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ANTIPYRETIC AND ANALGESIC ACTIVITY IN CRUDE ETHANOLIC EXTRACT OF *CALENDULA OFFICINALIS* LINN

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Crude extract of *Calendula officinalis* Linn. exhibited significant antipyretic (74.95% inhibition) and analgesic (27.42% inhibition) effects at a dose of 300 mg kg^{-1} 40 mg kg^{-1} respectively as compared to standard i.e. acetyl salicylic acid which exhibited 50.5% and 11.3% respectively. The extract not only reversed the induced hyperthermia but also affect the normothermia in rats. A dose of 20 mg kg^{-1} crude extract was found to be equipotent in its analgesic action to 40 mg kg^{-1} of acetyl salicylic acid. The extract was found to be non-toxic and showed a wide margin of safety through oral route.

Key words: *Calendula officinalis*; Antipyretic; Analgesic activity.

Introduction

Calendula officinalis Linn Compositeae (Kirtikar and Basu 1933;) Nadkarni 1954; Chopra *et al* 1980; Pamela 1991) commonly known as "Genda" or "Marigold" is found all over the world as an ornamental garden plant. The Plant has a long history of uses both medicinally and commercially (Barzynski 1954; Russo Maria 1972; Mamchur *et al* 1987; Vasudevan *et al* 1997; Eggensperger *et al* 1998) but is valued much for its medicinal uses in all system of treatments i.e. Unani, Eastern and Homoeopathic.

A survey of literature revealed that the plant is used as a remedy for epileptic fits, fever, muscular pain, kidney trouble (Kirtikar and Basu 1933; Nadkarni 1954; Robert 1968; Chopra *et al* 1980), inflammation (Grinkevich *et al* 1963; Fleichner 1985), bleeding piles, (Kirtikar and Basu 1933; Nadkarni 1954), cuts and wounds (Bose *et al* 1959; Fleichner 1985; Aulbach 1998).

Ample data is available about the chemical composition of *Calendula* plant which depicts the presence of terpenes, triterpenes, glycosides, sterols, alkaloides, tannins, phenolic compounds, flavonoides, pigments, phytosterin, essential oils, resin and 18-N paraffin (Gedeon 1951; Kurowska *et al* 1985).

For establishing and confirming the therapeutic role and effectiveness of *Calendula* plant in modern medicine, scientific experimental studies of the plant are needed. The present work is an attempt to establish and confirm the antipyretic and analgesic activities in the crude ethanolic extract of *Calendula* plant.

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Materials and Methods

Preparation of extract. Fresh mature fully grown plants of *Calendula* were taken, washed and dried in air. The plant (1.0 kg) was cut into small bits and soaked in 4.5 l of 95% ethyl alcohol for 120 h. The solvent was then filtered and concentrated *in vacuo,* which yielded 279.0g (27.90%), greenish brown coloured mass which was marked as crude ethanolic extract and was used during the whole study.

Animals. Healthy albino male rats (120-125 g) and mice (18-22g) were selected for toxicity, antipyretic and analgesic studies. For antipyretic studies animals with basal rectal temperature not exceeding 97.2°F, were selected and kept in an air-conditioned room having a temperature of 25°C. Animals were kept under observation for a period of 7 days with free supply of food and water. Each group comprised of six animals and each test was repeated thrice to confirm the results.

Toxicity studies. Crude ethanolic extract of *Calendula* plant dissolved in 1% Tween 80 was administered to a group of albino rats through intragastric route in different doses. Animals were observed for gross behavioral effects and mortality upto 48 h.

Antipyretic activity. The animals were divided into different groups as shown in Fig 1. Pyrexia was produced by injecting 0.1 ml $100g^{-1}$ TAB Berna vaccine subcutaneously (Dixit 1970) in experimental animals (Fig 1) Experiment was conducted in an air-conditioned room (25°C). Feed was withheld overnight and during the experiment while water was withheld only during the experiment. Change in temperature was noted after each 30 min time interval.

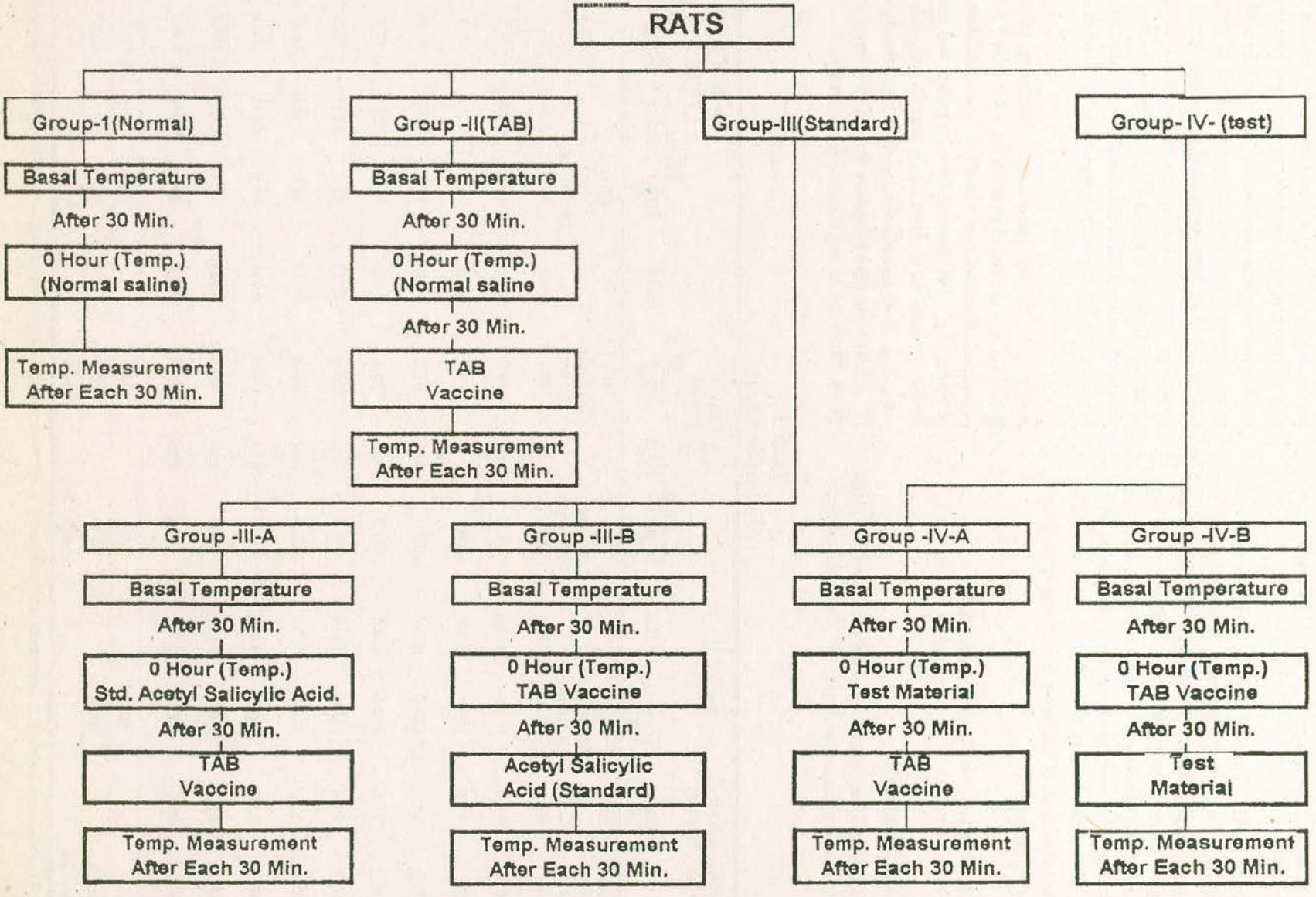

Fig 1. Flow sheet showing the procedure followed for drug administration.

Temperature Index (T.I), percentage of pyrexia and anti-pyrexia was calculated by the method of Okpanyi and Ezeukwis (1981).

Analgesic activity. Writhing test: Writhing in mice was induced by injecting intraperitoneally 300 mg kg^{-1} of 3% aqueous acetic acid solution. The number of writhing and stretching episodes were counted for a period of 20 min (Turner 1965). Test substance i.e. *Calendula* plant extract and standard i.e. acetyl salicylic acid in doses of 10, 20 and 40 mg kg^{-1} were administered orally 15 min prior to acetic acid injection. The percentage of inhibition in writhing episodes was calculated as follows:

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\text{Experimental}}{\text{Control}}\right)$$

Results and Discussion

Toxicity studies. Crude ethanolic extract of *Calendula* plant was well tolerated up to a dose of 10g kg^{-1}, through oral route. No mortality was observed during seven days observation period. A dose of 1.75g kg^{-1} and onwards caused a vasodilation followed by vasoconstriction pupillary dilatation, lacrimation, hypothermia, piloerection, hiccough, loss of writhing reflex, dryness of mouth, change of colour of tongue from pink to purplish blue, increased heart rate, rapid shallow breathing, drowsiness and frequent urination. Severity and depth of all these signs and symptoms were found to be dependent on strength and concentration of the extract used. A loss of 2.0 to 3.0°F body temperature was recorded during toxicity study.

Antipyretic activity. Results are tabulated in Table 1. TAB Berna vaccine produced pyrexia for more than 5 h in all experimental animals. Crude extract of *Calendula* plant in a dose of 300 mg kg^{-1} was found to be more potent and powerful in action as compared to standard i.e. acetyl salicylic acid in the same dose. Both test (Group IV, A&B) and standard (Group III, A&B) were able to reduce the existing fever in rats by 75.55, 74.95, 25.25 and 50.05% respectively.

Table 1
Antipyretic activity

Observation time(min)	Group-1 Normal	Group-II Tab		Group-III Standard				Group-IV (Test)			
				III-A		III-B		IV-A		IV-B	
S.No.	Temp °F	Temp °F	S.E±	Temp °F	S.E±	Temp °F	S.E ±	Temp °F	S.E ±	Temp °F	S. E ± °F
1. Basal temp.	97.0	96.9		97.0		97.1		97.1		97.1	
2. 0.0	--	Tab Vaccine		Std drug		Tab Vaccine		Extract		Tab vaccine	
3. 30	--	N.Saline		Tab Vaccine		Std drug		Tab Vaccine		Extract	
4. 60	97.2	98.2	0.014	98.0	0.018	97.3	0.011	96.9	0.017	96.3	0.015
5. 90	97.2	101.1	0.010	99.3	0.009	98.8	0.017	96.6	0.020	96.1	0.015
6. 120	97.1	101.1	0.066	100.3	0.018	98.5	0.015	96.6	0.018	96.3	0.015
7. 150	97.2	102.1	0.012	101.7	0.014	98.7	0.013	97.2	0.021	97.0	0.016
8. 180	97.3	102.4	0.009	101.5	0.016	99.4	0.013	97.2	0.016	97.3	0.010
9. 210	97.3	103.2	0.019	101.0	0.016	100.5	0.015	99.8	0.016	99.5	0.013
10. 240	97.2	103.4	0.016	100.9	0.015	101.0	0.017	99.9	0.016	100.0	0.018
11. 270	97.0	102.9	0.019	101.6	0.014	100.8	0.014	99.8	0.016	100.8	0.013
12. 300	97.2	102.8	0.011	101.8	0.011	100.5	0.013	99.7	0.017	100.7	0.011
13. 330	97.2	102.5	0.010	101.6	0.014	100.6	0.010	99.7	0.016	100.7	0.011
T.1	--	50.7		37.7		25.1		12.4		12.7	
% Pyrexia		100.0		74.75		49.50		24.45		25.05	
% Antipyretic				25.25		50.5		75.55		74.95	

T1, $\sum$ r-nb; T1, Therapeutic Index; $\sum$ r, Summation of all half hourly temperature readings after inducing pyrexia; nb, total number of readings (10)X basal temperature.

Table 2
Effect of *Calendula* extract on acetic acid induced writhing in mice

S.No.	Treatments	No. of animals	Doses (mg kg^{-1})								
			10 mg kg^{-1}			20 mg kg^{-1}			40 mg kg^{-1}		
			No. of writhing	% Inhibition	P. value 0.05(2)10	No. of writhing	% Inhibition	P.Value 0.05(2)10	No. of writhing	% Inhibition	P. Value 0.05(2)10
1.	Control (Normal Saline)	6	62	--	--	62	--	--	62	--	--
2.	Standard	6	62	0	P<0.05	59	4.8	P<0.05	55*	11.3	P>0.05
3.	Extract	6	57	8.0	P<0.05	52*	16.1	P>0.05	45**	27.42	P>0.05

Note: % Inhibition = 100 X (1-Test/Control), *Significant; ** Highly Significant.

Analgesic activity. Significant analgesic activity was observed in crude extract (Table 2). The activity was found to be more potent when compared with the reference substance (i.e. acetyl salicylic acid). The percent inhibition analgesia by the plant extract at 10, 20 & 40 mg kg^{-1} dose was 8.06, 16.12 and 27.42% respectively as compared to standard which was 0, 4.8 & 11.3%. The extract was found to be highly significant in a dose of 40 mg kg^{-1} as compared to standard.

Crude ethanolic extract of *Calendula* plant exhibited a potent antipyretic and analgesic activity. Oral administration of plant extract before and after the provocation of fever at a dose of 300 mg kg^{-1} was more effective (75.5 and 74.95% inhibition, Table 1 group IV A and B). Data also indicates that the plant extract not only reversed the induced hyperthermia but also affected the normothermia in rats (Table 1, Group IV A). It is evident from the study that a dose of 300 mg kg^{-1} of plant extract was more potent and powerful as compared to 300 mg kg^{-1} of acetyl salicylic acid.

Crude extract produced analgesic action on both chemical and mechanical stimuli. The percentage inhibition in analgesic activity was found to be dose dependent. A dose of 10, 20 and 40 mg kg^{-1} of plant extract exhibited 8.0, 16.1 and 27.42% inhibitation while standard i.e. acetyl salicylic acid exhibited 0.00, 4.8 & 11.3% inhibition respectively in same doses. A dose of 40 mg kg^{-1} of acetyl salicylic acid was found to be equipotent to 20 mg kg^{-1} of plant extract.

The results of the acute oral toxicity indicates a wide margin of safety and hence the plant can be regarded as non toxic particularly in therapeutic doses. Signs and symptoms observed during toxicity study also confirm its use as an antipyretic and analgesic agent.

Present days studies have also proved that carboxalic acids are used to allay paint inflammation and elevated body temperature (Goodman and Gilman 1985) Qualitative and quantitative study of *Calendula* plant revealed the presence of carboxalic acids (Kurowska *et al* 1985). Thus the antipyretic and analgesic activity exhibited by the plant extract may be attributed to the presence of organic acids in plants.

Further more literature citation reveals that the plant is used to cure gastric, deuodenal ulcers and other ulcerative conditions (Torjescu 1986). Therefore there is no doubt that the extract has no propensity to induce gastric or intestinal ulcers as is observed by the use of many analgesic and antipyretic drugs (Goodman and Gillman 1985).

The results obtained on *Calendula* plant gave only a rational basis to substantiate the use of plant as an antipyretic and analgesic agent.

Conclusion

From the study, it is concluded that full therapeutic potency of the plant is yet to be explored. Chemical investigations are warranted to identify and to isolate the active principle present in the plant extract responsible for the said activities.

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