ANALGESIAC, ANTI-INFLAMMATORY AND ANTIDIARRHOEAL EFFECTS OF *DATURA STRAMONIUM* HYDROALCOHOLIC LEAVES EXTRACT IN MICE

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ABSTRACT

Three experiments were performed, Exp-1 and Exp-2 were designed to study the antidiarrhoeal effect and the effect on enteropooling induced by castor oil for two treated groups (T1&T2) orally dosed with ***Datura stramonium*** leaves hydroethanolic extract at 50 and 100mg/Kg BW. compared with IP dosed atropine sulphate and control groups , each consist of 6 mice. Exp-1 results showed that both DS extract doses caused a dose dependent antidiarrhoeal effect manifested by significant decrease in charcoal intestinal travelling distance and percent (ITP) which is similar to atropine sulphate (0.1mg/kgBW IP) for the high DS extract dose. While Ex-2 results showed the superiority of DS extract via decreasing the intestinal castor oil enteropooling effect than atropine sulphate (0.3mg/Kg BW) possibily because the DS extract may have another active mechanism beside its antimuscarinic effect due to its tropane alkaloids content . In Exp—3 same dosed DS groups were used to study the analgesic effect by using hot plate method and anti-inflammatory effect that measured by using formalin test compared with Tramado HCL at 40 mg/Kg IP and Diclofenac(0.75mg/Kg BW IP)as reference drug. The results of hot plate indicate a dose dependent effect for both DS doses resembling that of tramadol HCL in their antinoiciceptive effect versus time indicating that the extract have a central analgesic effect probably by narcotic and non narcotic mechanism while the formalin results for both DS doses at the early and late phase indicate clearly their analgesic and anti inflammatory effect due to its phytochemical contents.

Keyword: *Datura ,analgesic , anti-inflammatory, antidiarhoeal.*

1. INTRODUCTION

One of the most important medicinal plants is *Datura* (thorn apple), which is an annual plant belongs to the family of Solanaceae. *Datura* plant is an important medicinal plant as it is a well known source of different phytochemicals, it is distributed throughout most of the part of the world and it is abundant in Iraq, This plant is rich in alkaloids which induce a stimulation of central nervous system and depression of the peripheral nerves typical for parasympathetic. Its medical effects characteristics include spasmolytic, antispasmodic, anticholenergic and narcotic (Roddick, 1991). Also *Datura* plant rich in secondary metabolites which have antibacterial activity by different mechanisms (Saadabi *et al*., 2006).

Datura stramonium (Family: Solanaceae), it is indigenous to Caspian region and in United States, South America, France, Germany, Hungary and Middle East. The main active constituents of plant are atropine and scopolamine. Dry leaves consists 0.25 % of alkaloids of *stramonium*, it is used as an aphrodisiac, medicinal, psychotropic, sacred and antispasmodic (Kulkarni , 2005).The present study aimed to evaluate the analgesic , anti-inflammatory , anti- motility and anti-enteropooling effects of DS leaves hydroalcoholic extract in mice as favorable characters to formulate and prepare anti-diarrhoeal drug.

2. MATERIALS AND METHODS

Datura Stramonium (DS)Leaves were collected from local garden in Abu Graib.The plant authenticated by comparing with herbarian voucler specimen. The plant leaves were air dried under shade , powdered mechanically and stored in airtight container.

Extraction; Hydroalcoholic extraction of DS leaves powder was prepared according to Harborne (1984) in which 60 grams of the plant powder was put in 1000 ml flask then 200 ml of 25% ethanol was added , mixed and extracted by magnetic stirrer at 40°C for 48 hours ,then filtered by using whatman paper No1 to get rid of residues. The filterate dried up by using incubator at 40°C and the dried extract weighed and kept at -20°C in sterile tight container. The net weight of dry extract of DS leaves was 3g making the yield 5%.

Animals; Nearly hundred of Swiss albino mice weighing 20-30 gram of both sexes. The animals were grouped according to the design of each experimental study . The animals have been kept in cages housed at standard condition of light and ventilation and have freely access to standard rodent diet and tap water. The animals were kept for a week for acclimatization before subjecteted any experiment.

Experimental design; Two experiments (Exp-1 &Exp-2) were designed to study the anti-diarrhoeal effect of hydroalcoholic extract of DS leaves on intestinal transit time and castor oil induced enteropooling in mice. The third experiment designed to study the analgesic effect of DS extract in mice by using hot plate method as well as the possible anti-inflammatory effect by using formalin test.

Preparation of DS extract doses: Dried DS extract was dissolved in 5% ethanol and the concentrations were adjusted for both extract doses to be given orally to mice at a dose volume of 0.1ml /10gmBW.

Exp-1: the effect of DS hydroalcoholic extract on intestinal transit time in mice

The effect of normal intestinal propulsion in mice was tested by using charcoal meal method described by Ayethen *et al* (1989), in which overnight 24 fasted mice divided into four groups each consist of 6 mice subjected to the following treatment.

-Control group received 55 ethanol at a dose 10 ml/kg PO.
-Atropine sulphate treated group given I.P at a dose of 0.1mg/kg BW.
-T1 group:- DS extract at oral dose of 50 mg/Kg.BW.
-T2 group:-DS extract at oral dose of 100mg/Kg.BW.

Thirty minutes later ,the animals were administered orally with freshly prepared standard charcoal test meal (0.2ml per mouse of 10% activated charcoal suspension in gum agacia),After 30 minutes , the animals were sacrificed and small intestine was isolated and the following parameters measured.

-The distance traversed by charcoal meal from the pylorus to the ileocaecal junction was measured.
-The length of the entire small intestine was also measured , then the distance traveled by charcoal was measured and expressed as intestinal Transit percent(ITP) according to the following equation (Jabbar *et al* .,1999)

$$\text{Intestinal Transit percent} = \frac{\text{The distance traversed charcoal meal (cm)}}{\text{Total length of small intestine (cm)}} \times 100$$

Exp-2 the effect of DS extract on castor oil induced enteropooling in mice

Intraluminal fluid accumulation was determined by method described by Robert *et al* (1976) and Boominathan *et al*(2005). Overnight fasted twenty four mice were divided into four groups each of 6 mice consist of two DS extract dosed group (T1&T2) and control group as in exp-1 compared with Atropine sulphate dosed group at 0.3 mg /Kg BW given intraperitoneally .One hour later all groups administered castor oil (1ml P.O.) then after 2 hours the mice were sacrificed. The two ends of intestine were tied with thread .The intestinal content was removed by milking into a graduated tube and the volume determined .The intestine was reweighed and the differences between full and empty intestine was calculated.

Exp-3- analgesic and anti-inflammatory of DS extract in mice:

A- Hot plate method : This test is used to measure the analgesic effect of drug or herbal extract by applying thermal pain stimuli to animal then measure the pain reaction time (latency) as threshold for acute pain .Twenty four albino mice of both sexes weighing 20-30 g .equally divided into four groups subjected to the following dosing regimen.

Control:- orally dosed with 5% etanol at 10ml/Kg.BW.
Tramadol HCL treated group: dosed IP 40mg/Kg.BW.
T1 and T2 groups same as in experiment-1 and experiment-2 .

Mice were placed on hot plate maintained at 55± 1°C. The pain reaction time (lanecy pe second) between placing the animal on hot plate and kickhng ,jumping ,licking or holding hind limbs was measured for each tested mouse. A cut of time of 30 seconds was followed to avoid any thermal injury to the paw (Wolf and Mcdonald,1994). Pain reaction time (latency) was recorded before and at 30 , 60 , 90 , 120, and 150 minutes after treatment inorder to

assess the analgesic dose of extract and the time effect response.The prolongation of latency time of treatment groups were compared with the value of control one. The percetage of antinociceptive maximal possible effect(MPE) was calculated from the formula(Guisti, *et al*,1997)

$$\%\,MPE = \frac{\text{Test latency} - \text{predosing latency}}{\text{Cut of time} - \text{Predosing latency}}$$

MPE= percentage of antinociceptive Maximal Poosible effect.
Test latency = latency after drug treatment.
Predosing latency = latency before drug treatment (zero time).
Cut time = 30 seconds.

B-Formalin test ;- Twenty four albino swiss mice weighed 20 -30 g divided equally into foure groups consist of same DS extract dosed groups (T1&T2) as in hot plate and control group compared with Diclofenac sodium group at dose of 0.75 mg/Kg BW IP.subcutaneous injection of 10 µL formalin 25 solution into the right paw of hind leg of mice induced biphasic nociceptive response(lickining and flinching of the injection paw (Tjolson *et al* ,1992).An early phase during the first five minutes followning formalin injection due to direct stimulation of nociceptior neurogenic pain(Quiescent phase). The second (late) phase startining 15 minutes after formalin injection due to inflammatory process (Ammanlon *et al* ,2008 and Lee Bars *et al* .,2001). The gap between the two phases (early and late phase) showed diminution of nociceptive response.The animals were placed indivdually in glass cylinder for clear observation of the paw during the period of test that last for 60 minutes.
Statistical analysis:- Data were analysed statistically by using one and two ways analysis of variance (ANOVA) with least significant differences (LSD) to compare groups means .probability level $P \leq 0.05$ was considered statitically significant.

3. RESULTS

Exp-1:- The results of the effect of hydroalcoholic DS leave extract on intestinal transit are listed in table(1).The extract at two doses significantly decreased($p \leq 0.05$) the distance traveled by charcoal meal and cosequently the percentage of intestinal transit in(T1&T2)in dose dependent manner incomparison with control. However atropine sulphate 0.1mg/Kg.BW IP caused significant reduction in charcoal meal traversing distance and ITP in comparison with T1 as well as control groups but not with T2 group that showed nearly similar results.

Table -1: The effect of hydroalcoholic DS leaves extracts and atropine sulphate on distance (cm) and intestinal transit percent of charcoal meal in mice**.**

Treatment group n= 6	Dose	Total length of intestine (cm)	Distance travel by charcoal meal (cm)	Intestinal Transit Percent (%ITP)
Control 5% ethanol	10ml/kg BW PO	51.65	29.17±0.82 A	49.82±1.55 A
Atropine sulphate	0.1mg/KgBW IP	48.90	16.60± o.56 C	34.13±0.93 C
T1 hydroalcoholic DS extract	50mg/KgBW PO	49.70	18.4±0.40 B	37.02±1.1 B
T2 hydroalcoholic DS extract	100mg/Kg BW PO	50.26	16.1±0.37 C	32.03±1.0 C

-Different letters denote stastical difference at $p \leq 0.05$ between groups.

Exp-2 : The results of the effect of DS leaves hydroalcoholic extracts compared to atropine sulphate and control group on castor oil induced enteropooling in mice are listed in table 2 that showed all treated group (T! ,T2 & atropine) caused a significant reduction (≤0.05) in weight and volume of intestinal content in comparison with control group. There is no significant change in the parameters recorded between atropine sulphate and T1group , but T2 group recorded a significant reduction ($p \leq 0.05$) than these two groups.

Table-2 :- The effect of different DS hydroalcoholic extracts and atropine sulphate on castor oil induced enteropooling in mice.

Group n= 6	Dose	Weight of intestinal control (g)	Volume of intestinal content(ml)
Control 5% ethanol	10ml/Kg BW PO	3.45±0.19 A	3.07±0.18 A
Atropine sulphate	0.3mg/Kg BW PO	2.2±0.15 B	2.0±0.12 B
T1 hydroalcoholic DS leaves extract	50mg/Kg BW PO	2.1±0.18 B	1.9±0.18 B
T2 hydroalcoholic DS leaves extract	100mg/Kg BW PO	1.7±0.12 C	1.2±0.15 C

-Different letters denote stastical difference at p≤0.05 between groups.

Exp-3:- Hot plate: The results of analgesic effect of DS leaves hydroalcoholic extract of both doses 50 and 100 mg/kg BW given orally compared with Tramadol HCL (40mg/Kg BW. IP) and control group that tested by measuring the pain reaction time to thermal stimuli using hot plate method are listed in table 3 showed that TramadolHCLgroup exhibted after 30 minute of treatment a significant increase (p≤0.05) incomparison with T1 and control groups but not with T2 group in pain reaction time with maximum effect recorded at 60 minute that is significantly and gradually decreased at 90 , 120 to reach the lowest result at 150 minute after treatment . Same pattern with nearly same results of pain reaction time as that of tramadol HCL was noticed in T2 group which were significantly higher than T1 group results throught out all experimental period . Both DS leaves extract groups (T1&T2) showed a significant increase in pain reaction time incomparison with control group throughout all experimental .

Table -3: Change in pain reaction time (latency) per second after different periods of DS leaves extract and tramadol treatments in mice.

Time / Group N=6	O	+30 min	60 min	90 min	120min	150 min
Control 5% etanol 10 ml/KgBw orally	5.0 ±0.1 A a	5.2±0.2 C a	5.5±0.2 C a	5.4±0.1 C a	5.6±0.2 C a	5.4±0.1 B a
Tramadol HCL I.P 40mg/Kg	5.2±0.2 A e	17.3±1.2 A ab	20.7±1.5 A a	16.1±1.1 A b	10.5±0.8 A c	7.1±0.3 A d
T1 hydroalcoholic DS extract 50mg/Kg orally	4.9±0.1 A d	13.4±1.1 B ab	16.8±1.1 B a	12.1±0.9 B b	7.7±0.4 B c	5.2±0.2 B d
T2 hydroalcoholic DS extract 100 mg/Kg orally	5.1±0.2 A e	17.1±1.4 A ab	20.1±1.6 A a	15.8±0.8 A b	9.5±0.5 A c	6.8±0.2 A d

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

All treated groups (Tramodol ,T1 and T2) showed nearly same pattern of antinociceptive MPE % effect versus time with maximum effect after 60 min as they recorded 47.4 , 60.3 ,62.5 MPE % for T1 and T2 and tramadol HCL respectively which gradually decreased at 90 and 120 minutes untile they reacth at 150 minutes after treatmant (1.2 , 6.8 , and 7.7 MPE % for T1 ,T2 and tramadol respectively (graph-1).

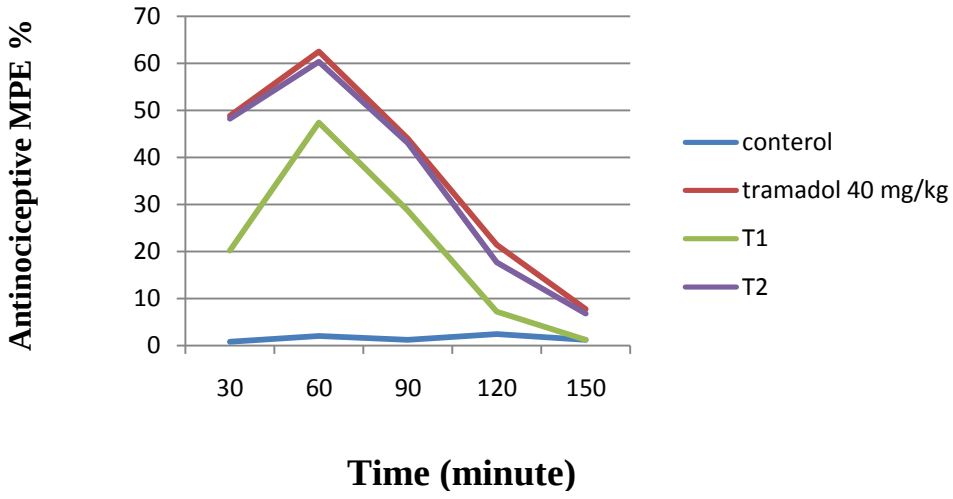

Figure 1:Changes in antinociceptive MPE% in different groups and periods.

Formalin test:- The results of formalin test listed in table 4 revealed that there were significant reduction ($p \leq 0.05$) in nociceptive response between different treated groups (T1 ,T2 .Diclofenac) and control one , Also between early and late phase for all treated groups. Diclofenac group showed nonsignificant differences with T2 group while they are both showed significant reduction in nociceptive responses than T1 group (DS extract 50 mg/Kg) in early phase of experiment (0-5 minutes). Same patteren noticed in late phase (15-45 min) more significant reduction $p \leq 0.05$ than early one in response (No of licking and flicking) between treated groups and with that of control one .The DS extract treated groups (T1and T2) showed a dose dependent reduction in antinociceptive responses both in early and late phase.

Table-4:Nociceptive responses (No of licking and flicking)in mice treated with Diclofenac and DS hydroalcoholic extracts using formaline test.

Time Group N=6	Nociceptive responses(number of licking and flicking)	
	Early phase(0-5)minutes	Late phase (15-45)minutes
Control orally 5% ethanol 10 ml/Kg	42.5 ±2.5 A a	45.3 ± 2.1 A a
Diclofenac I.P 0.75 mg/Kg	29.9 ±1.2 C a	17.5 ±1.0 C b
T1 orally DS leave hydroalcoholic extract 50 mg/Kg	35.0 ±1.3 B a	25.2 ±1.5 B b
T2 orally DS leave hydroalcoholic extract 100 mg/Kg	30.1 ±1.0 C a	18.2 ±1.1 C b

-Different capital letters denote significant differences ($p \leq 0.05$)between groups
- Different small letters denote significant differences ($p \leq 0.05$) within groups.

4. DISCUSSION

Activated charcoal is a non-absorbable agent that obstacle absorption of chemicals and drug due to its adsorption properties.Thus gastrointestinal motility test with activated charcoal was carried out to find the effect of DS leaves hydroalcoholic extract on perstaltic movement.Datura Stramonium dried leaves reported to contain 0.25% of tropane alkoloid with main active constitutent are atropine ,hyoscyamine and scopolamine , which all known pharmacologicaly and therapeutically to have antimuscarinic effect causing clearly antispasmodic and antidiarrheal effect when used in herbal medicine (Osni *et al*,2011)

In exp-1, The DS extract at both doses were significantly decreased the traveling distance and IT percent of charcoal meal in dose dependent manner nearly similarly to that of atropine sulphate at IP dose of 0.1mg/Kg

especially for DS extract at dose 100mg /kg which is clearly indicate the presence of active ingredients enough to cause such similar intestinal antimotility effect as that of atropine.

While in exp-2:Castor oil cause accumulation of water and electrolytes in intestinal loop.Both doses of DS leaves hydroalcoholic extract produced a dose dependent reaction in intestinal weight and volume in mice treated with castor in a manner better than atropine sulphate results especially for T2 dose of 100mg/Kg B.W. Ricinolic acid markedly increase the PGE2 in portal venous and gut lumen and also causes increase secretion of water and electrolytes into the small intestine (Belber and Juan,1979). It seem that atropine sulphate decrease intestinal enteropooling due to its antimuscarinic effect on receptors which decrease the intestinal secretion.While the superiority of DS extract at high dose may be due to the combined effect of anticholinergic component of tropane alkaloids (atropine ,Scopolamine and hyosciamine) with similar antimuscarinic effect as atropine and /or possibly the inhibtion of prostaglandin biosynthesis by othe DS extract active ingredients like tannin,flavenoid , phenol ,steroid that repoted to be present in the plant which may abolish the ricinolic acid induced secrotary effect in intestine.

In the third experiment the hot plate results recorded that both DS leaves extract groups(T1&T2) showed significant increase in pain reaction time in dose dependent manner indicating the presence of analgesic effect in both extract doses. Same MPE% patteren with nearly same results of pain reaction time as that of tramadol HCL were recorded .These similarity in MPE% versus the time of all treated drug indicate possible similarity in the kinetic and dynamic between tramadol and both DS extract doses with possible maximum drug concentration at active site and so maximum effect recorded at 60 minute. Tramadol is a synthetic centrally acting analgesic , it has both weak opioid agonist with selectivity for μ receptor and no opioid as a weak inhibitor of monoamine neurotransmitter noradrenaline and serotonine uptake(Scott *et al* ,2000) .AL-Jadder,(2011) reported in her study on tramadol antinociceptive effect in mice nearly same MPE% pattern as in the present study for the dose 40mg/Kg using same pain stimuli(hot plate) for testing. Antinociceptive effect attributed to its central opioid effect and inhibitor reuptake at the B level of spinal cord(Triscott *et a*l, 2008).

Same narcotic effect was recorded for Datura stramonium since it is used in herbal medicine reported to affect the nervous system mostly severely causing narcotic symptoms and dilated pupils(Alaani;2011)..DS usually used as sedative ,analgesic and an ointment made with powdered leaves allay pain of hemorrhoids. So it is possible that DS hydroalcoholic leaves extract contain some active ingredients with narcotic effect as well as other reported active ingredients with non-narcotic analgesic effect like flavinoids ,tannin,alkaloid ,phenols and glycosides(Al-ani,2011 .; Osni *et al*,2011).

It is difficult to separate the influence of anti-inflammatory from the analgesia in animal by using hot plate so formaline test has such advantages and also may give an indication on the mechanism of analgesic effect of the plant extract in tested animal. Also it is more realistic since pain stimulus is continuous as in most clinical pain. The early phase may be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase seemed to be resulted from the combination of an inflammatory reaction in the peripheral tissue and functional damages in the dorsal horn of spinal cord may be initiated by the C-fiber barrage during the early phase .The diminution of pain response that occurred after the first phase of formalin test(interphase period) may be due to active inhibitory processes (anti-analgesia) triggered by the initial pain (OK Lee *et al*.,2000).

The result of formalin test indicated that both DS extract treated groups(T1andT2)showed dose dependent antinociceptive responses both in early and late phase similarity to that of Diclofenac treatment especially for T2 indicating the presence of enough active ingredient in hydroalcoholic extract of DS leaves like flaviniods ,phenols,Alkaliods and steroids that reported to have analgesic as well as anti-inflammatory effect. Probably other substances in relieving pain through elevation of pain threshold or diminshing inflammatory response by inhibiting some chemical pain mediatters such prostaglandin , leukotriens , serotonin and histamine((Sharma *et a*l,2010)) This is interesting results since one of the favorable character of anti-diarrhoeal drug its analgesic and anti-inflammatory effect(Bennet and Brown,,2003).

5. REFERENCES

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