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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANALGESIC ACTIVITY OF *DATURA STRAMONIUM* L. VAR. TATULA, LEAF EXTRACT COLLECTED FROM COASTAL BELT OF ODISHA

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ABSTRACT: A wealth of experience on the application of plant and plant products in promoting health has accumulated over centuries, and the information widely exists for modern scientific exploration on drug invention. *Datura stramonim* is belongs to the family solanaceae is mostly found throughout the globe expect the colder region or arctic regions and traditionally the extracts obtained from the leaves or seeds of the plant are used in various types of ailment as well as pathological condition like asthma (smoke extract), cancer, microbial infections etc. Similarly the hydro alcoholic extracts are used traditionally as a potent analgesic during surgery or bone setting. The aim of the present investigation is to evaluate the analgesic activity of hydro alcoholic leaf extract of *Datura stramonium* in albino rats. The experiment was performed by inducing *Datura stramonium* extracts in two different concentrations to test groups by using hot plate method, tail flick method and formalin induced nociception method for analgesic study and compared with Tramadol HCL at 40 mg/Kg IP, Pentazocin 20mg/kg and Diclofenac 0.75mg/Kg body weight intraperitoneally as reference drugs respectively. The results of hot plate method indicates a dose dependent effect for both the doses of *Datura stramonium* resembling that of Tramadol HCL in their anti nociceptive effect versus time indicating that the extract have a central analgesic effect probably by both narcotic and non narcotic mechanisms while the results of formalin induced nociception model for both the doses of the early and late phase confirms clearly their analgesic and anti nociceptive effect, which may be due to various phytochemical contents present in the leaf of the plant *Datura stramonium* .

KEYWORDS: *Datura stramonium*, Analgesic activity, Anti noiciceptive, Formalin

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1. INTRODUCTION

According to the International Association for the Study of Pain (IASP), Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to humans or animals. It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus. Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can occur independently of any obvious predisposing causes e.g. trigeminal neuralgia or persistent long after the precipitating injury has healed e.g. phantom limb pain [1]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics i,e NSAIDs eg. salicylates and corticosteroids eg. hydrocortisone. All of these drugs known to possess unwanted and toxic effects. Moreover synthetic drugs are very expensive to develop since, for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested where the cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs [2-3]. Generally NSAIDs causes adverse effects and the use of these drugs as analgesic agents have not gain importance hence, drugs with no such effects been searched all over the globe. During this process, plant-based drugs used in the traditional medicines is concern since they are easily available, cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plants and plant based medicines to cure different ailments [4].The purpose of this investigation is to explore the analgesic and anti-inflammatory activity of the plant *Datura stramonium* belongs to the family solanaceae are mostly found throughout the world expect the colder region or arctic regions. The leaves and seeds are mostly shows the pharmacological activity. *Datura stramonium* is an annual plant. The stem is herbaceous, branched and glabrous or only lightly hairy when cultivated the plant reaches a height of about one meter. The branching stems are spreading, leafy, stout, erect, smooth and pale yellowish green in color, branching repeatedly in a forked manner. Leaves are hairy, big, simple dentate, oval glabrous, apposite veins of leaves are pale black, stalked, 4-6 inch long, ovate and pale green. The upper surface is dark and grayish green, generally smooth, the under surface paler, and when dried, minutely wrinkled. The plant reportedly contain various chemical constituents like 3α,

6β-ditigloyloxytrop, tigloidine, apohyoscine, hyoscine, 3α-tigloyloxytropan, norhyoscine, meteloidine, hyoscimine, cuscohygrine and tropine, Scopolamine and a mixture of two unidentified alkaloids daturanolone and fastusic acid, atropine, fastunine, fastudine, fastusidine, daturanolone and fastusic acid [5].

2. MATERIALS AND METHODS

Collection of plant materials

Leaves of the plant *Datura stramonium* (DS**)** Leaves were collected from the costal belt of Brahmapur, Odisha in the month of February-March, 2017 and authenticated by Dr. K. B. Satapathy, P.G. Department of Botany, Utkal University, Bhubaneswar, Odisha, India. After authentification, the leaf part of the plant was collected in bulk quantity, washed under running tap water to remove the adhering dirt and shade dried at room temperature.

Preparation of extracts

The dried leaves were powdered mechanically by using a mechanical grinder and stored in airtight containers. The powdered plant materials were first weighed and extracted successively[6] with petroleum ether (60-80^{0}C) and Hydro alcohol (ethanol and water 50:50) the extracts were concentrated by evaporating the solvent under reduced pressure using Rotary evaporator (IKA Rv 10 V digital). The yield of the petroleum ether and hydro alcoholic extracts were found to be 2.83 and 19.64 % w/w respectively.

Preliminary phytochemical screening of extracts

The preliminary phytochemical screening of petroleum ether and hydro alcoholic extract of *D. stramonium* was performed according to the method described by Gupta et al.[7] The tests were based on the visual observation of colour change or formation of a precipitate after the addition of specific reagents.

Experimental design

Wistar albino rats weighing 150-200 g of either sex were used. The experimental protocol was approved by the Institutional Animal Ethics Committee and animals were maintained under standard conditions for an acclimatization period of 15 days before performing the experiment. The condition of the animal house was approved by Committee for the purpose of Control and Supervision on experiments on Animals (Regd. No. 192/ CPCSEA, 22-05-2000).The acute oral toxicity study[8] was done according to OECD guideline at dose range 100 to 2000 mg/kg. No mortality of animals was observed at the dose range and hence two different doses 50 and 100 mg/kg was taken for screening of analgesic activity study.

a. Hot Plate Method

The study was done using the effect of hot plate induced pain in rats[9] mature mice were randomly divided into four groups (1-4) of 6 mice per group, fasted for 12 hours with clean drinking water. Group 1 served as control group treated with 5% ethanol at 10ml/Kg body weight orally, group 2

standard group treated with Tramadol HCL 40mg/Kg body weight intraperitoneally and group 3 and 4 test groups received extract of *D. stramonium* at 50 and 100 mg/Kg body weight orally.

The pre drug treatment (PRT) was assessed by placing each rat upon a heated metal plate (Hot plate) maintained at the temperature of about 55± 1^0C. The pain reaction time (latency per second) between placing the animal on hot plate and reflexes like kicking ,jumping ,licking or holding hind limbs was measured for each tested rats. A cut of time of 30 seconds was followed to avoid any thermal injury to the paw by the help of a digital stop watch. Pain reaction time (latency) was recorded for each group of animals at 0, 15, 30 and 60 minutes before and after treatment in order to assess PRT of the normal, standard drug and extract in different concentration and time effect response. The prolongation of latency time of treatment groups were compared with the value of control group animals.

b. Tail Flick Method

The experiment was carried out by measuring the tail withdrawal time from hot water[10]. Rats were randomly divided into four groups (1-4) of 6 rats per group and fasted for 12 hours. Group 1 served as control group treated with 5% ethanol at 10ml/kg body weight orally, group 2 standard group treated with Pentazocin 20mg/kg body weight intraperitoneally, group 3 and 4 test groups received extract of *D. stramonium* at 50 and 100 mg/kg body weight orally After 30 min of pentazocin administration and 1hr of extract administration, about 3-5cm of the tail of each rat was dipped into a water bath for 0, 15, 30 and 60 min. containing warm water maintained at the temperature of 50±10^0C and the time taken by the rats to flick the tail considered as the pain reaction time (PRT) and was recorded for all the rats.

c. Formalin Induced Nociception

Twenty four albino rats weighed 150-200 gm of either sexes divided equally into four groups i,e Group 1 served as control group treated with 5% ethanol at 10ml/kg body weight orally, group 2 standard group treated with Diclofenac sodium 0.75 mg/kg body weight intraperitoneally, group 3 and 4 test groups received hydro alcoholic extract of *D. stramonium* at a dose range of 50 and 100 mg/kg body weight orally respectively, after 30 min of diclofenac sodium administration and 1hr of extract administration subcutaneous injection of 10 µL formalin 25 solution into the right paw of hind leg of rat. The biphasic nociceptive response like biting, licking and flinching of the injected paw was recorded using a digital time-out stopwatch as total licking time (s) per 5 min observation period for a total duration of 2 h following injection of formalin. The control group compared with standard group. An early phase during the first five minutes following formalin injection due to direct stimulation of nociceptior neurogenic pain (Quiescent phase). The second (late) phase started 15 minutes after formalin injection due to inflammatory process [11]. The gap between the two phases (early and late phase) showed diminution of nociceptive response. The animals were placed individually in glass cylinder for clear observation of the paw during the period of test that last for

45 minutes.

Statistical analysis

All the results are expressed as mean ± SEM. Comparison was made between the test and control groups. The data were statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett's t-test and p value less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Table-1: Phytochemical screenings of crude extracts of *D. stramonium* leaves

Sl. No	Chemical Constituents	Crude Extracts	
		Petroleum ether extract	Hydro alcoholic extract
1	Flavonoid	+	+
2	Cholesterol	+	-
3	Tannins	+	+
4	Glycosides	+	+
5	Alkaloids	+	+
6	Phenols	-	+
7	Saponins	+	+
8	Proteins	+	+
9	Carbohydrates	+	+
10	Terpenoids	+	+

+ = the presence and - = the absence of chemical constituents

Preliminary phytochemical screening of extracts

Phytochemical screening study is intimately related to the needs of finding bio-active chemical constituents from medicinal plant extracts. The phytochemical screening test was conducted for both petroleum ether and hydro alcoholic crude extract of *D. stramonium* leaves and summarized in table 1. The results obtained from this study pointed that the presences of flavonoids, cholesterols, alkaloids, Phenols, tannins, carbohydrates, saponins, proteins, glycosides, and terpenoids in the plant extracts. However, phenols were not detected in petroleum ether extract and cholesterol is absent in hydro alcoholic extract. According to some of the previous studies, a qualitative phytochemical screening test of water and ethanol extract of *D. stramonium* extract showed the presence of different class of chemical constituents such as saponins, flavonoids, alkaloids, phenols, steroids, and glycosides[12].

Table-2: Reaction Time of Hydro alcoholic Extract of *D. stramonium* using Hot plate model.

Groups	Extract/drug	Dose (mg/kg)	Reaction Time (Sec) Mean±SEM			
			Basal	15 min	30 min	60 min
1	Control	---	4.6±0.49	5.66±0.49	5±0.36	4.66±0.66
2	Tramadol HCL	40	4.83±0.30	7.66±0.21**	10.6±0.33**	10.6±0.33**
3	*D. stramonium*	50	4.6±0.33	05±0.36	5.6±0.23*	5.6±0.33*
4	*D. stramonium*	100	04±0.25	06±0.51	10±0.51**	10.3±0.42**

Each values are represented as Mean ± S.E.M. n=6, * p<0.05, **p<0.01 as compared to control group.

Hot plate response in albino rats

Analgesic activity was investigated by Hot plate method. The reaction time was taken as the parameter for the evaluation of analgesic activity. There was no significant difference has been observed in reaction time of the control group at different time interval. Further there is no significant difference has been observed when treated groups are compared with control at basal point. The standard drug tramadol hydrochloride at 40 mg/kg showed the significant difference (P<0.01) against control at 15, 30, 60 min respectively (Table 2).The hydro alcoholic extract of D. stramonium at a dose of 50 mg/kg showed a significant difference (P<0.05) how ever 100 mg/kg dose shows no significant difference in results when compared with the control group animals (P<0.01) at 15, 30 and 60 min time interval.

Table-3: Reaction Time of Hydro alcoholic Extract of *D. stramonium* using Tail flick model.

Groups	Extract/drug	Dose(mg/kg)	Reaction Time (Sec) Mean±SEM			
			Basal	15 min	30 min	60 min
1	Control	---	04±0.33	4.3±0.1	4.6±0.19	4.6±0.30
2	Pentazocin	20	05±0.36	7.3±0.33**	10±0.36**	10.6±0.42**
3	*D. stramonium*	50	05±0.36	06±0.36**	6.3±0.33*	7.3±0.33**
4	*D. stramonium*	100	05±0.33	06±0.23**	7.6±0.33**	10±0.23**

Each values are represented as Mean ± S.E.M. n=6, * p<0.05, **p<0.01 as compared to control group.

Tail flick response in albino rats

Analgesic activity was investigated by tail flick method. The reaction time was taken as the parameter for the evaluation of analgesic activity. There was no significance difference has been observed in reaction time of the control group at different time interval. Further there is no

significant difference has been observed when treated groups are compared with control at basal point. The standard drug pentazocin at a dose level of 20 mg/kg showed the most significant difference results (P<0.01) against control at 15,30, 60 min respectively (Table 3).The hydro alcoholic extract of *D. stramonium* at a dose of 50 and 100 mg/kg shows significant difference (P <0.05) and (P<0.01) respectively at 30 min time interval compared to control group. Then after 60 min of induction of *D. stramonium* extract at a dose level of 50 and 100 mg/kg showed significant pain reduction effect (P<0.01) when compared to the control group.

Table-4: Reaction Time of Hydro alcoholic Extract of curcuma *D. stramonium* on Nociceptive responses.

Group	**Nociceptive responses (number of licking and flicking)**	
	Early phase (0-5) minutes	**Late phase (15-45) minutes**
control group orally 5% ethanol 10ml/Kg	42.5 ±2.5	45.3 ± 2.1
Diclofenac sodium 0.75 mg/kg	29.9 ± 1.2**	17.5 ± 1.0**
***D. stramonium*50 mg/kg**	35.0 ± 1.3*	25.2 ± 1.5**
***D. stramonium*100 mg/kg**	30.01 ± 1.0***	18.2 ± 1.1***

Each values are represented as Mean ± S.E.M. n=6, * p<0.05, **p<0.01 as compared to control group.

Formalin Induced Nociception

The results of formalin induced nociception test is listed in table 4 revealed that there were significant reduction (p≤0.05) in nociceptive response between The standard and hydro alcoholic extract of *D. stramonium* treated groups as well as control one , Also between early and late phase for all treated groups. Standard group showed nonsignificant differences with the group treated with *D. stramonium* 100 mg/kg, while both the standard and higher dose treated group showed significant reduction in nociceptive responses than the group treated with *D. stramonium* 50 mg/kg in early phase of experiment (0-5 minutes). Same pattern noticed in late phase (15-45 min) more significant reduction p≤0.05 than early one in response (No of licking and flicking) between treated groups and with that of control one .The *D. stramonium* extract treated groups (50 and 100 mg/kg) showed a dose dependent reduction in anti-nociceptive responses both in early and late phase.

DISCUSSION

Pain is a symptom of most of the ailments that requires treatment with analgesics [13]. Severe pain due to several infected and non infected diseases needs the use of strong analgesics, means opioid drugs. The addiction liability of opioids led to intensive research for compounds without this side effect. Many approaches have been used to differentiate the various actions of strong analgesics by developing animal models not only for analgesic activity but also for addiction liability. Painful stimuli can consists of direct stimulation of the efferent sensory nerves or stimulation of pain

receptors by various means such as heat or pressure. The role of endogenous peptides such as enkephalins and endorphins gives more insight into brain processes and the action of centrally acting analgesic drugs. Herbal drugs having analgesic activity may contain the chemical constituents like glycosides, alkaloids, flavonoids, saponins, tannins, terpinoids. From the phytochemical screening, we observed that the analgesic activity of hydro alcoholic leaf extract of *D. stramonium* may be attributed due to the presence of various phyto-constituents and other bioactive compounds[12]. The present study demonstrated that hydro alcoholic leaf extract of *D. stramonium* has intrinsic analgesic activity due to presence of flavonoids, alkaloids, Phenols, tannins, carbohydrates, saponins, proteins, glycosides and Terpenoids. Further studies are required to know the mechanism of action and actual chemical constituents that are present in the crude extracts of this plant leaves which are responsible for its analgesic activity. Most of the NSAIDs have also analgesic activity. Antipyretic analgesics causing analgesia by blocking impulse generation of pain peripherally while the narcotic analgesics block synaptic transmission of impulses signaling pain in the CNS were differentiated by some researchers[14]. Today, the classification of central and peripheral analgesics is definitively too simplified [15] but provides a guide for differentiation by pharmacological methods. Hot plate method is originally described by Woolfe and MacDonald in 1944 [16]. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance [17]. Mixed opiate agonists antagonists can be evaluated if the temperature of the hot plate is lowered to 49.5ºC [18]. It is known that centrally acting analgesic drugs elevate the pain threshold of rodents towards heat. The above findings indicate that *D. stramonium* may be acting centrally. The extract was found to significantly increase the tail flick reaction time in rats. Originally tail flick method was developed by Schumacher and Goodell in 1940 [19] for quantitative measurement of pain threshold in man against radiation and for evaluation of analgesic opiates. Later on, the procedure has been used by many researchers to evaluate analgesic activity in animal experiments by measuring drug induced changes in the sensitivity of mice or rats to heat stress applied on their tails. This test is very useful for discriminating between centrally acting morphine-like analgesics and non-opiate analgesics. Conformation of analgesic activity of *D. stramonium* has been done by formalin-induced paw licking method. The formalin test in rats has been proposed as a chronic pain model which is sensitive to centrally active analgesic agents by Dubuisson D, Dennis in 1977 [20]. The formalin test was selected because of several advantages including the ability to mimic human clinical pain conditions, sensitivity to agents having mild analgesics property, production of tonic stimulus and sensitivity to NSAIDs [21]. According to this method, drugs acting through central mechanism inhibit the early response called neurogenic phase where as those acting peripherally are good effective in the late phase known as inflammatory phase.

4. CONCLUSION

In the present study the hydro alcoholic extract was prepared from leaves of *D. Stramonium*and its analgesic effect was studies in different established models in rats. Toxicological studies reveal that *D. stramonium* is safe and does not alter the normal physiological and behavioral process up to a moderate dose level. Administration of hydro alcoholic extract of leaves of *D. stramonium* showed a remarkable analgesic activity in hot plate method, tail flick method and formalin induced nociception model. The above results are comparable to the research work previously done by several researchers which confirms that the hydro alcoholic leaf extract of *D. stramonium* inhibited early and late phases of the pain and possesses a significant, analgesic activity which was confirmed in our study.

5.ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

Authors have no conflicts of interest.

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