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Farrah R Jaafar
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Saba Jaafar Ajeena
Food Sience Department Agriculure, College Baghdad University, University of Baghdad, Baghdad, Iraq

Saadia Saleh Mehdy
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Kufa, Iraq

Correspondence
Farrah R Jaafar
Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Anti-inflammatory impacts and analgesiac activity of aqueous extract *Datura innoxia* leaves against induced pain and inflammation in mice

Farrah R Jaafar, Saba Jaafar Ajeena and Saadia Saleh Mehdy

Abstract
Two experiments were performed, the first one was to investigate the main phytochemicals contents of *Datura innoxia* (DI) aqueous leaves extract and the second was to investigate the analgesic and anti-inflammatory impact of different doses of such extract against chemically and physically induced pain in mice. The result of experiment one indicated the presence of following phytochemical; flavonoids alkaloids, saponin, phenol, tannins and glycosides.
In experiment two, investigate the analgesic and anti-inflammatory effects of DI aqueous leave extract in four treated groups each of 6 animals that consist of two DI extract treated gatherings (T1&T2) orally dosed with DI leaves aqueous extract at 100 and 200mg/Kg BW. compared with a group dosed Diclofenac (0.71mg/Kg BW)and Meloxicam(0.3mg/kg). The fourth group acts as a control one. in indicated that there were significant analgesic effects in dose dependent manner that means the extract possibly has central and peripheral effects. The high DI dose showed nearly the same analgesic effectas Diclofenac at dose 0.71mg/kg, T2 group and Diclofenac (0.71mg/Kg BW) group (6.7 ± 0.2 and 7.6 ±0.2) respectively after 30 minutes of administration and the effect of analgesia continued in both T2 and Diclofenac group (6. 9 ± 0.18 and 6.3 ± 0.3) respectively at 60 minutes after gives and effect appeared at 90 minute T2 and Diclofenac group (5. 7 ± 0.2 and 5.8 ± 0.12). while T1 showed pain reaction time of (5.8 ± 0.2 and 5.8 ± 0.12 and 4.6 ± 0.3) respectively at 30, 60, 90 minutes revealed less effective than T2 and Diclofenac group and all these three groups were in significant comparison with the control group that in pain reaction time (4.2 ± 0. 2 and 4.4 ± 0.1 and 4.3 ± 0.2) in different periods.
The results appear caused a significant decreasing (P<0.05) T1 (54 ±2.5) T2 (37± 3.2) and Diclofenac (0.71mg/Kg BW) (29 ± 1.2) comparison with the control group (70±1.5) in writhing test. In formalin test the results showed that both *D. innoxia* doses in early and late phase T2 (25.0±1.0,12.10±1.30) T1 (30±1.2,20.1±1.55) Meloxicam (0.3mg/kg) (24.7±1.0,11.10±1.20) and control (38.5± 2.20, 39±2.10) recorded their analgesic and anti inflammatory effects, this is probably due to the active phytochemical contents that posses both analgesic and anti-inflammatory effects.

Keywords: Aqueous extract *Datura innoxia* leaves, anti-inflammatory impacts, analgesiac activity

1. Introduction
The medicinal plants contain substances in their parts that can be used for therapeutic purpose or as a precursor for the synthesis of other useful medicines. In another words, these plants possess therapeutic properties or exert beneficial pharmacological effects on the animal body so they are generally designated as "Medicinal Plants" [18]. Although there were no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. The genus Datura of the family: Solanaceae distributed in the Middle and South of Iraq and normally flowered between August and September [18]. The common species that are found in Iraq are *Datura innoxia* Mill, Datura metel Linn and Datura stramonium Linn [4, 23]. Although it has been known for its extensive toxicological effects, the Datura has been thoroughly used in the herbal therapy of many diseases. Study on components of Datura have prescribed widely as a curative for wide range of diseases such as asthma, eye disorder and glucoma, cancers, pyschatric disorders, neurological disorders, shock, tuberculosis, motion sickness, paralysis, arthritis, rheumatism, peritonitis, orchitis, hydrocele, hemorrhoids, spasms, worm infestation, dandruff, alopecia, venereal diseases and depression in sexual activity [3, 21]. *Datura innoxia* is considered an alternative treatment for Parkinson's disease [30]. The present study applied on leaf aqueous extract of DI to evaluate its pain reliving and anti-inflammatory effects in mice as preferred characters to formulate and compose an active herbal analgesic, anti-inflammatory herbal drug.

2. Materials and Methods

D. innoxia leave were collected from Collage of Agriculture farms during September after 4 weeks of germination of *Datura innoxia* plant. The plant classification was done in Ministry of Agriculture/ State Board for Seeds Testing and Certification (S.B.S.T.C) in Abu Graib /Baghdad at certificate. Aqueous extraction was done according to Harborne [16]: sixty grams of dried powder plant leaves were put in pyrex flask (500ml) containing (200ml) distilled water. mixed by magnetic stirrer at 40 °C for 24 hours then filtered to get rid of residue and placed in an incubator at 40 °C to produce a dried extract. The dry extract acquired was kept in a refrigerator at 20 °C. The first experiment was conducted during the period from November to December 2015 whereas the second was conducted during the period from February to April 2016.

2.1 Animals

Swiss albino mice with body weight ranged from 25 to 30 g either sexes were used were provided by Veterinary Medicine college, University of Baghdad from animal house. They were left for fourteen days at standard housing conditions for acclimatization.

2.2 Experimental design

1-Exp-.1 Phytochemicals Analysis of DI leave Aqueous extract and its crude powder were tested according to following methods:

Flavonoids: Flavonoids was tested following method of Jaffer *et al*, [18] Alkaloids were detected according to method of Odebiyi and Sofowora, [24] Saponins, Glycosides, Resins, Tannins: these tests were tested by method described by Harborne [16].

Steroids and Terpenoids: were detected according to method of Savithramma *et al*. [29].

Phenolic: conten was done following method described by of Harbornem [17].

Exp-2. A queous DI extract analgesic and anti-inflammatory Activity

A- Hot plate test (thermal): 24 albino Swiss mice were utilized to perform this test divided into four gatherings (6 for each). Group 1: Treated orally with refined water just and served as control, Group 2: Treated orally with 100 mg/kg B.W of DI aqueous extract, Group 3: Treated orally with 200 mg/kg B.W of DI aqueous extract, Group 4: Treated orally with0.71 mg/Kg Diclofenac. Thirty minutes after administration, every mouse in all gatherings was dropped tenderly on a plate and kept up at 55ºC ± 1ºC. This temperature produces pain thermal reflex in mice that can be measured by noticing their pain response times30, 60 and 90 min after oral DI extract dosing treated groups, specifically pain reflex recorded licking and flicking of their paw and jumping [19].

B-Writhing test (Acetic acid-induced writhing test)

This test done performed 24 mice allocated to four group dose as in hot plat method and was done according to[34] the total number of writhes was counted after intraperitoneal injection of acetic acid (70% solution) at dose volume (10ml/kg). The extracts were administered orally at 100 and 200 mg/kg doses 30 min prior to acetic acid injection. Number of writhing and stretching was observed and recorded and expressed as percentage inhibition according to equation.

C- Formalin test

The formalin test for nociception, which is predominantly used in mice, involves moderate, continuous pain generated by injured tissue. The response to formalin shows an early and a late phase, The early phase, which starts immediately following injection of formalin lasts approximately after 5 min and is probably due to direct chemical stimulation of nociceptors. The second phase, which lasts to 40 min, starts approximately 15 min following the formalin injection and suggsts that peripheral inflammatory processes are involved [15]

Twenty four albino swiss mice were divided equally into four groups (6 mice for each group) Solutions of eqouse extract of *D. innoxia* at doses of (100,200 and mg/kg), meloxicam (0.3 mg/kg) as comparative drug and as control dosed with 0.1ml/10g B.Wdislled water). All were administered orally, 30 min before injection of formalin.

Formalin (25%) 10 µL was administered subcutaneously (SC) into the dorsal surface of the right hind paw of the mice, The responses were measured for two distinct phases as far as nociceptive reaction to be specific jumping (it is viewed as one pain related behaviors of the formalin model portrayed by spontaneous, fast and brief shaking or No. of licking and flicking of injected paw) and licking of the infused paw [5, 20] (No. of licking and flicking of injected paw), the initial 5 min after formalin injection is known as acute or early phase of formalin induced pain and the duration between 15 and 45 min as the late phase [35]

2.3 Statistical analysis

Statistical analysis was applied by two ways ANOVA with least significant differences (LSD) to compare groups means. Probability level $P<0.05$ was considered statistically significant

3. Results

3.1 Exp-1 Phytochemicals Detection of The active components of *Datura innoxia*

The result indicated the presence of the following phytochemicals in DI aqueous extract: alkaloid, flavonoids, phenol, steroids, resins, tannins, saponins and glycosides while terpenoids coumarins was absent in crude extract. The presence of terpenoid in plant powder and the absence in crude extract may be due to evaporation of terpenoids during extraction process. In addition, the evaporation process during extraction may affect on concentration of some phytochemicals which appear in the results of the present study (Table 1).

Table 1: Phytochemical analysis of *Datura innoxia* leaves in crude *D. innoxia* powder and its aqueous extract according to analysis procedures

Test	*D. innoxia* curd powder	*D. innoxa* Aqueous extract	Results
Flavonoid	+	+	Yellow Color
Saponnins			
Stirring	++	+	Big Foam
Silver Nitrate	++	+	Silver Mirror
Mercuric Chloride	+	–	Silver Color
Steroid	++	+	Blue-Green Color
Terpenoid	+	–	Brown Color
Resins	+	+	Appearance of Turbidity
Phenol	++	++	Blue –Green Color
Glycosides			
Fehling. T.	++	+	Red Precipate
Benedict´s T.	++	+	Red Precipate
Alkaloid			
Mayer´s	++	++	White Precipitate
Dragendroff	++	++	Orange Precipitate
Peccric acid	++	++	Green Precipitate
Coumarins	_	_	
Tannins			
Lead Acetate	++	++	Gelatin-White Color
Ferries chloride	+	+	Blue –Green Color

+ = present, ++ = more present according to colour intensity, – = not detected

Exp- 2 Analgesic Activity and Anti-inflammatory Activity

3.1.a-Hot plate test

The results are recorded in table (1) in which statistically significant increase ($p<0.05$) was observed in the pain reaction time against induced thermal pain by hot plate in the two treated groups (T1, T2) administered with aqueous *D. innoxia* extract at dose and at time dependent manner. The highest analgesic doses DI were recorded at 30, and 60 min for both DI leave extracts.

Table 1: Effect of the aqueous extract of DI on pain second after different doses and periods in hot plate test

Groups N= 6 Times	T1 orally DI leave aqueous extract 100 mg/Kg	T2 orally DI leave aqueous extract 200 mg/Kg	Diclofenac P.o 0.71 mg/Kg	Control D.W
Zero time	C 3.9 ± 0.18 a	C 4.1 ± 0.2 a	C 4.2 ± 0.2 a	A 4.1± 0.1a
After(30minute)	A 5.8 ± 0.2 c	A 6.7 ± 0.2 b	A 7.6 ± 0.2 a	A 4.2 ± 0.2 d
After(60minute)	A 5.8 ± 0.12 b	A 6.9 ± 0.18 a	B 6.3 ± 0.3 a	A 4.4 ± 0.15 c
After(90minute)	B 4.6 ± 0.3 b	B 5.7 ± 0.2 a	B 5.8 ± 0.12 a	A 4.3 ± 0.2 b
LSD	0.80			

-Different small letters denote significant differences ($p<0.05$ between groups.

-Different capital letters denote significant differences ($p<0.05$) within group between periods.

3.2.b- Writhing test

Results of the writhing test are recorded in Table (2) which revealed that both extract doses caused a significant decreasing ($P<0.05$) in the number of acetic acid-induced writhes (abdominal constriction and stretching of hind limb). D.I extract showed a significant decreasing ($P<0.05$) in writhes effect at dose 200 mg/kg, more than that at dose 100 mg/kg, also recorded significant decreasing ($P<0.05$). On the other hand, Diclofenac also exhibited the significant inhibition ($P<0.05$) of acetic acid-induced writhes at dose 0.71 mg/kg. as compared with other treated groups and control one.

Table 2: Analgesic Activity by Acetic Acid Induced Writhing in Mice treated with aqoauses DIextract and Diclofenac

Groups N= 6 mouse	No. of writhes within 30 min
Control orally D.W 0.1 ml/Kg	A 70± 1.5
Diclofenac. P.O 0.71 mg/Kg	D 29±1.2
T1 orally DI leave aqueous extract 100 mg/Kg	C 54±2.5
T2 orally DI leave aqueous extract 200 mg/Kg	B 37±3.2

-Different capital letters denote significant differences ($p<0.05$) between groups.

-L.S.D=4.6

3.3.c- Formalin test: The results of formalin test is recorded in Table (3) showed that there was significant decrease ($p<0.05$) in nociceptive response between different treated gatherings T1, T2, Meloxicam and control one group manifested by reduction in the number of liking of injected limb, Also between results of early and late phases for all treated group

Table 3: Analgesic and anti- inflammatory effect tested by formalin test in Mice treated with aqoauses DIextract and Diclofenac

Group N=6	Nociceptive response (number of licking and flinching)	
	Early phase (0-5) minute	Late phase(15-45)minute
Control orally D.W 0.1 ml/Kg	A 38.5±2.20 a	A 39.0±2.10 a
Meloxicam(0.3mg/kg)	C 24.7±1.00 a	C 11.10±1.20 b
T1 orally DI leave aqueous extract 100 mg/Kg	B 30.0±1.20 a	B 20.1±1.55 b
T2 orally DI leave aqueous extract 200 mg/Kg	C 25.0±1.00 a	C 12.10±1.30 b
LSD	4.40	

-Different capital letters denote significant differences (p<0.05) between groups
- Different small letters denote significant differences (p<0.05) within group periods

4. Discussion

One of the substantial medicinal plants with wide medicinal applications is *Datura innoxia* consider one of important herbal medicine since it is a good source of multiple phytochemicals (secondary metabolites). The distribution of the plant is wide world; however, it is abundant in Iraq. While we explain the importance of the Dantura, there are still insufficient studies that address its toxicological and chemotherapeutic effects [2].

The valuable medicinal effects of plant material primarily results from its secondary products which is usually attributed to the combination of the metabolites rather than a single compounds [27].

4.1 Exp-1 Phytochemicals screening analysis

The findings of this study are that alkaloids, saponins, tannins, flavonoids, phenols and resins found in *D. innoxia* leave powder and aqueous extract. However, coumarins and terpenoids was absent in crude extract. The finding of terpinoid in curde powder but not in the extract may be attributed to evaporation of terpenoids during the extraction process [8]. The results of phytochemicals analysis are comparable to studies results of [5] which have been done on the same extract. Phytochemical screening is which can lead to isolation and comprehended the pharmacological properties of the bioactive phytochemicals [32] by reported the presence of phenol, steroids, alkaloids, resins, flavinoids, glycosides, saponins and tannins in *D. innoxia* leave.

4.2 Exp-2 Analgesic and anti-inflammatory Activity

Presently, the pain relieving activity has been done to assess whether the analgesic effects of the extract are caused by peripheral or central mechanism. The hot plate induced pain test is believed to testify the central mechanisms [11].

In this experiment, a central model that has selectivity for opioid-determined analgesics in the hot plate test was used. It is selective parameter which has ability to analyze centrally acting drugs such as opiate drugs as this test is sensitive to centrally acting drugs [10] in the hot plate test, that has selectivity for opioid-determined analgesics [1]. Results indicated central analagic effect positively proportional with DI extract dose [29, 22, 9].

The precise mechanism of action is not fully known, however, it is thought the basic mechanism of its anti-inflammatory, antipyretic and analgesic action is by inhibiting of cyclooxygenase COX1 &COX2 and subsequent suppression of prostaglandin synthesis [37] or possibly due to the reported narcotic effect of Dutura plants which might give central acting analgesic effect.

The experimental results obtained in this present study indicated that the extract dose dependently reduced acetic acid induced writes.

The analgesic effect produced by the Datura extract central mechanisms involving these receptor systems including opiate, dopaminergic descending noradrenergic and serotonergic systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain [12].

These most of DI phytochemical compounds are recognized for their anti-inflammatory, analgesic effects since it can inhibit the enzymes implicated in the productions of the chemical mediators of pain and inflammation as well as their antioxidant activity [26].

The formalin test is effective and reliable models for nociception and is valid for different types of analgesic drugs. The test make a distinct biphasic response, hence, multiple analgesic can act differentially in the early and late phases. In this test, the centrally acting medications, for example, opiates inhibited both phases similarly, while peripherally acting medications just.

In the formalin test which have two particular phases, the initial phase is neurogenic and the final is inflammatory phase [35].

There are other pain mechanisms mediated by followings: few mediators, for example, histamine, kinin, serotonin and prostaglandins are discharged from the harmed cells, which participate in the inflammatory response and can invigorate the nociceptors and induction of pain [33] or by suppressing conducting of nerve impulses along nerve fibers that are responsible for propagation and transport of impulses. In additions, it is not exclude the possibility of the extracts induced antinociception in the late phase can partially mediated by peripheral mechanism. In this test, the centrally acting medications, for example, opiates inhibited both phases similarly, while peripherally acting medications just inhibited the second stage [13, 28].

The D.I Aqueous leave extract shown to possess antinociceptive effects appeared in all the nociceptive models signaling to the presence of both centrally and peripherally mediated pain relieving activities. The action of the extract mentioned above as analgesic might be related to its important constituents [flavonoids, tannins, steroids, alkaloids, phenols and terpenoids) that have been reported to reduce inflammatory process and pain [25], falvonoids reported to have both analgesic and anti-inflammatory activity [14].

There have also been reported previously the role of tannins in anti-nociceptive activity [38]. On the other hand, alkaloids are well identified for their activity to inhibit pain perception [36, 6]. Moreover, since the extract suppresses both peripheral and central mechanisms of pain. It was finding that all treated animal by extract showed depression and other signs like tranquilzation and may possibly posing central narcotic analgesic action symptoms of depression to surrounding[tranquilzation) and have been noticed in animal both D.I Aqueous leave extract groups indicate possibilly presence of naracotic analgesi central effect.

5. Conclusion

From the present study results it can be concluded that aqueous extract have definitive analgesic and anti-

inflammatory effects with possibly many mechanism of action involved mainly centrally possibly due to its phytochemical compounds contents.

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