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Evaluation of anti-inflammatory activity of Electrohomeopathic drug (spagyric essence) Angiotico -2 in rats

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Abstract
This particular research was carried out to evaluate *in vitro* anti-inflammatory activity of Electrohomeopathic medicine Angiotico - 2 (A-2) in Wistar rats. Literature study shows no scientific report has been available until date on the anti-inflammatory of A-2. Therefore the current analysis focuses to prove scientifically the anti-inflammatory activity of Electrohomeopathic medicine A-2. The anti-inflammatory property of A-2 was evaluated using carragenan induced mice oedema animal models of albino rats of either sex. The *in vivo* anti-inflammatory activity of Electrohomeopathic medicine A-2 showing potent anti-inflammatory properties as that compared to standard drug Ibuprofen.

Keywords: electrohomeopathy, angiotico-2, anti-inflammatory

Introduction
There are specific research activities developed in different regions of world on the use of herbal medication over some stretch of time. This includes Ayurveda, Siddha, Unani, Chinese, Homeopathy, Electrohomeopathy and other traditional or may be folklore medical system. In 1898, a pioneer Italian Count Cesare Mattie developed a new system of medicine and entitled as Electrohomeoapathy. He adopted the concept from a Swiss priest Parcelsus who was also a pioneer of chemical medicine. Parcelsus used the method of preparing the natural substances by different methods of extraction, distillation, fermentation, cohobation and the final mixture containing a combination of a different of ingredients with more powerful but similar effect. The philosophy of Count Mattie was that as the individual is complex and his disease can only be cured by the use of complex remedies. These complex remedies could restore the normal function of different organs and biochemical constituents into its perfect status. Consequently, in accordance with Count Cesare Mattie the principle of Electrohomeopathy is "Complexia Complexes Curantor" [1]". Afterwards, eminent persons like Krauss (1914), N. L. Sinha (1920), Gidden (1956) and many more constructively facilitated their effort to make popular this system of medicine.

In this Electrohomeopathy system, cohobation process is used to prepare spagyric essences from 114 medicinal plants for the treatment of different diseases [2, 3]. C.C. Mattie allocated all 114 plant medicines in the individual league lean on their curative properties and entitled as Scrofolso, Canceroso, Angiotico, Fabrifugo, Vermifugo, Venereo, Limphatico, Pettorale and a series of Electricities [4].

Angiotico - 2 or A-2 belongs to the blood remedy league and comprises spagyric essence of different plant combination like *Achilia millefolium* (Asteraceae), *Avena sativa* (Poaceae), *Hammamelis verginica* (Hammamelidaceae), *Hydrastis Canadensis* (Ranunculaceae), *Sanguinaria Canadensi s* (Papaveraceae). It is widely used by local practitioners to treat all kinds of inflammation. However, literature survey does not reveal any scientific report on wound healing activity of A-2. So the present study is an attempt to evaluate anti-inflammatory efficacy of A-2.

Material and Methods
Animals
Wistar rats weighing 150-200 gm of either sex were obtained from the animal house of School of Pharmaceutical Sciences, SOA (Deemed to be University). All the animals were housed in polypropylene cages maintaining temperature of 25±5°C with relative humidity 55-65% and 12 hr light and dark cycle. They were accessed with a free standard food diet and water and also remained fasting overnight prior to the day of the experiment. All the experimental procedures were performed in morning hours, according to the current guidelines for the care of laboratory animals and ethical guidelines investigations for experimental pain in the animals. An approval was obtained from the animal ethical committee of School of Pharmaceutical Sciences, SOA University, Odisha, India. (Registration No.1171/C/08/CPCSE) to meet the experiment protocol

Chemicals & Instruments
Carrageenan chemical was obtained from Sigma Chemical Co. (St Louis, Mo, USA) Ibuprofen (Brufen junior suspension from Abbot India Ltd.) is purchased from a local chemist. The instrument Vernier calliper was purchased from Precision India Ltd.

Acute Oral Toxicity
An acute toxicity study was carried out using albino rats (150-200g) as per OECD toxicity guideline 420. A-2 was administered as a starting dose level of 5mg/kg to one rat. Sighting study was carried out up to 2000mg/kg and no evidence of toxicity was found and further main study was performed using 5 rats. As a

result, it was found that there are no sign and symptoms of toxicity at 2000mg/kg. It was concluded that the drug can be unclassified as per GHS toxicity ranking. Special attention was given during the first 4 hours to all the animals for toxicity signs and behavioural changes, then up to 24 hours and finally kept aside and observed for following 14 days. As the dose of 2000 mg/kg was found to be safe for all animals, so 1/10th of this dose, i.e. 200 mg/kg was taken in the study [5].

Carrageenan induced paw oedema model

The carrageenan was induced by injecting 0.1ml of 1% w/v carrageenan suspended in 1% of CMC solution and injected in the sub-plantar region of the left hind paw of individual rats. For the evaluation of Carrageenan induced paw oedema test, the rats were divided into 3 groups containing 6 in each.

Group 1: Control - Carrageenan induced

Group 2: Control - Carrageenan + Standard (Ibuprofen orally40mg/kg)

Group 3: Control - Carrageenan + Test (Electrohomeopathy A-2 orally 200mg/kg)

The paw thickness of each rat was measured with vernier calliper and noted before the induction of carrageenan and after the injection again the thickness of paw was measured at different time intervals like 1 hr, 2 hr, 3 hr and 4 hr. The anti-inflammatory activity was calculated as percentage of inhibition of oedema in the test and standard groups and compared to the carrageenan induced control group. Ibuprofen, a potent anti-inflammatory drug, was used as standard. The percentage of inhibition of oedema was calculated by using the formula: [6]

$$\% \text{ of inhibition} = \frac{T_0 - T_t}{T_0} \times 100$$

Where as T_o is the thickness of the paw of control at the corresponding time when T_t is the paw thickness of the test drug at the same time.

Results

Anti-inflammatory activity

It was observed that the carrageenan induced group showed a significant increase in the thickness of the left hind paw with the increase in the time interval, whereas the standard and test group produced a significant decrease in the paw thickness when compared to the control group. i.e. 69% (Standard) and 66% (A-2) at 3 hr. [Table-1]

Table 1: *In vivo* anti-inflammatory activity of Electrohomeopathy A-2 on carrageenan induced paw oedema in Wistar rats

Groups	Change in thickness of hind paw (mm) in specific interval of time				% inhibition
	0 hr	1 hr	2 hr	3 hr	
Control (Carrageenan 0.1 ml of 1% w/v)	5.48 ± 0.07	7.56 ± 0.05	9.02 ± 0.13	10.24 ± 0.08	
Standard (Ibuprofen 40 mg/kg) + Carrageenan (0.1 ml of 1% w/v) +	5.16 ± 0.50*	4.52 ± 0.04*	3.84 ± 0.10*	3.20 ± 0.10*	69
Test A-2(200mg/kg) + Carrageenan (0.1 ml of 1% w/v)	5.28 ± 0.06*	4.62 ± 0.10*	4.64 ± 0.40*	3.46 ± 0.09*	66

Statistical Analysis

The data were represented as the mean of three replicate determination ± standard deviation. SD. Results were analysed by one-way ANOVA variance followed by multiple comparison of tukey's t-test. *P<0.05 values are considered as statistically significant when compared to control.

Discussion

Inflammation can be defined as the localized physical condition which mainly characterized by cellular injury, capillary dilation, leucocyte infiltration, redness, swelling, loss of functions and pain which serves as the mechanism of initiating of various noxious substances and of damaging tissues in the human body. [7] Inflammation occurs when a physical agent triggers an immune reaction resulting in tissue injury, ischemia, cell death, cancer and tissue degeneration [8, 9]. Therefore, anti-inflammatory agents are needed to counter the inflammation. Repeated or prolonged use of synthetic anti-inflammatory agents may produce moderate to severe adverse effects like gastric lesion, gastrointestinal damage, cardiovascular failure or may be a renal failure. As the plant medicines are comparatively safe, non-toxic, and effective, it may be a valuable substitute for synthetic anti-inflammatory agent. In traditional medicinal system like Ayurveda, Unani and Siddha, a large number of medicinal plants were used as anti-inflammatory agents for the treatment of human disease.

There are a number of anti-inflammatory plants that could help to attain alike results without the harmful effect [10]**.** Still today more than 70% of the developing world's population still depends on alternative systems of medicine [11]. Electrohomeopathy medicine A- 2 is a competently herbal formulation and is being extensively used by local practitioners for its anti-inflammatory action. The information is collected from local Electrohomeopathic practitioners regarding the efficacy & the safety of A-2 and it is quite amazing. The toxicity study also does not reveal any toxic result and the % inhibition of oedema in the paw of rates as compared to control and standard also encourages for further research on Electrohomeopathy.

Carrageenan induced paw oedema model is one of the common and suitable animal models for the evaluation of inflammatory activity. It is generally represented in the biphasic curve for the time period of the carrageenan induced paw oedema in rats [12]. In the first phase of inflammation the oedema at the hind paw occurs within one hour of carrageenan induction, which mainly releases the neurotransmitters like serotonin and histamine resulting in inflammation i.e. increase in the thickness of paw oedema [13]. The second phase of inflammation is observed at 3rd hour when cyclooxygenase (COX) enzyme releases and develops prostaglandin synthesis [14]. Usually the anti-inflammatory drugs mainly inhibit the COX enzyme, thereby blocking the synthesis of prostaglandins and producing anti-inflammatory actions. Both the Standard ibuprofen and Electrohomeopathic medicine A-2

successfully counter all phases of inflammation, which is clearly reflected by the percentage of inhibition of the paw oedema model of rats. The Electrohomeopathy medicine A-2 might mimic the mechanism of ibuprofen to block the synthesis of prostaglandin or may have its own mechanism which is subject to further research.

Conclusion

The above result indicates that A-2 possesses significant anti-inflammatory activities. Therefore, this scientific study justifies the use of A-2 medicines for healing of all kinds inflammations by local Ectrohomeopathiic practitioners. Further research is essential to isolate the phytochemical constituent which is principally responsible for the significant action of A-2.

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