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CHARACTERISATION OF ANTINOCEPTIVE AND ANTI OXIDANT EFFECTS OF CERTAINS ANTHRARUFIN (1, 5 DIHYDROXY ANTHRAQUINONE)

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ABSTRACT
Anthraquinone compounds are one of the abundant polyphenols found in fruits, vegetables, and herbs. However, the in vivo anti-inflammatory activity and molecular mechanisms of anthraquinones have not been fully elucidated. We investigated the activity of anthraquinones using nociceptive experimental conditions. 1, 5 Dihydroxy Anthraquinone one of the major anthraquinones ameliorated various inflammatory acetic acid-induced abdominal writhing without displaying toxic profiles in body and organ weight, gastric irritation, or serum parameters. Our data strongly suggest that anthraquinones act as potent anti-inflammatory and antinociceptive components in vivo, thus contributing to the immune regulatory role of fruits and herbs.

Keywords: Anthraquinones, Anti-inflammatory activity, Antinociceptive effects, Immune regulation, 1, 5-Dihydroxyanthraquinone

INTRODUCTION

Phytochemicals have always been a source of curiosity and challenge to the chemists and a potential treasure trove for pharmacologists [1]. The isolation, identification and structure determination of chemical constituents from plants have fascinated the scientists for more than two centuries. The advancement in synthetic chemistry has matched the same enthusiasm resulting in synthesis of many complicated chemical molecules which were considered impossible to synthesize and could be obtained only from nature. Medicinal plants play a vital role as sources of active anti-inflammatory ingredients in the prevention of various inflammatory diseases. Rhein, a bioactive constituent of anthraquinone, has been well recognized for its excellent anti-inflammatory activities and therapeutic effects on arthritis [2]. The present study is concerned with exploring the potential pharmacological actions of a few quninone derivatives. In a similar fashion the recent concepts on inflammation, mediators of inflammation, the mechanism of action, therapeutic uses and adverse effects of steroidal and nonsteroidal anti inflammatory drugs have been reviewed subsequently. The review of literature also includes a brief description on the chemistry of quninone and their biological effects with special emphasis on anti nociceptive, anti inflammatory actions and possible mechanisms mediating these effects.

MATERIALS AND METHODS

The anthrarufin (1, 5-dihydroxyanthraquinone) used in the study were synthesized adopting standard procedures at Research Organics, Chennai. Melting point,Thin layer chromatography (TLC), U.V spectra and I.R spectra of the synthesized compounds were compared with the standard samples and were found to be similar.

Animals

Wistar albino rats ranging between 120-160 g of either sex were used. Rats were maintained in stainless steel cages under constant conditions of temperature (23 ± 2 ˚C), relative humidity (60 ± 2%) and lighting (12 h light / dark cycle). The animals had free access to water *adlibitum* and fed with pellet diet (Lipton India Ltd., Mumbai, India.) except 1 h before and during the experiments. All experimental procedures were carried out in strict accordance with the guidelines prescribed by the committee for the Purpose of Control and Supervision on

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Experimentation on Animals and were approved by the Institutional Animal Ethics Committee (IAEC) from Pallavan Pharmacy college, Kanchipuram (2308/PO/Re/S/2024/CCSEA).

Drugs and Chemicals

The following drugs and chemicals were used. Acetic acid (E. Merck), Carboxy methyl cellulose (Glaxolaboratories, Bombay) Morphine sulphate (Ghazipur), Naloxone hydrochloride (Endolabs, USA), Diclofenac sodium injection (Novartis), Carageenan (Sigma), L–Arginine (Sigma), L NitroArginine Methyl Ester (Sigma), Aspirin (S.D.Fine Chemicals), Formalin (S.D.Fine Chemicals), Diagnostic kits for the assay of interleukin-6,Tumour necrosis factor alpha, and cyclooxygenase (Cayman chemicals,USA) [3].

Drug Administration

Anthrarufin (1,5-dihydroxyanthraquinone) derivatives were prepared as a uniform su spensionin 1% carboxy methyl cellulose (CMC) and injected by subcutaneous (s.c) route in doses of 3, 6, 12, 25, 50, 100 and 200 mg/kg, 60 min prior to the test procedure. The route of administration and time interval for various tests were based on an earlier study on a few Quinone.

Preliminary screening and acute toxicity

A preliminary acute toxicity testing was carried out in mice. The different anthrarufin (1,5-dihydroxyanthraquinone) tested were 1,5 DHA. The animals were treated with different doses of various anthrarufin (1,5-dihydroxyanthraquinone) and continuously monitored for two hours to detect any behavioral or autonomic changes and were under observation for two weeks to record any mortality. The highest dose employed was 2g/kg S.C.

Assessment of antinociceptive activity

Three methods were employed to assess the antinociceptive activity [4].

Acetic acid induced abdominal constriction assay

This assay procedure is considered very sensitive with minimal noxious stimulus.The number of abdominal constrictions (writhings) in mice for aperiod of 15minutes was counted following intraperitoneal (i.p) injection of 0.6% acetic acid in a dose of 10 ml/kg[5]. Any significant reduction in the number of abdominal constrictions when compared with vehicle treated animal was considered as antinociceptive response. Separate groups of animal received different anthrarufin (1,5-di hydroxyanthraquinone) in doses of 3, 6, 12, 25, 50, 100 or 200 mg/kg s.c, sixty minutes before acetic acid challenge. Morphine (5mg/kg s.c) was included as a Reference drug for comparison and administered 30 minutes before acetic acid challenge. The abdominal constrictions induced by acetic acid after anthrarufin (1, 5-di hydroxyl anthraquinone) administration was compared with that of the vehicle treatment. The percent inhibition of abdominal constrictions produced by different pretreatments was calculated using the formula:

$$\%\text{Inhibition} = \frac{C-T}{C} \times 100$$

C=Number of abdominal constriction in vehicle treated group

T= Number of abdominal constriction in DHA treatment group.

Antioxidant Activity – DPPH scavenging activity

2, 2 diphenyl-1-picryl hydrazyl (DPPH) is a stable free radical, showing a deep violet colour, characterized by a 456 absorption band in ethanol solution at 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, the free radical DPPH is reduced to corresponding hydrazine[6]. DPPH assay is considered a valid and easy method to evaluate the scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical scavenging assays. 3 ml of reaction mixture contain in 200 µl of DPPH and 2.8 ml of different anthrarufin (1,5-di hydroxyl anthraquino neat various concentrations (4-250µg/ml) in ethanol was incubated at 37^0C for 30 min and absorbance of the test mixture was read at 517 nm using a spectrophotometer. A standard antioxidant vit E (4-250µg/ml) was used for comparison. The percentage inhibition of DPPH radical was calculated comparing the results of the test with that of the blank using the following formula.

$$\text{Percentage scavenging} = \frac{\text{Absorbance of blank}}{\text{Absorbance of Test}} \times 100$$

RESULTS

Preliminary screening and Acute Toxicity

A Preliminary acute toxicity testing was carried out in mice. Nomortality was observed in mice upto a dose of 2 g/kg s.c for the anthrarufin (1,5-dihydroxyanthra quinone) tested.

Antinociceptive Activity

Aceticacid induced abdominal constrictions inmiceafteri.p.injectionof acetic acid was found to be 32.5. In morphine (5 mg / kg s.c) treated animals, no abdominal constriction response could be observed and thus producing 100% inhibition of this nociceptive behavior [7, 8].

Acetic acid abdominal constriction assay

Anthrarufin (1,5dihydroxyanthraquinone) A dose dependent reduction in abdominal constriction was

observed after treatment with different doses (3 — 200 mg / kg s.c) of DHA [9].The reduction in abdominal constrictions was significant with 3 mg/kg s.c. of DHA [10]. A maximum reduction was observed with 200mg/kg. The ED_{50} of DHA in this assay procedure was calculated from the dose response curve andfoundtobe13.8 mg/kgs.c [11]. DHA in different doses (3 — 200 mg/kg s.c) decreased the number of abdominal constrictions induced by acetic acid in mice. The percentage inhibition of nociception by different doses of DHA is presented in. A maximum antinociceptive effectwas observed with200 mg/kg of DHA [12]. The ED_{50} of DHA in this assay was calculated from the dose response curve and found to be 12mg/ kgs.

A significant reduction in the number of abdominal constrictions wasobserved by 50 mg / kg of Anthrarufin when compared to the vehicle treatment [13]. Consequently the percentage inhibition of this nociceptive response also remained the same (77%) during the observation period [14]. The reduction remained almost unchanged during the subsequent four days observation and ranged between 8.3 and 9.2. The percent inhibition of nociception was also almost uniform and ranged from 71.3 to 73.96 during the observation period. The findings of the present study indicate that, subcutaneous administration ofAnthrarufin produced consistent and dose related antinociception when assessed by acetic acid-induced visceral nociception in mice [15]. In a dose of 200mg/kg all the tested Anthrarufin exhibited nearly 100 percent inhibition of abdominal constriction response. It is pertinent to note that morphine in a dose of 5mg was able to produce near 100 percent inhibition of abdominal constrictions. Although the dihydroxy Anthrarufin apparently are less potent than morphine, maximum efficacy (100 percent inhibition) was achievable with all the tested dihydroxy Anthrarufin.

Table 1: Effect of Anthrarufin (1,5-Dihydroxyanthraquinone) on Acetic Acid Induced Abdominal Constrictions In Mice
Values are expressed as mean ± SEM from 6 rats. Significant at **p <0.05 as compared to control group using one way ANOVA followed by Dunnett's multiple range test.

Treatment	Concentration(µg/ml)						
	4	8	16	32	64	125	250
	% Inhibition						
1,5-DHA	28.46 ±0.21	34.00±0.28	42.32 ±0.58	49.30 ±0.27	55.63±0.24	60.63±.21	70.55±0.33
1,5-DHA	25.46 ±0.21	33.28±0.53	37.44 ±0.31	47.74 ±0.39	53.78±0.49	65.4±0.33	75.25±0.52
1,5–DHA	28.35±0.27	44.42±0.58	53.22±0.65	59.34±0.40	62.53±0.45	69.22±0.41	73.44±0.56
1,5-DHA	24.28±0.82	32.68±0.32	41.61±0.26	47.60±0.29	53.52±0.23	57.59±0.19	70.64±0.42
VITE	33.34 ±0.28	37.82±0.27	49.30 ±0.27	58.34 ±0.25	65.33±0.21	74.32±0.26	85.48±0.20

Table 2: DPPH Scavenging Activity Of Anthrarufin (1, 5-Di Hydroxyanthraquinone.
Values are expressed as mean ± SEM from 6 rats. Significant at **p <0.05 as compared to control group using one way ANOVA followed by Dunnett's multiple range test.

Treatmentmg/kg s.c	Number of Abdominal Constrictions
Vehicle	32.5±0.22
Morphine5	0±0235*
Anthrarufin (1,5-dihydroxyanthraquinone)	
3	23.8±0.31*
6	21.5±0.56*
12	16.2±0.31*
25	11.8±0.31*
50	5.5±0.22*
100	0.50±0.22*
200	0±0231*

CONCLUSION

Remarkable achievements have been made in modern medicine in the development of several effective drugs to treat pain and inflammation. Morphine and many opioids and a variety of NSAID are used for many decades for their beneficial effects. Eventhough, these drugs offer considerable relief, their inherent side effects limit their regular use in chronic pain or inflammatory diseases. Recent investigations on anthrarufin compounds have identified them as novel therapeutic agents in many diseases. The combination of analgesic and antioxidant properties without provoking gastric ulceration make them unique in therapeutic utility. The present study has identified highly effective compounds among anthrarufin family to possess anti nociceptive and antioxidant properties. Future organized clinical investigations on

these lines shall reveal the utility of these compounds either alone or as adjuncts to the currently employed analgesic drugs.

REFERENCE

1. Ardenghi JV, *et al.* Analysis of mechanism of antinociceptive action of nigaichigoside F1 obtained from *Rubus imperialis* (Rosaceae). *J. Pharm. Pharmacol.*, *58*, 2006, 1669-1675.
2. Arivudai Nambi R, *et al.* Anti-inflammatory activity of flavones and its hydroxyl derivatives – A structure-activity study. *Indian J. Pharm. Sci.*, *58*, 1996, 18-21.
3. Arivudai Nambi R. A study on the role of insulin and altered glycemic state in the gossypin-induced delay in small intestinal transit; possible mechanisms. Ph.D. Thesis, The Tamil Nadu Dr. M.G.R. Medical University, 2000, Chennai.
4. Baumann J, *et al.* Flavonoids and related compounds as inhibitors of arachidonic acid peroxidation. *Prostaglandins*, *20*, 1980, 627-639.
5. Benko S, *et al.* Brain oedema and subpleural haemorrhage in experimental P-avitaminosis. *Physiol. Chem. Phys.*, *2*, 1970, 110-116.
6. Beretz A, *et al.* Old and new natural products as the source of modern thrombotic drugs. *Planta Medica*, *57*, 1991, 68-72.
7. Calcagnetti DJ, *et al.* Analgesia produced by centrally administered DAGO, DPDPE, and U50488H in the formalin test. *Eur. J. Pharmacol.*, *153*, 1988, 117-122.
8. Calcutt NA, *et al.* Tolrestat treatment prevents modification of the formalin test model of prolonged pain in hyperglycemic rats. *Pain*, *58*, 1994, 413-416.
9. Carr A, *et al.* The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Radic. Biol. Med.*, *28*, 2000, 1806-1814.
10. Davis MC, *et al.* Chronic stress and regulation of cellular markers of inflammation in rheumatoid arthritis: Implications for fatigue. *Brain Behav. Immun.*, *22*, 2008, 24-32.
11. Deeks JJ, *et al.* Efficacy, tolerability, upper gastrointestinal safety of celecoxib for treatment of osteoarthritis and rheumatoid arthritis; systemic review of randomized controlled trials. *BMJ*, *325*, 2002, 619-626.
12. Enomoto S, *et al.* Inhibitory effect of C.S. traditional folk medicines on aldose reductase (AR) and haematological activity and on AR inhibitory activity of quercetin-3-O-methyl ether isolated from *Citrus laurifolius* L. *Biol. Pharm. Bull.*, *27*, 2004, 1140-1141.
13. Esposito E, *et al.* Role of nutritional antioxidants in the prevention and treatment of neurodegenerative disorders. In *Nutrient-Drug Interactions*, edited by Kelly Anne Meckling, CRC Press, Taylor and Francis, Baca Raton, London, New York, 2007, 130-157.
14. Gabor M, *et al.* Effect of hesperidin methyl chalcone on capillary resistance in the internal organs of the guinea pig and the rat. *Acta Physiol. Acad. Sci. Hung.*, *34*, 1968, 221-226.
15. Harborne JB, *et al.* The natural distribution of the phenolic aglycones. In *Biochemistry of Phenolic Compounds*, Academic Press: New York, 1964, 77-127.
16. Laughton MJ, *et al.* Inhibition of mammalian 5-lipoxygenase and cyclooxygenase by flavonoids and phenolic dietary additives. *Biochem. Pharmacol.*, *42*, 1991, 1673-1681.