 Carragenan induced rat paw edema

After 30 m notes of carragenan administration (0.1 ml of 1% suspension).

Standard and *WC* extracts was administered and the paw volume was observed at different time intervals. It was formed that significant of paw volume after administration of WCAQ, WCET (200 mg/kg) and standard drug Diclofenac 25 mg/kg (P<0.001) was observed.

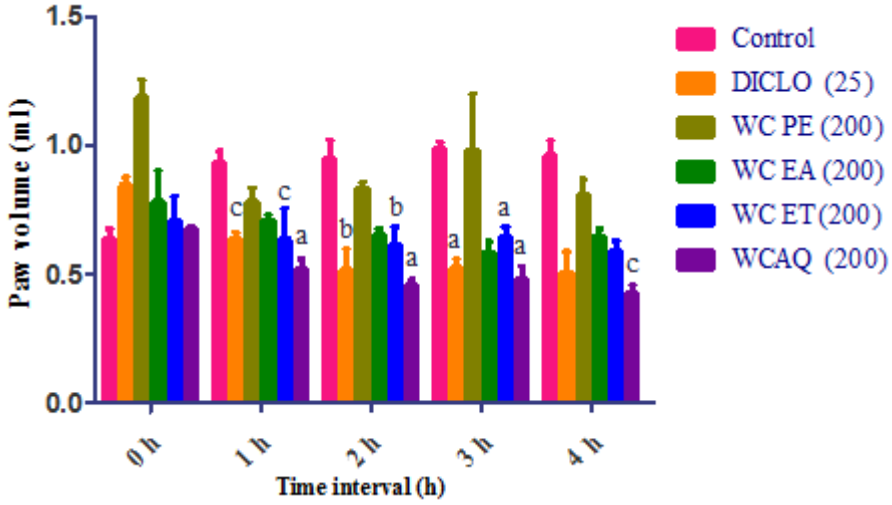

Fig 3: Effect of fruit extracts of *WC* on Carragenan induced inflammation in rats. Data expressed as mean ± S.E.M. Two way ANOVA followed by Bonferroni post test. a: P<0.001, b: P<0.01 and c: P<0.05. Values in parentheses indicate the dose in mg/kg (n=6).

3.4 Antipyretic activity

3.4.1 Effect on pyrexia

No significant decrease in body temperature was observed in any treatment group.

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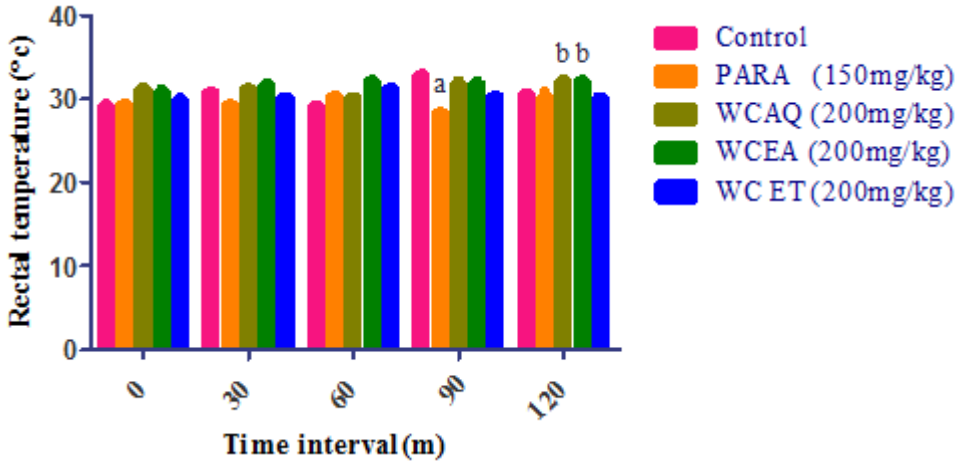

Fig 4: Effect on milk induced pyrexia. Data expressed as mean ± S.E.M Two way ANOVA followed by Bonferroni post test. a: P< 0.05 and b: P<0.01. Values in parentheses indicate the dose in mg/kg (n=6).

IV. Discussion

Acetic acid-induced writhing (abdominal constriction) in the mice is globally used model, for the study of peripheral analgesic action. In Acetic acid-induced writhing model, abdominal contraction are observed due to acute inflammatory reaction with production of prostaglandins E_2 and F_2 in the peritoneal fluid (Vyas S et al., 2008). In our finding, treatment of Withania coagulans extract is antagonized. Acetic acid-induced writhing may be due to suppression of prostaglandins E_2 and F_2. In the central pain model i.e. Hot plate induced analgesia, analgesia is caused by direct activation of nociceptors, centrally acting drugs like morphine and other opioids class of drugs can act by inhibiting nociceptors (Placios-Espinosa et al., 2008). In case of acetic acid-induced abdominal contraction, all the extracts of W.C i.e. AQ, EA and ET shows significant ($p<0.001$) decrease in abdominal contraction and in central pain model i.e. Eddy's hot plate, it is found that animals treated with WCAQ and WCET shows significant ($p<0.001$) increases in reaction time, it indicates that W.C extracts shows analgesic and anti-inflammatory effect in dose dependent manner and these effects may be due to mediation of opioid receptor and inhibition of inflammatory reaction respectively.

Anti-inflammatory activity of W.C is evaluated by the carragenan–induced paw edema model. This model is also used globally to screen the drugs having anti-inflammatory potential.

The inflammatory mediator such as histamine, serotonin and kinins are released in first phase. The prostaglandins are released in second phase and then slow reacting substance are released after 3h (Chakra borty et al., 2004), (Vinegar et al., 1969), (TianYQ et al., 2011, Wang D et al., 2010).

WCAQ, WCET shows significant ($p<0.001$) reduction in paw withdrawal latency from 1 to 3 h, WCPE and WCEA does not shows anti-inflammatory activity during the time interval. Withanolides which is present in the W.C is reported for the COX-2 enzyme inhibition (Nithya et al., 2010). Hence the analgesic and anti-inflammatory activity of W.C may be due to COX-2 enzyme inhibition and presence of steroids. Thus the present study shows that W.C extract i.e. AQ and ET possess anti-inflammatory, peripheral and central analgesic action and these finding supports the traditional use of withania coagulans seed.

V. Conclusion

The analgesic. Antipyretic, anti-inflammatory activity obtained support the traditional use of *W.C* for pain and inflammation.

Based on the results on various animal models, it can be concluded that various extracts of *W.C* could be a potent analgesic, moderate anti-inflammatory and posses no anti-pyretic activity.

Further studies for the involvement of actual phytoconstituent for its analgesic and anti-inflammatory are necessary to explore.

VI. SUMMARY

The analgesic. Antipyretic, anti-inflammatory effects of *Withania coagulans* was studied in animals. In analgesic effect that extract was found to be more effective in acetic acid induce writhing but not in hot plate which clearly indicates its peripheral action. The extracts of WC were found to be effective in inflammation induced by carragenan. No decrease in rectal temperature was observed in animals treated with WC. Based on the result obtained we can conclude that WC could be an alternative treatment for pain occurring due to varied reasons.

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