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Evaluation of analgesic, antipyretic and ulcerogenic effect of Withaferin A

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Abstract
Withania somnifera, popularly known as Ashwagandha is widely considered to be an integral part of Ayurvedic and Indigenous medical systems for over centuries for the treatment of various ailments. Withanolides (steroid lactone), are the major active constituents present in the roots and leaves of *Withania somnifera*. In the present study, withaferin A (active component of *Withania somnifera*), a steroid lactone was examined for its analgesic, antipyretic and ulcerogenic properties employing different experimental models in mice. For comparison purpose, non steroidal anti-inflammatory drug indomethacin was used as standard. The analgesic activity was measured using the acetic acid induced-abdominal constriction and hot plate tests. The antipyretic and ulcerogenic effects were assessed using the yeast-induced pyrexia test and gastric ulceration respectively. It was found that withaferin A (20/30mg/kg b.wt. i.p.) at both the doses produced significant analgesic and antipyretic effect in comparison to standard drug indomethacin. In addition, withaferin A (30/40mg kg.b.wt. p.o.) fed animals showed absence of gastric damage at different dose levels after 16 hrs fasting, whereas indomethacin (20mg/kg/b.wt. p.o) administered mice produced remarkable gastric ulceration. The results clearly indicate that withaferin A possesses potent analgesic and antipyretic properties without causing any gastric damage. supporting previous claims of its anti-inflammatory effect. It might be a useful contribution to highlight the mechanism of action of *Withania somnifera* as an arthritic drug. However, there is a need for further studies in order to confirm these results with more details.

Keywords: *Withania somnifera*; withaferin A; analgesic; antipyretic; ulcerogenic; indomethacin.

INTRODUCTION

Withania somnifera also designated as Ashwagandha, Indian Ginseng and Winter Cherry has been considered to be an integral part of Ayurvedic and Indigenous medical systems for over 3000 years (Andallu and Radhika, 2000). Withanolides, the active constituents of *Withania somnifera* Dunal (Solanaceae) are a group of pharmacologically active compounds present in roots and leaves. The chemistry of withanolides has been well studied and they are basically steroidal lactones. Withanolides are similar to ginsenosides (the active constituents of Panax ginseng) in structure and activity (Grandhi *et al.*, 1994). Withaferin-A (steroidal lactone), one of the best studied withanolide found in the leaves and roots of *Withania somnifera* L. Dunal (family-Solanaceae) has been reported to be inhibitor of angiogenesis, protective in certain types of cancers with strong antioxidant properties (Ali and Shuaib, 1997).

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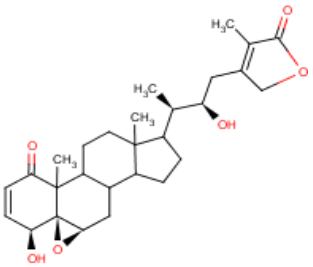

Figure 1: Structure of withaferin A (4β, 27-dihydroxy-1-oxo 5β, 6β, epoxy with a 2-24 dienolide) .

Moreover, in our earlier study, *Withania somnifera* root powder and its active principle withaferin A (Fig. 1) have been tested and reported to have anti-

inflammatory effect on monosodium urate crystal-induced inflammation (Rasool and Varalakshmi, 2006; Sabina *et al*., 2008). Recent studies also reported that withaferin A has anti-inflammatory effect by inhibiting the proinflammatory gene expression (Singh *et al.*, 2007). The present study aims at identifying the analgesic, antipyretic and ulcerogenic properties of withaferin A at different doses in standard mice models. The non steroidal anti-inflammatory drug indomethacin was used for comparison purpose.

MATERIALS AND METHODS

Animals

The study was performed with cross breed Swiss Albino mice, of either sex, weighing 25-30 gms. Animals were purchased from S.L.R.T.C., Karigiri, Vellore, India. Mice were acclimatized for a week in temperature controlled room with a 12 hour dark-light cycle and fed commercially available pelleted feed (Hindustan Lever Ltd., Mumbai, India) and water *ad libitium.*

Test drug

The commercially available Withaferin A (a fine white powder, >95% purity by HPLC, mol.wt. 470.61) was purchased from Natural Remedies Ltd., Bangalore, India and stored at -20^{o}C. Indomethacin was purchased from Tamil Nadu Dadha Pharmaceuticals Ltd., Chennai, India. A homogenous suspension of Withaferin A and indomethacin were made with 0.5% carboxy methyl cellulose in phosphate buffered saline. Fresh solution was prepared before each experiment. All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

Dosage

Based on our preliminary studies with different dosages of Withaferin A, it was found that 30mg/kg/b.wt produced significant anti-inflammatory effect by reducing paw swelling in monosodium crystal-induced inflammation (experimental model for gouty arthritis) in mice (Sabina *et al.*, 2008) and up to 50mg/kg b.wt, no mortality was observed in mice during our examination period. But further increase in dosages show reduction in survival of mice (LD50- around 80mg/kg/b.wt). Hence, 20/30mg/kg/b.wt was considered for analgesic and antipyretic studies and higher dosages 30/40 mg/b.wt for ulcerogenic effect.

Analgesic test: Acetic acid-induced writhing response in mice

This test was performed using the method described by Witkin *et al*. (1961). The muscular contractions were induced in mice by intra peritoneal injection of 0.6% solution of acetic acid (10 ml/kg b.wt.). Immediately after administration of acetic acid, the animals were placed in glass cages and the number of "stretchings" per animal was recorded during the following 30 minutes period. A significant reduction in the number of writhings by any treatment as compared to control animals was considered as a positive analgesic response. Withaferin A (20/30 mg/kg b.wt. i.p.) and indomethacin (10 mg/kg b.wt. i.p.), suspended with 0.5% carboxy methyl cellulose in phosphate buffered saline, were administered intra-peritoneally 30min before the acetic acid injection.

Hot-plate reaction time in mice

In order to check the temperature withstanding power of the animal, the hot plate reaction time was tested by the method of Williamson *et al*. (1996). Mice were placed individually in a glass beaker, placed on a thermostatically controlled hot plate maintained at 55°C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The time taken for the mice to react in this fashion was obtained using a stopwatch. The animals were first tested for the paw-lick or jump response and only those that reacted after 4sec were used for the experiment. Mice were tested in groups of six per dose, 30min after Withaferin A (20/30mg/kg b.wt, i.p) or indomethacin (10mg/kg b.wt, i.p). Control animals received equal volume of normal saline and the experiment was repeated.

Antipyretic test: Yeast induced pyrexia

The mice were injected subcutaneously with 10ml/kg of 20% aqueous suspension of bakers yeast and the rectal temperatures were recorded initially and at 18h. Withaferin A (20/30mg/kg b.wt) and indomethacin (10mg/kg b.wt) were administered intra-peritoneally after the 18hrs reading. When the increase of temperature was at its peak, it was measured at hourly intervals up to 5h after administration of test drugs as per the method of Mukerjee et *al*. (1996).

Ulcerogenic test: gastric ulceration

Animals were kept fasting for 16h and the test compounds were then administered orally. Withaferin A was administered at dose levels of 30/40mg/kg b.wt. and indomethacin at a dose of 20mg/kg b.wt separately to check the ulcerogenic activity. Animals were killed 3h after the administration of the drugs and the stomachs were removed, cut along the lesser curvature and the gastric mucosa was washed with normal saline and scored according to the scale, 0: no lesion, 0.5: hyperemia, 1: one or two lesions, 2: severe lesions, 3: very severe lesions, 4: mucosa full of lesions (Cashin *et al*., 1977).

Statistical analysis

Results were expressed as mean±S.D and statistical analysis was performed using ANOVA to determine significant differences between groups, followed by Student's Newman-Keul's test. P<0.05 implied significance.

RESULTS

Analgesic test

In the acetic acid induced writhing method, Withaferin A treatment similar to indomethacin, produced a significant reduction in the number of abdominal constrictions in mice. This reduction was dose related and found to be maximum with 30mg/kg. b.wt. (Fig. 2). In the hot plate method, as shown in Fig. 3, the Withaferin A treatment increased the reaction time and showed more significant analgesic activity at 30mg/kg. b.wt.

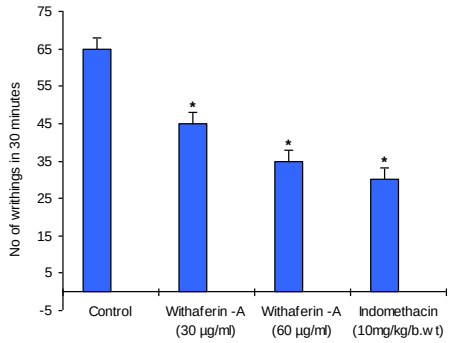

Figure 2: Effect of Withaferin A and indomethacin on acetic – induced writhing response in mice.
Comparisons are made with control groups. Values are expressed as mean± S.D. (n=6). Symbols represent statistical significance at: * p<0.05.

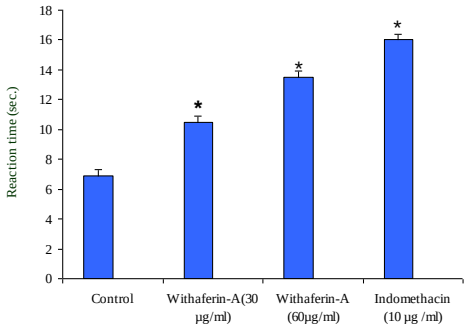

Figure 3: Effect of Withaferin A and indomethacin on hot plate reaction time in mice
Comparisons are made with control groups. Values are expressed as mean± S.D. (n=6). Symbols represent statistical significance at: * p<0.05.

Antipyretic test

Administration of Brewer's yeast to rats produced significant increase in rectal temperature of mice 18 hr. after injection (Fig. 4) (p<0.05). Withaferin A at the dose of 30mg/kg/b.wt caused a more significant reduction in reaction in rectal temperature.

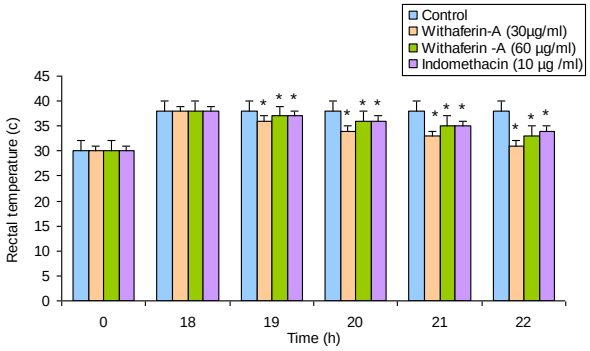

Figure 4: Effect of Withaferin A and indomethacin on yeast-induced pyrexia in mice
Comparisons are made with control groups. Values are expressed as mean± S.D. (n=6). Symbols represent statistical significance at: * p<0.05.

Ulcerogenic test

Mice administered with Withaferin A at both the dose levels (30/40mg/kg.b.wt) were found to be devoid of significant gastric lesions as compared to the standard anti-inflammatory agent indomethacin (20mg/kg/b.wt) which shows remarkable gastric lesions (Fig. 5)

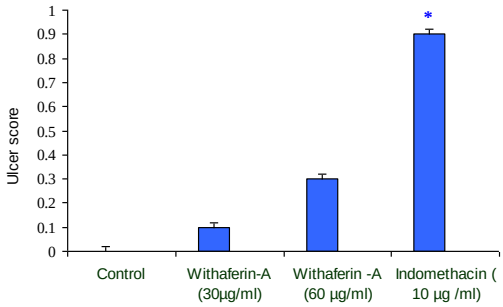

Figure 5: Ulcerogenic action of Withaferin A and indomethacin in mice
Comparisons are made with control groups. Values are expressed as mean± S.D. (n=6). Symbols represent statistical significance at: * p<0.05.

DISCUSSION

A number of anti-inflammatory drugs are available in the markets that are used for treatment for various inflammatory disorders. These drugs generally show analgesic and antipyretic effect that is often associated with gastric damage. Therefore, an attempt is made in the present study to evaluate the analgesic and antipyretic effect of Withaferin A and to explore whether it is accompanied with any significant gastric damage.

The main symptoms of the body against any inflammatory response include pain and increase in temperature. Therefore any anti-inflammatory

compound must essentially possesss analgesic and antipyretic properties (Kasahara *et al.*, 1985). The analgesic effect of Withaferin A was evaluated using two different tests: acetic acid induced writhing response and hot plate reaction time in mice.

The acetic acid induced writhing response is used to screen for both peripheral and centrally acting analgesic activity. The abdominal constrictions observed in this study are because of irritation of peritoneal cavity caused by administration of acetic acid. Acetic acid is supposed to release prostaglandins E2 and F2α in the peritoneal fluid that excite pain nerve endings (Deraedt *et al.*, 1976). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria *et al.*, 2008). Collier *et al.* (1968) suggested that these endogeneous mediators of pain are sensitive to non-steroidal anti-inflammatory drugs and opioids. In the present study, the analgesic action of Withaferin A can be attributed to the blockade of release of the endogeneous mediators of pain i.e. the prostaglandins. It suggests that Withaferin A has some inhibitory action on the cyclooxygenase pathway which is actually involved in the synthesis of prostaglandin biosynthesis.

The hot plate reaction test is used specifically to screen the central nervous system acting analgesic activity of a drug. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity. The opioid agents exert their analgesic effects via supra spinal and spinal receptors (Nemirovsky *et al*., 2001). In the hot plate test, Withaferin A (30/60 mg/kg b.wt.) showed a significant analgesic action 30 min. after its administration (Fig. 3). From the results it can be inferred that Withaferin A showed a significant analgesic effect in both hot plate reaction test and acetic acid writhing response. Analgesic effect of Withaferin A in both models confirms that it has been acting through both peripheral and central mechanism.

Antipyretic activity is a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis (Vane, 1987). The yeast induced pyrexia test in mice was done to investigate the anti-pyretic effect of Withaferin A. It is well known that pyretic activity involves stimulation of the region in the hypothalamus that controls body temperature; via prostanglandins synthesized within the central nervous sytem and that the blood-brain barrier prevents drug molecules or other chemicals from entering the central nervous system (Zakaria *et. al.*, 2008). Several investigators have used this method to record pyrexia 15 to 18 hrs, after yeast injection, and then administered the antipyretic drugs to be studied (Asha and Pushpagandhan, 1999). It was found that Withaferin A caused a significant reduction in the rectal pyrexia similar to the standard drug Indomethacin (Fig. 4). This result seems to support the view that Withaferin A has some inhibitory effect on prostaglandin biosynthesis because prostaglandin is believed to be involved in regulation of body temperature.

Production of gastric lesions is one of the signifcant side effects of non-steroidal anti-inflammatory drugs (Pagella *et al.*, 1983). The ulcerogenic action of indomethacin especially in an empty stomach has already been established (Rasool *et al.*, 2008). Indomethacin-induced ulceration mostly affects the glandular (mucosal) part of the stomach (Nwafor *et al*., 1996). Although the underlying etiologic mechanisms of indomethacin-induced gastric mucosal lesions are still unclear, but indomethacin is known to inhibit the cyclooxygenase enzyme (COX) responsible for the production of prostaglandins, involved in general house keeping activities, e.g. maintainence of gastric mucosal integrity (Rasool and Varalakshmi, 2006). Inhibition of COX-1 enzyme may result in the formation of ulcers in many human and hence the selective inhibition of COX-2 enzyme by compounds has a major advantage over non-selective non-steroidal anti-inflammatory drugs (Smith *et al*., 2000). In the present study it was found that Withaferin A has produced significantly less number of gastric lesions as compared to the standard drug indomethacin (Fig. 5). This suggests that the Withaferin A is devoid of any ulcerogenic potential.

CONCLUSION

The result of the present study clearly indicates that Withaferin A has remarkable analgesic and antipyretic properties accompanied with absence of significant gastric damage, supporting previous claims of its anti-inflammatory effect (Sabina *et al.*, 2008). It might be a useful contribution to highlight the mechanism of action of *Withania somnifera* as an arthritic drug, but there is a need for further studies in order to confirm these results with more details.

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