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Investigation of Anti-nociceptive Activity of Neem (*Azadirachta indica*) *A. Juss* on Acetic Acid Induced Writhing in Rats

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Abstract:

Background: Plants represent the principal means of therapy in traditional medicine and the plant kingdom has long served as a prolific source of useful drugs.

Objective: This study was undertaken to investigate the anti-nociceptive activity of *Azadirachta indica A.juss* leaf extract in experimental animals.

Methods: Three doses of methanolic extract of *Azadirachta indica* leaf (100, 200, and 400 mg/kg) were administered intraperitoneally (i.p.) to investigate their potential anti-nociceptive activity using acetic acid induced writhing in rats compared to morphine and diclofenac sodium as standard drugs.

Results: The methanolic extract of *indica* at a dose of 400 mg/kg produced 72.01% protection against writhing induced by acetic acid. This result points to approximately equal protection exhibited by 25mg/kg diclofenac sodium. Co-administration of *indica* (400 mg/kg) and diclofenac (25 mg/kg) produced 100% protection as diclofenac sodium (50mg/kg) and morphine (2.5, 5 mg/kg).

Conclusion and recommendation: On the basis of results obtained, the use of *indica* leaf extract as antinociceptive seems to be promising. Bioassay guided fractionation of the methanolic extract of *indica* leaf is recommended for safety and efficacious use. Further work on determination of active ingredient(s) and mechanism of action is also needed.

الخلاصة

خلفية: تعتبر النباتات من وسائل العلاج الرئيسية للطب التقليدي وظلت لأمد طويل المصدر الرئيسي لأدوية مفيدة.

الهدف: أُجريت هذه الدراسة لإستقصاء النشاط المسكن للألم لمستخلص أوراق شجرة النيم في الحيوانات المعملية.

الطرق: تم إعطاء ثلاث جرعات من المستخلص (100, 200 و 400 ملجم لكل كيلوجرام) لإستقصاء آثارها المتوقعة لتسكين الألم الناتج من حمض الخليك المسبب للانقباطات البطنية عند الجرذان مقارنة بالأدوية المرجعية مورفين و دايكلوفيناك صوديوم.

النتائج: أحدث المستخلص الميثانولي للنيم عند الجرعة 400 ملجم لكل كيلوجرام 72.01% حماية ضد الإنقباضات البطنية المسببة بواسطة حمض الخليك. أشارت هذه النتيجة إلى حماية مساوية للدايكلوفيناك 25 ملجم لكل كيلوجرام. أدي الإستخدام المشترك لمستخلص النيم (400 ملجم لكل كيلوجرام) ودايكلوفيناك (25 ملجم لكل

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5 ملجم لكل 0و كان ذلك مثل تأثير الجرعة الأعلى للديكلوفيناك (كيلوجرام) الي حماية بنسبة 100%
5 ملجم لكل كيلوجرام). كيلوجرام) والمورفين (2.5 و0للديكلوفيناك)
الإستنتاج والتوصية:
إستناداً على النتائج المتحصلة يبدو أنّ النيم يمكن أن يعتبر مصدر متوقع جديد فعال كمسكن للآلام. نوصي بأن تجرى الإختبارات الحيوية الموجهه للتجزئة للنيم لتحديد المادة أو المواد الفعالة وتوضيح آلية عملها.

Key Words: *Azadirachta indica*, Acetic acid induced writhing, Anti-nociceptive activity.

Introduction:

Azadirachta indica A.juss commonly known as neem is native of India and naturalized in most of tropical and subtropical countries including Sudan. The chemical constituents of many biological activities can be extracted from the plant including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is the most biologically active compound found in *Azadirachta indica*. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and Nimbin [4,5] Substances derived from natural products have been utilized for centuries for various purposes including the treatment of pain. Opium, for example, has been used since the earliest records of time, some 7000 years ago. Not until the 19th century where individual components of different natural product remedies identified and purified. Today, drug discovery has become a complex field far beyond the use of only natural products. However, natural products have dominated the drug industry for many years and several marketed drugs are based on isolates from such natural products. [1]

Pain can be defined as somatic sensation of acute discomfort, a symptom of some physical hurt or disorder, or even emotional distress. It is a crucial aspect of the body's defense mechanism and it is a part of a rapid warning relay instruction the motor neurons of the central nervous system (CNS) to minimize physical harm.[2]

Due to having adverse side effects, like gastric lesions, caused by non-steroidal anti-inflamatory drugs (NSAIDs), tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to world health organization (WHO) still about 80% of the world population rely mainly on plant-based drugs.[3]

Reported biological and pharmacological activities of *indica* include antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, larvicidal, fungicidal, antiulcer, spermicidal, anthelminthic, antidiabetic, immunomodulating, molluscicidal,

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nematicidal and insecticidal [6,7]. This study was undertaken to investigate the anti-nociceptive activity of *indica* leaf extract in experimental animals.

Materials and Methods:

Plant material:

Indica leaves were collected from the garden of the Faculty of Pharmacy, University of Gezira in September 2012. The plant material was identified by the Department of Pharmacognosy, Faculty of Pharmacy - University of Gezira, Sudan.

Preparation of extract:

One hundred grams of coarsely powdered leaves of *indica* were extracted by maceration using pure methanol in a conical flask for 72 hours, filtered and evaporated by a rotary evaporator at 60 °C to greenish semi-solid mass, which was kept in refrigerator until use.

Drugs:

Diclofenac sodium 75mg/5ml (Shanghai, Sudan) and morphine 10mg/ml (Darou Pakhsh, Iran) were used as standard drugs. Acetic acid (Central Drug House (P) LTD, India) was used to induce writhing in rats.

Preparation of working solutions:

Indica methanolic extract dissolved in distilled water was used. The required concentrations of acetic acid (0.6 %), diclofenac sodium (25, 50 mg/kg) and morphine (2.5, 5 mg/kg) were prepared by dilution with distilled water.

Experiments:

In the present study rats (150-200gm) of both sexes were used. Three groups of rats (n=5) received the methanolic extract of *indica* (100, 200 and 400 mg/kg i.p.). For the control, five groups of rats (n=5) were used whereas, group one was untreated (negative control), while the remaining four groups were treated with standard drugs (positive control), two groups received morphine (2.5 and 5 mg/kg i.p.) that known to act centrally and peripherally to relieve pain and the other two groups received diclofenac sodium in doses of (25 and 50 mg/kg i.p.) that known to act only peripherally. A single group of rats (n=5) received a combination of *Azadirachta indica* (400 mg/kg) and diclofenac (25 mg/kg) intraperitoneally. Thirty minutes later each rat received acetic acid (0.6% i.p.) in a dose of 10 ml/kg, which known to induce writhing in rats[8]. Abdominal writhes observed 10 minutes after acetic acid administration for a period of twenty minutes.

Table (1) represents mean number of writhes ± standard deviation (SD) . Statistical difference was analyzed by one way analysis of variance (ANOVA). The combined therapy of neem and diclofenac was analyzed using Student's *t*-test. Multiple comparisons were made using dunnett test and considered significant if P-value < 0.05. Percentage of protection from writhes induced by acetic acid was calculated as followed:

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$$\% \text{ protection} = \frac{MWut - MWt}{MWut} \times 100$$

Where:
MWut = Mean number of writhes of an untreated group (negative control).
MWt = Mean number of writhes of a treated group.

Results and Discussion:

Table (1) represents the total number of writhes induced in rats by acetic acid 10 minutes after administration during an observational period of 20 minutes.

Table 1: Effect of *indica*, morphine and diclofenac on acetic acid induced writhing in rats.

Treatment	Dose (i.p.)	Mean of writhing ±SD	Protection %
Negative control (untreated)	0.00	53.4±25.02	0.00
indica (test)	100 mg/kg	26 ±18.75	51.5
	200 mg/kg	24.6 ±14.59	54.1
	400 mg/kg	15 ±12.43	72.01*
Diclofenac sodium (Standard drug)	50 mg/kg	0 ±0	100**
	25 mg/kg	14.6 ±2.96	72.26*
Morphine (Standard drug)	5 mg/kg	0 ±0	100**
	2.5 mg/kg	0 ±0	100**
Diclofenac sodium+ *indica* (1:16)	25 mg/kg+400 mg/kg	0 ±0	100**

P-value<0.05 * P-value<0.01**

Results showed that, the methanolic extract of *indica* at a dose of 400 mg/kg produced 72.01% protection against writhing induced by acetic acid approximately equal to the effect of diclofenac (25 mg/kg). Experimental control agents, diclofenac in a dose of 50 mg/kg and morphine (2.5 and 5 mg/kg) showed 100% protection. Co-administration of *indica* (400 mg/kg) and diclofenac (25 mg/kg) produced a synergized activity of 100% protection. The anti-nociceptive activity of *indica* leaf extract coincides with

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what was reported by Khanna *et al*, 1995; Khosla *et al*., 2000; Sharma *et al*., 2010; Dinda *et al*., 2011 and Kausik *et al*., 2002. [6,9-12]

Acetic acid induces pain by releasing endogenous substances that excite pain nerve endings centrally and peripherally and the observed abdominal constriction produced by acetic acid is related to the sensitization of nociceptive receptors to prostaglandins. Diclofenac and other non-steroidal anti-inflammatory drugs (NSAIDs) are known to inhibit the number of writhes by inhibiting cyclooxygenase in peripheral tissue, thus interfering with the mechanism of transduction in primary afferent nociceptors by blocking the effect or the synthesis and/or release of inflammatory mediators (prostaglandins). Morphine acts by combining with opioid receptors, which are found in the central nervous system and the peripheral sites.[8]

Conclusion and Recommendation:

The use of *indica* leaf extract seems to be a promising antinociceptive agent. Bioassay guided fractionation for the methanolic extract of *indica* should be investigated for the determination of active ingredient(s), and to elucidate the mode of action.

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