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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY BY METHANOL EXTRACT OF *MUCUNA PRURIENS* (L.) DC

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ABSTRACT

Mucuna pruriens belongs to family *Fabaceae* is covered with fuzzy hair and used as vermifuge but when plant is older, hair are completely removed. Loose orange hairs surrounded the seedpods. When seedpods come in contact with skin it produces severe itching. In the current study, using the Carrageenen–induced rodent's paw edema model, performed experiments. The results showed the maximum effect at the dose of 500mg/kg in comparison with control and standard drug. Analgesia is measured by latency to withdraw the mice tail clearly out of water (in seconds) before and after administration of extract (300, 500 and 1000 mg/kg-orally) and compare with standard drug Acetylsalicylic acid (300 mg/kg-orally). In acetic acid induced writhing test extract and Acetylsalicylic acid were given before the administration of acetic acid (i.p) and count the number of writhes. The main purpose of this work is to conclude that the extract of *Mucuna pruriens* exhibited significant analgesic and anti-inflammatory activities and beneficially use as an analgesic and anti-inflammatory agent for the relief of pain and edema.

INTRODUCTION

Mucuna pruriens (L.) DC or velvet bean family *Fabaceae* (Ekanem *et al*., 2004). It has various medicinal activities in all parts of plant (Adepoju & Odubena, 2009). Reddish-orange hair are surrounded the seed pods. When seedpods come in contact with skin it produces severe itching. Mucunain and serotonin are responsible the itching.

Oval, shiny black or brown seeds of *Mucuna pruriens* that contain L–DOPA, used in Parkinson 'disease (Lieu *et al*., 2010) with high protein and energy contents (Pugalenthi *et al*., 2006) and tryptamine alkaloids mucanine, prurienidine and mucananine (Misra & Wagner, 2005) fat and fatty acids, protein (globulins, lectins), water, fiber and minerals as well.

Plant hairs used as vermifuge but when plant is older, hair are completely removed (Parakash *et al*., 2001). It has anti-oxidative and anti-microbial (Rajeshwar *et al*., 2005b), anti-neoplastic (Rajeshwar *et al*., 2005a), aphrodisiac (Upadhyay and Butiyan, 2000), anti-diabetic (Majekodunmi *et al*., 2011), anti-fungal and anthelmintic activities (Devi & Kumar, 2011).

It reduces the blood sugar (Rathi *et al*., 2002) and increases the ovulation in women, sperm count and testosterone level in the body (Upadhyay Butiyan, 2000). *Mucuna pruriens* neutralizes the toxic effect of snakebite (Meenatchisundaram & Michael, 2010) and restores antioxidant level by reducing the lipid peroxidation (Shukla *et al*., 2007). The protective effect of plant seed extract against snake venom -induced cardiovascular depressant has been demonstrated (Guerranti *et al*., 2002). It also reported that *Mucuna pruriens* decreases the pain sensation (Shad *et al*., 2008). Removal of pain stimulus resolves unpleasant pain sensation (Raj, 2007). The objective of the present study is to investigate the pain and edema reducing activity of methanol extract of *Mucuna pruriens*.

MATERIALS AND METHODS

Extraction procedure: The seed of *Mucuna pruriens* were kept under shade for drying and soaked in methanol for a period of ten days. The methanol was filtered and a thick gummy mass was then evaporated under low pressure and dried, using a rotary evaporator and concentrated mass was stored in an air tight vial for the screening of anti-inflammatory and analgesic activities (Chowdhury *et al*., 2004).

Animals: Albino rats (160-200 g) and albino mice (25 to 30 g) of both sex were used and they were maintained under controlled conditions in five groups (n = 7) at 22±1^{o}C with light / dark cycle of 12 hours (Light on from 07.00 a.m to 07.00 p.m) at the Department of Pharmacology, University of Karachi and had access to water and food.

Materials: *Mucuna pruriens* seed extract (300, 500 and 1000 mg/kg-orally) as a treated drug and acetyl salicylic acid (300 mg/kg-orally) as a reference drug (Singh *et al*., 2010) were administered according to the body weight for anti-inflammatory and analgesic activities (Bhutia *et al*., 2010). 0.1 ml of 1% carrageenen (Hajhashemi *et al*., 2003) and acetic acid (10 ml/kg i.p) were used (Akuodor *et al*., 2011) for the induction of edema and writhing respectively.

Carrageenen-induced hind paw edema method: The animals were pretreated with *Mucuna pruriens* (300, 500, 1000 mg/kg orally), 30 minutes before administration of carrageenen (0.1 ml of 1%). Carrageenen was administered into the sub plantar aponeurosis of right hind paw of each rat through a needle. Edema of carrageenen -injected foot in term of ml was measured using plethysmometer (UGO Basile- Italy) and then note the readings at 1, 2, 3, 4 and 5 hours after carrageenen injection. The animals received the acetyl salicylic acid (300 mg orally) as a standard drug (Winter *et al*., 1962).

Tail Flick method: The mice were treated with *Mucuna pruriens* (300, 500, 1000 mg/kg orally) and acetyl salicylic acid (300mg orally) after 30 minutes of tail immersion. The animals received acetyl salicylic acid as a reference drug (Hemamalini *et al*., 2010). The lower 2-3 cm of mice tail was immersed in 51°C hot water. The time taken by the mice to flick the tail from hot water was noted and determined tail flick latency difference (TFLD). Repeat the procedure at 30, 60, 90, 120, 150, 180 and 210 minutes of interval. Saline (0.9% NaCl) was used for control (Luiz *et al*., 1988: Hajhashemi *et al*., 2002).

Writhing test: The mice were pretreated with *Mucuna pruriens* (300, 500, 1000 mg/kg-orally) and acetyl salicylic acid 30 minutes before the administration of acetic acid (10 ml/kg-i.p) (Koster *et al*., 1959: Saleem *et al*., 2011). The animals received the acetyl salicylic acid (300mg-orally) as a standard drug. Each animal was placed in a plastic cage and count the no. of writhes for twenty minutes after administration of acetic acid and calculates the percent inhibition of number of writhes.

$$\text{Inhibition (\%)} = \frac{Vc - Vt}{Vc}$$

Vc = Mean value of number of writhes for control animals
Vt = Mean value of number of writhes for treated animals

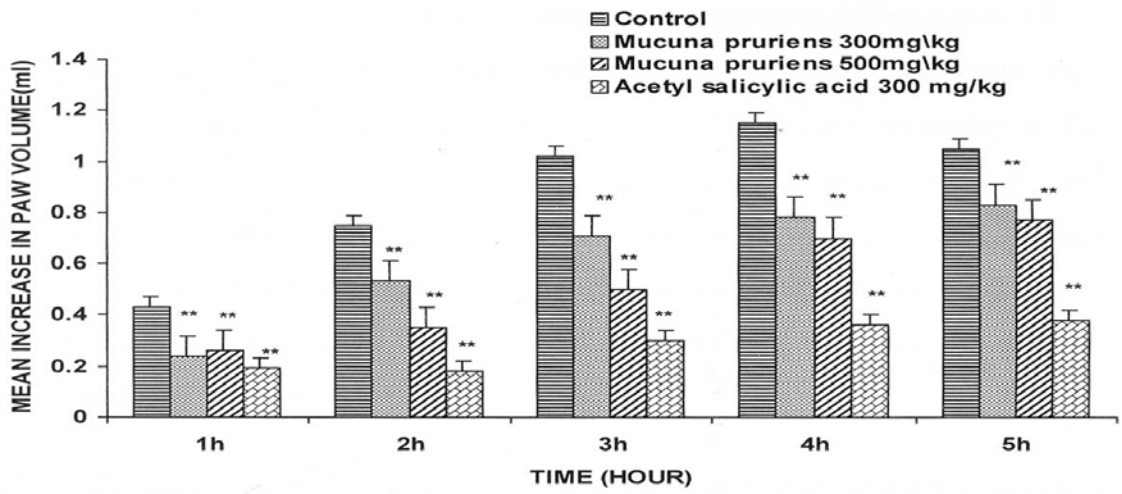
Analysis of data: All results were given as average value ± standard deviation (st.dev). The significance of difference between averages was determined (Newman, 1939: Keuls, 1952) and the data obtained from the present study was analyzed for p-value at <0.05 and <0.005 following the one way ANOVA.

Results

The anti-inflammatory effects of methanol extract of *Mucuna pruriens* on carrageenen-induced paw edema in rats are given in Fig. 1. The result showed that the plant extract possess significant anti-inflammatory activity. *Mucuna pruriens* is highly significant at 300mg/kg with percentage inhibition of edema was 44.18% at 1hour, 29.3% at 2hour, 30.3% at 3hour, 32.1% at 4hour and 20.9% at 5hour. At the dose of 500mg/kg, percentage inhibition of edema was 39.5% at 1hour, 53.3% at 2hour, 50.9% at 3hour, 36.5% at 4hour and 26.6% at 5hour. The standard drug acetyl salicylic acid produced 55.8% at 1hour, 76.0% at 2hour, 70.5% at 3hour, 68.6% at 4hour and 63.8% at 5hour. The results of different doses of *Mucuna pruriens* extracts and standard drug acetyl salicylic acid are shown in Fig. 1.

Figure 2 showed the result of *Mucuna pruriens* at 300 mg/kg, which was highly significant at 30 to 180 minutes (tail immersed to 8.0 seconds in water bath at 51^{o}C) as compared to Acetyl salicylic acid, which is highly significant at 30 to 210 minutes (tail immersed to 7.0 seconds in water bath at 51^{o}C). The result of the plant extract at 500 mg/kg was highly significant at 30 to 180 minutes as compared to Acetyl salicylic acid, which showed significant results at 30 to 210 minutes. Similarly the plant extract at 1000 mg/kg produced highly significant increase in latency time at 90 to180 minutes. Acetyl salicylic acid also showed highly significant result at similar time interval.

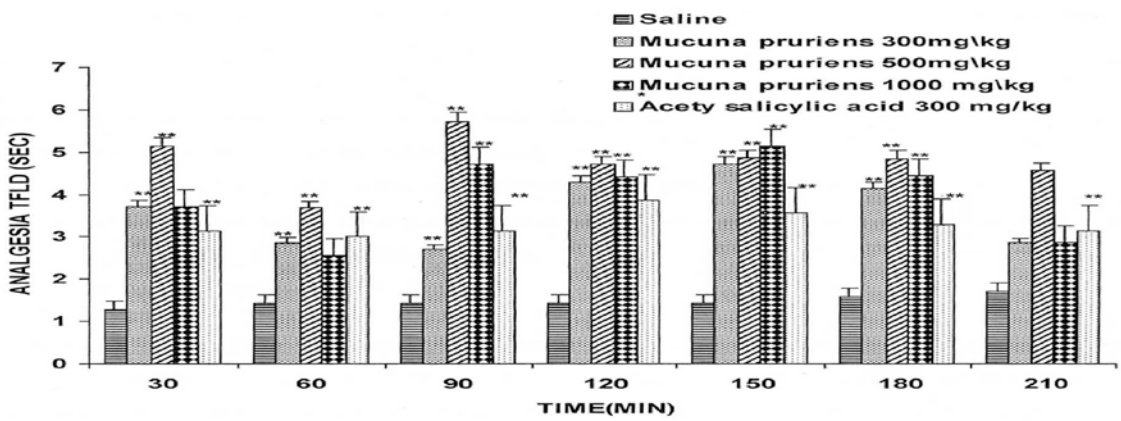
In the acetic acid induced writhing test both Acetyl salicylic acid and various doses of the plant extract showed significant dose related inhibition of number of writhes. In the control animals the number of writhes induced by acetic acid was 16.0 ± 0.32. Acetyl salicylic acid at the dose of 300, 500 and 1000 mg/kg reduced the mean number of writhes to 9.0 ± 0.31, 7.0 ± 0.25 and 6.0 ± 0.25 respectively with percent inhibition of 43.7, 56.2 and 62.5% in Figure 3. The administration of *Mucuna pruriens* (300, 500 and 1000 mg/kg) induced a significant dose dependent decreased in mean number of writhes. The maximum activity of the *Mucuna pruriens* was observed at 1000 mg/kg in which number of writhes reduced from 16.0 ± 0.32 to 6.0 ± 0.27 with percent inhibition of 62.5%.



n = 7

**p <0.01 as compared to standard.

Fig. 1. Anti-inflammatory effect of *Mucuna pruriens* on carrageenan induced rat paw edema.

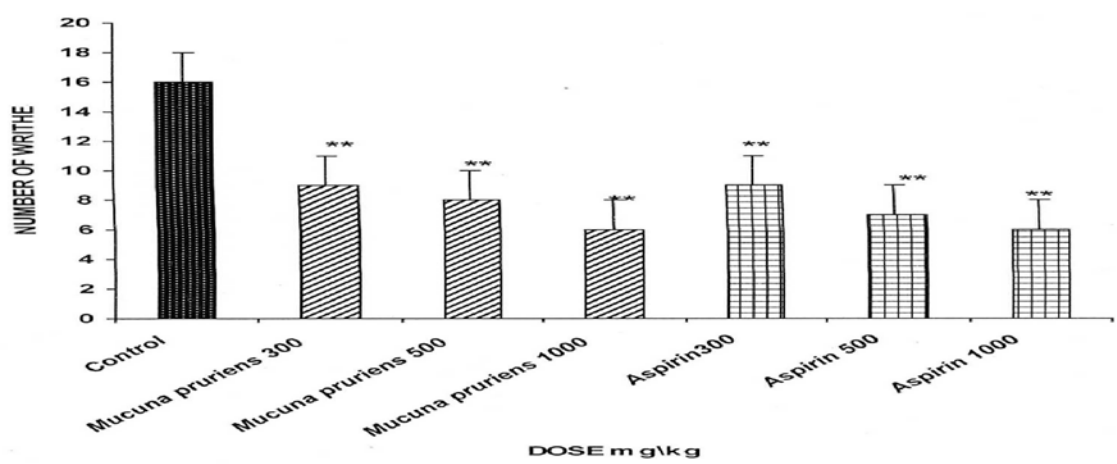


n = 7

*p < 0.05 as compared to standard.

**p <0.005 as compared to standard.

Fig. 2. Analgesic effect of *Mucuna prufiens* and aspirin in mice tail flick method.



n=7

**p< 0.005 as compared to standard.

Fig. 3. Analgesic effect of *Mucuna prufiens* and aspirin in acetic acid induced writhing test in mice.

Discussion

Mucuna pruriens seed extract involved reducing the inflammation (Rajeshwar *et al*., 2005c) and current study also showed significant inhibitory effect against carrageenen-induced rat paw edema. Other herbs include ginger (Jia Yong-Liang *et al*., 2011), licorice, turmeric, pokeroot and saffron (Hosseinzadeh & Younesi, 2002) are also reduce inflammation (Spellman, 2006). Many anti-inflammatory chemicals are also derived from marine sources including the omega-3 essential oils (Tull *et al*., 2009). Other plant chemicals, belong to plant chemical family bioflavonoid, that are known to reduce edema include carotenoids and catechins (Proefrock, 2003).

Edema is mediated by the release of bradykinin, serotonin and histamine after administration of carrageenen at 1st hour and then release of prostaglandin. This method has been used to determine the effect of NSAIDs that inhibit the cyclo-oxygenase enzyme that is involved in the prostaglandins synthesis and Acetylsalicylic acid used for anti-inflammatory activity (Subramanian *et al*., 2008). It may be possible that the *Mucuna pruriens* has any anti-inflammatory chemical compound. Further study is required to establish the mechanism and identify the anti-inflammatory components.

Use of herbal drugs is most popular in the under developed countries. Herbal medicines have pharmacological potential against most of the diseases such as *Hygrophila spinosa*, *Gmelina asiatica* and *Curcuma longa* etc are known analgesic herbal drugs and *Mucuna pruriens* was selected because of its therapeutic values (Katzenschlager *et al*., 2004) and, opioids and salicylates have well known adverse and side effects (Ami *et al*., 2011). *Mucuna pruriens* decreases the pain sensation (Lauk *et al*., 1939). Plant seed extract may act like acetyl salicylic acid brings its effect via inhibition of the enzyme cyclo–oxygenase and hence decreases prostaglandins synthesis which leads to inhibition of pain and inflammation. Enzyme cyclo–oxygenase is involved in the complex chain reactions resulting the synthesis of prostaglandins by the conversion of arachidonic acid that is the precursor of prostaglandins. Cyclo–oxygenase has two isozymes COX_1 and COX_2 in which COX_2 plays an important role for the synthesis of prostaglandins in inflammatory cells.

Mucuna pruriens was produced significant analgesia by acetic acid induced writhing. The effect of the investigated plant seed extract reduces the number of writhes, suggesting its peripheral action of analgesia. In this study acetic acid was selected because it has similar pain responses as human clinical pain. Prostaglandins E_2 (PGE_2) and prostaglandins F_2-Alpha ($PGF_{2\alpha}$) in peritoneal mass are produced by acetic acid when administered intraperitonialy resulting local inflammation (Shaikh *et al*., 2012).

Herbal drugs study with potent anti-inflammatory and analgesic activities are necessary because of their lesser adverse and toxic effects (Fayyaz *et al.,* 1992) but modern pharmaceutical drugs are mostly narcotics and non – narcotics with severe side effects (Ami *et al*., 2011).

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