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PHYTOCHEMICAL & PHARMACOLOGICAL ACTIVITY OF DATURA *METAL & CALENDULA OFFICINALIS*

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Abstract:
Analgesics from Natural Sources: There are various medicinal plants available in nature which are having analgesic activity. The medications most frequently involved were NSAIDs (30%), painkillers (15%, especially paracetamol, on its own or in association with a weak opiate), ENT medication (12%), benzodiazepines (7.5%) and digestive anti-acid preparations (7.5%). were noted in 9% of cases. In both model Hot Plate Method & Tail flick method we select 4 group Control, Standard, Datura Metal linn & Calendula officinalis (10% gel) and Datura Metal linn & Calendula officinalis (15% gel). In the Hot plate model comparative study show Datura Metal linn & Calendula officinalis (15% gel) show more effectiveness than Datura Metal linn & Calendula officinalis 10% gel after 15 minute of application while Datura Metal linn& Calendula officinalis 10% gel show most effective after 60 minute of application.
In the tail flick model the comparative study show Calendula officinalis (10% gel show more effective than Datura Metal linn 10% gel after 30 minute of application while Datura Metal linn (10% gel show most effective after 60 minute of application.

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1. INTRODUCTION:

Analgesics from Natural Sources: There are various medicinal plants available in nature which are having analgesic activity, also they are being used traditionally by ancient medicinal practitioner[1].

The medications most frequently involved were NSAIDs (30%), painkillers (15%, especially paracetamol, on its own or in association with a weak opiate), ENT medication (12%), benzodiazepines (7.5%) and digestive anti-acid preparations (7.5%). were noted in 9% of cases. We also looked for the most frequent associations between substance and effect: for NSAIDs, the most frequent related effects were gastrointestinal pain, bleeding, and also renal or pancreatic failure. For painkillers, nausea, vomiting, and bleeding were observed, and also states of confusion or pancreatitis. With benzodiazepines, asthenia, confusion and memory disturbances were observed [2].

2. MATERIAL & METHODOLOGY:

2.1 Plant Material

Seeds of Datura metal linn. And leaves & flowering tops of Calendula Officinalis were collected at full bloom stage from plant nursery of Bhopal region M.P. After cleaning and washing thoroughly the plant parts are dried under shade protected from sun light, for 10 to 14 days then dried material were stored in a dry air tight container to avoid contamination and damage.

The Plant were identified and authenticated by Dr. Shaba Naaz H.O.D. Department of Botany, Safiya science college, Bhopal M.P. (Sample voucher 180,181) for further reference a voucher specimen has been deposited in the department.

2.2 Extraction of Plant Material

The extraction and isolation of the seeds of *Datura* and leaves of *calendula officinalis* was carried out by using the maceration method with methanol and water. Firstly, 80g of both powdered drug was accurately weighed and macerated with 200ml of each water and ethanol in 1000ml beaker covered it with plastic stock or aluminium foil. Extraction was conducted for seven days with occasional stirring it. The extract was filtered through filter paper. The Filtered extract was concentrated to dryness through the instillation process to get a concentrated product and also to separate alcohol and water concentrated product was kept a side for solidification of the product. Weight the final product and calculate the yield (the yield was found to be 3.75).

Figure 1Drug kept for Maceration

2.3 Laboratory Animals

The investigation of analgesic activity of the herbal preparation was conducted on albino rat weighing between18-22g were be housed under 12 hour light/dark cycles at 23+2°c. The animals were be access to food and water ad libitum. All animal experiments were carried out in accordance with CPCSEA guidelines and institutional animal ethical clearance. A total of 24 animals will be divided in to 6 groups (N = 6) for Analgesic Activity[3].

2.4 Drugs & Chemical

Acetic acid, dipyrone, formalin, glutamate, morphine sulphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Naloxone was obtained from Ark pharma (Carros, France). Dipyrone, glutamate and morphine were prepared in saline (0.90% Nacl) and contained 2% dimethyl sulfoxide. In all the pharmacological test naloxone was administered 15 min before the administration of the extracts of *Crassocephalum bauchiense*. Formalin stock solution was prepared in phosphate buffer solution (phosphate buffer solution concentration in mm: Nacl 137, KCl 2.70 and phosphate buffer 10). Acetic acid was prepared in saline (0.90% NaCl). Capsaicin stock solution (10−2 M) was prepared by successively dissolving capsaicin in 10% ethanol, 10% Tween 80 and 80% NaCl 0.90%. The stock solution was further diluted in saline upon administration to 80 mg/ml

2.5 Pharmacological analysis

2.5.1 Acute toxicity study of crude extract: For this purpose, female albino rat (150-200g) will be used. Fixed dose method (OECD guideline no. 425) of CPCSEA will be adopted[4].

An acute oral toxicity study was performed, followed by the Organization for Economic Cooperation and Development (OECD) guidelines 425 of chemicals. Ten Albion rats (100–250 g weight) were randomly divided into two groups (n = 5). Group 1 served as the control group and received 1 ml/kg 0.5% carboxymethylcellulose (CMC), while group II was treated with a dose of 2,000 mg/kg SF5. First, the animals were orally treated with a single SF5 dose of 2,000 mg/kg. The animal was critically observed for 24 h. If no morbidity and mortality were observed, then the other four animals were given their respective dose. Toxicity was observed for 14 days in total. Gross observations made to detect the toxicity were general behaviour, skin, fur, and eye changes, secretions from the mucous membrane, respiratory and autonomic or CNS disturbances, morbidity, and mortality. After 14 days, female rats were anesthetized using isoflurane (2–3%) diluted with oxygen, and blood was collected by cardiac puncture [5].

2.5.2 Hot Plate Method

The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch. Firstly, experiment was carried out on the standard group (without application of any preparation) and readings were noted. After it test preparations, in different concentrations was applied to the test group1 and test group 2. For comparative studies topical preparation of diclofenac sodium gel 1% was applied to the paw of mice. The latency is recorded 15, 25 and 35 minutes after application of topical preparations, values which exceed the value before administration for 50% or 100% can be regarded as positive and *ED*50 values will be calculated [(6-9)].

Figure 2 Hot plate instrument

2.5.3 RADIANT HEAT METHOD TAIL FLICK

Originally, the method was developed by Schumacher et al.(1940),Wolffetal. (1940) for quantitative measurements of pain threshold in man against thermal radiation and for evaluation of analgesic activity.

Mice are placed into cages leaving the tail exposed. A light beam is focused to the proximal third of the tail. Within a few seconds the animal flicks the tail aside or tries to escape. The time until this reaction occurs is measured.

Before administration of the test compound or the standard the normal reaction time is determined. The animal is put into a small cage with an opening for the tail at the rear wall. The tail is held and by opening of a shutter, a light beam exerting radiant heat is directed to the proximal third of the tail. For about 6 s the reaction of the animal is observed. The mouse tries to pull the tail away and turns the head. With a switch the shutter is closed and time is noted. The test compound in different concentrations and the standard are applied topically. The animals are submitted to the same testing procedure after 15, 25 and 35 min. For each individual animal the reaction time is noted[10].

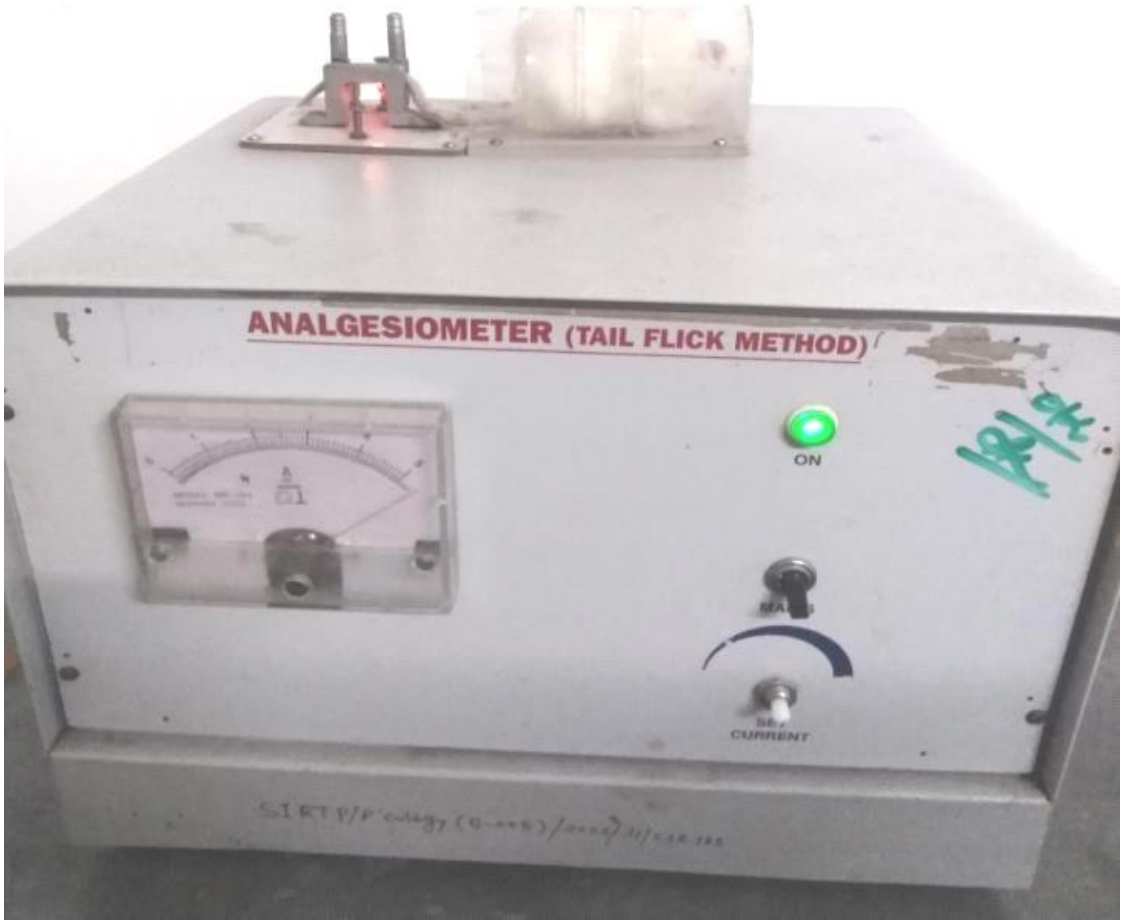


Figure 3 Radiant Heat Instrument

3. RESULTS:

3.1 Phytochemical test

Table: 01 Phytochemical evaluation of *Datura Metal*

S. No.	Name of Test	Ethanol
	Test for Carbohydrate	99.99%
1	Molish Test	+ve
2	Fehling's Test	+ve
3	Benedict's Test	+ve
	Test for Protein and Amino acid	
4	Biuret Test	+ve
5	Ninhydrin Test	+ve
	Test for Glycosides	
6	Borntrager Test	+ve
7	Killer-Killani Test	+ve
	Test for Alkaloids	
8	Mayer's Test	+ve
9	Wagner's Test	+ve
	Test for Saponins	
10	Froth test	-ve
	Test for Flavonoids	
11	Lead Acetate Alkaline reagent	+ve
	Test for Triterpenoids and Steroids	
12	Liebermann-Burchard Test	+ve
13	Salkowski Test	+ve
	Test for Tannin and Phenol	
14	Ferric Chloride Test	+ve
15	Gelatin Test	+ve
16	Lead Acetate Test	+ve

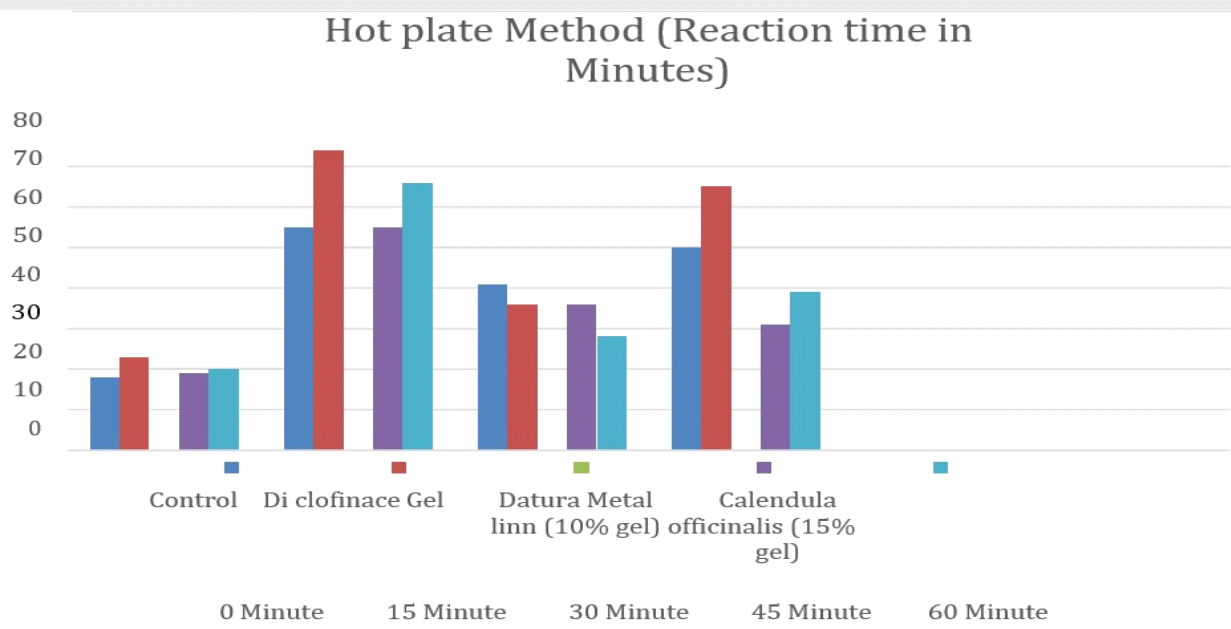
Table: 02 Phytochemical evaluations of *Calendula officinalis*

S.No.	Name of Test	Ethanol
	Test for Carbohydrate	99.99%
1	Molish Test	+ve
2	Fehling' Test	+ve
3	Benedict's Test	+ve
	Test for Protein and Amino acid	
4	Biuret Test	-ve
5	Ninhydrin Test	-ve
	Test for Glycosides	
6	Foam test	+ve
7	Killer-Killani Test	+ve
	Test for Alkaloids	
8	Mayer's Test	-ve
9	Wagner's Test	-ve
	Test for Saponins	
10	Froth test	-ve
	Test for Flavonoids	
11	Lead Acetate (Alkaline Reagent)	+ ve
	Test for Triterpenoids and Steroids	
12	Liebermann-Burchard Test	+ve
13	Salkowski Test	+ve
	Test for Tannin and Phenolic Compounds	
14	Ferric Chloride Test	+ve
15	Gelatin Test	+ve
16	Lead Acetate Test	+ve

3.2 Evaluation of Analgesic activity of *Datura Metal, Calendula Officinalis* Gel (Hot Plate Method)

Table 3: Analgesic effect of *Datura Metal gel ss Calendula Officinalis gel by* Hot Plate Method

S.No	Treatment	Hot plate method (Reaction time in seconds)				
		0 Min.	**15 Min.**	**30 Min.**	**45 Min.**	**60 Min**
1.	Control	18±52	23±33	14±22	19±37	20±16
2.	Standard	55±12	74±08	41±66	55±33	66±36
3.	*Datura Metal linn & Calendula officinalis* (10% gel)	41±26	36±66	38±33	36±29	28±03
4.	*Datura Metal linn & Calendula officinalis* (15% gel)	50±66	65±41	30±56	31±16	39±19

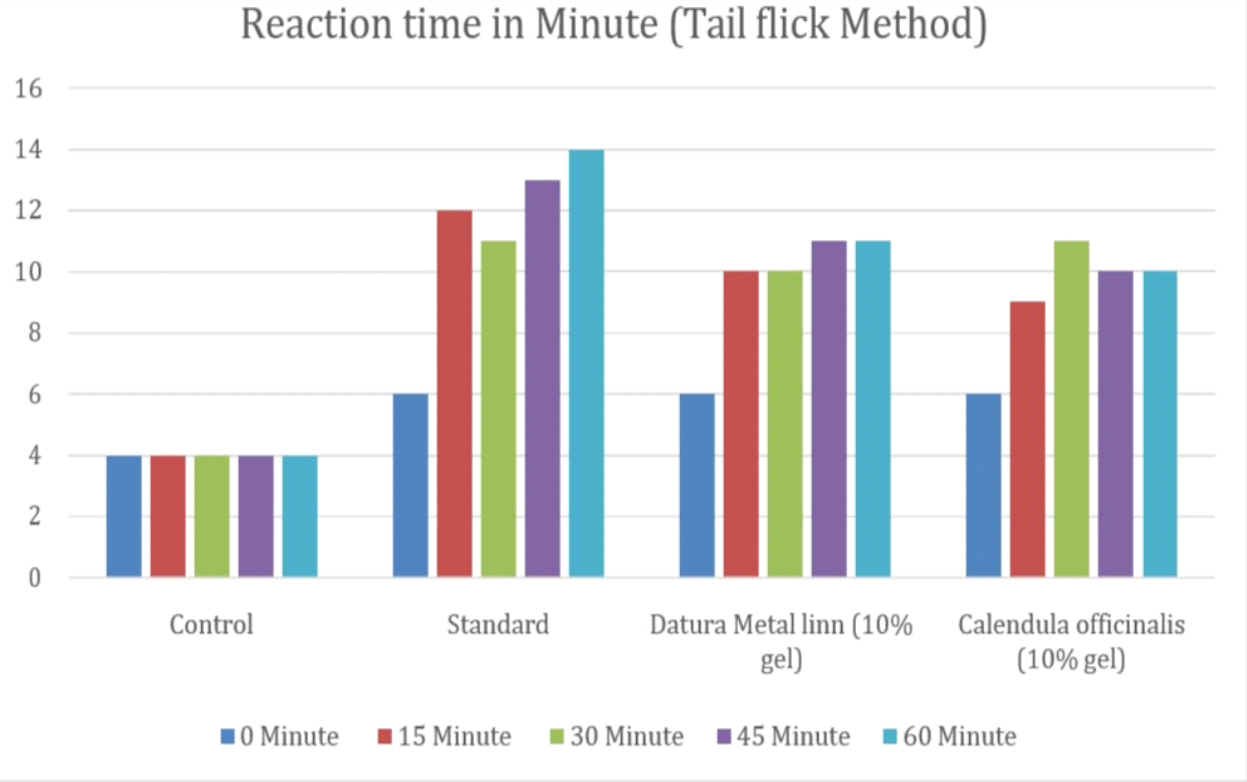


Graph 1: Reaction time in minute in different group of animals using Hot plate Method

3.3 Evaluation of Analgesic activity of *Datura Metal, Calendula Officinalis* Gel (Tail Flick Method)

Table 4: Analgesic effect of Datura Metal gel & Calendula Officinalis by Tail flick Method

S.No	Treatment	Tail flick method (Reaction time in seconds)				
		0 Min.	**15 Min.**	**30 Min.**	**45 Min.**	**60 Min**
1.	**Control**	**4.20±0.50**	**4.50±0.25**	**4.42±15**	**4.58±20**	**5.70±30**
2.	**Standard**	6.5±**0.3 3**	12.04±**0.15**	11.22±**0.20**	12.83±**0.35**	13.02±**45**
3.	*Datura Metal linn & Calendula officinalis* (10% gel)	**6.42±0.22**	10.20±**0.30**	10.30±**32**	11.05±**0.30**	11.00±**15**
4.	*Datura Metal linn & Calendula officinalis* (15% gel)	**6.35 ±0.22**	9.5±**0.30**	11.00±**31**	10.00±**20**	10.20±**10**



Graph: 2 Reaction time in minutes (tail flick method)

Two-way ANOVA test was performed for statistical analysis. One factor is time in minutes and another factor is the no. of responses shown by the albino rats during the activity. In both studies, the data show the fail the null hypothesis. It means results are significant in both method hot plate methods. tail flick method studies.

4. CONCLUSION:

There are various medicinal plants available in nature which are having analgesic activity, also they are being used traditionally by ancient medicinal practitioner.

The phytochemical results of *Datura Metal l*inn hydro alcoholic solvent show the presence of Glycosides, Alkaloids, Flavonoids, Triterpenoids and steroids, Tannins and Phenol while, Ethanolic extract of *Datura Metal* shows the presence of Protein and amino acids, Glycosides, Alkaloids, Saponins and Flavonoids, Triterpenoids and Steroids, and Tannins and Phenols. The phytochemical results of *Calendula officinalis* in hydro alcoholic solvent show the presence of Glycosides, Saponins, Flavonoids, Triterpenoids, Steroids and Phenols. Ethanolic extract of *Calendula officinalis* shows the presence of Glycosides, Triterpenoids and Steroids.

In both model Hot Plate Method & Tail flick method we select 4 group Control, Standard, *Datura Metal linn & Calendula officinalis* (10% gel) and *Datura Metal linn & Calendula officinalis* (15% gel)

In both model the Standard drug diclofenac show more effective medication for the treatment of the Pain. In the Hot plate model comparative study show Datura Metal linn & Calendula officinalis (15% gel) show more effectiveness than Datura Metal linn & Calendula officinalis 10% gel after 15 minute of application while Datura Metal linn& Calendula officinalis 10% gel show most effective after 60 minute of application.

In the tail flick model the comparative study show *Calendula officinalis* **(**10% gel show more effective than Datura Metal linn 10% gel after 30 minute of application while Datura Metal linn (10% gel show most effective after 60 minute of application.

The Datura metal show the presence of phytochemical compounds like alkaloids may be give analgesic

activity while the presence of the flavonoids it may be give anti-oxidant and anti- inflammatory activity.

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6. Conflict of Interest: There is no conflict of Interest.

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