ANTI-PYRETIC ACTIVITY OF TARAXEROL ACETATE

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ABSTRACT

Objectives: To investigate the anti-pyretic effect of Taraxerol acetate, a pentacyclic triterpene isolated from the aerial parts of Artemisia roxburghiana.

Methods: The plant materials were shade dried and ground into powder which was extracted 3 times by soaking with 24 L of methanol for two weeks. Preliminary fractionation of the crude extract, obtained from combine extract, was done by using solvents of increasing polarity: hexane, chloroform, ethyl acetate and n-butanol. The ethyl acetate fraction was subjected to silica gel column chromatography with hexane to yield taraxerol acetate. The structure of isolated compound was confirmed by comparing their corresponding NMR and mass spectral data with those previously reported. Yeast induced pyrexia model was used to carry out the anti-pyretic activity of Taraxerol acetate in Wistar adult rats.

Results: TA produced a significant decrease in the temperature of rats. The anti-pyretic effect of TA was dose dependent. When compared to standard drug, initially its action was similar to that but its effect reduced (decreased) earlier than aspirin.

Conclusion: Taraxerol acetate has a significant anti-pyretic effect in addition to its gastroprotective effect making it a safer anti-pyretic agent.

INTRODUCTION

Everywhere inflammation is associated with systemic manifestations which are collectively called the systemic inflammatory response syndrome or the acute phase response[1]. It consists of several pathological and clinical changes, the most prominent of which is fever. Fever is the increase in body temperature usually by 1- 4 °C[2] and is present mainly in that inflammation which is associated with infection. Pyrogens are the substances responsible to cause fever and are of two types. The exogenous pyrogens which are actually the bacterial products such as lipopolysaccharides (LPS) cause the stimulation of the leukocytes to release cytokines. The cytokines such as IL-1 IL-6 and TNF are called endogenous pyrogens[3,4]. These endogenous pyrogens cause an increase in the cyclooxygenase enzymes and more production of PG especially PGE_2 in the vascular as well as perivascular cells of the hypothalamus[5]. PGE_2 stimulate the production of cAMP, a neurotransmitter in the hypothalamus, whose function is to reset the temperature set point at a high level. A new hypothesis is put forward by Blatteis et al[6], according to which the febrigenic message from the site of injury (periphery) is conveyed to the ventromedial preoptic (VMPO) area of the hypothalamus by a neural route rather than a humoral one. This neural route specifically involves the vagus nerve and PGE2 and not the cytokines (like IL-1, IL-6 and TNF). The LPS produced by bacteria reach the liver kupfer cells, where it activates complement cascade to produce anaphylatoxin (C5a). The C5a causes production of PGE_2 by the activities of COX-1 and COX-2. PGE_2 causes depolarization of the afferent fibers of the vagus nerve and the message is relayed to the nucleus tractus solitaries (NTS), from where the impulse is conveyed to the VMPO through the ventral noradrenergic bundle to release norepinephrine (NE) in the preoptic area. Norepinephrine elicits two distinct core temperature (Tc) rises; the 1st one is rapid in onset, PGE_2 independent and α_1 adrenoceptor mediated; while the other one is delayed, PGE_2 dependent and α_2 adrenoceptor mediated.

Taraxerol acetate (TA) is a pentacyclic triterpene having an acetate group at C-3 hydroxyl group of taraxerol. Taraxerol acetate is found to have gastroprotective effects[7], anticancer[8], antiviral[9], antiulcer[10], anti-inflammatory, anti-microbial[11] and anti-leukemic activity[12]. So far no work has been done to explore its antipyretic activities. Therefore this study was undertaken to investigate the antipyretic effects.

MATERIALS AND METHODS

Plant material

The aerial parts (green stems with leaves) of Artemisia roxburghiana were collected from the University campus of Hazara University Mansehra, Pakistan. The authenticated voucher specimen (no 3486) was then deposited at the Herbarium of Hazara University, Mansehra Pakistan. The plant materials were shade dried and ground into powder.

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Extraction and isolation

The powdered plant materials (17 kg) were extracted 3 times by soaking with 24 L of methanol for two weeks. Combine extract were filtered and evaporated under reduced pressure to yield 1.5 Kg of the crude extract. Preliminary fractionation of the crude extract was done by suspending the crude extract in water and extraction using solvents of increasing polarity: hexane, chloroform, ethyl acetate and nbutanol. Remaining unfractionated crude extract was taken as aqueous fraction. The ethyl acetate fraction (160 g) that showed the highest PTP1B inhibitory activity was subjected to silica gel column chromatography with hexane containing increasing percentage of ethyl acetate used as eluents. Fractions obtained from the 4.0:6.0 to 0:10 hexane: EtOAc mixtures were further subjected to CC under the same conditions to yield taraxerol acetate (1.71 g, RF 0.75 in hexane: ethyl acetate 0.5:9.5; Figure 1). The structure of isolated compound was confirmed by comparing their corresponding NMR and mass spectral data with those previously reported. Being a pentacyclic triterpene we aimed to carry out its anti-pyretic activity.

Animals

For various in-vivo studies Wistar adult rats were used. They were of either sex, weighing 180±10g. They were kept 10 per cage with 12h light-dark cycle under the standard laboratory conditions and fed with water and rat feed. All the established ethical principles for the laboratory animals were observed. All the rats, before the commencement of experiments were acclimatized to the laboratory environment for 7 days.

Yeast induced pyrexia model for anti-pyretic activity

This model was used to investigate the antipyretic effects of the test sample. The animals were kept fasting over night[13]. In the dorsum of all rats, 10 ml/kg body weight of 15% (w/v) yeast suspension was injected subcutaneously to induce pyrexia. 17 hours after the injection of yeast the rectal temperature of each rat was recorded using a clinical thermometer. The animal that did not show a minimum increase of 0.5°C in their rectal temperature were excluded from the study. Then the animal were divided into 4 groups (n = 5). The negative control received saline in a dose of 10 ml/kg body weight, the positive control group was given Aspirin in a dose of 150 mg/Kg body weight as a standard drug, and to the test animals, TA is given in the dose of 30 and 60 mg/Kg body weight. The rectal temperature of each animal is again recorded at 30, 60, 90 and 120 minutes after the administration of TA. The mean of the rectal temperature for each group was determined and compared with the temperature taken before the administration of TA (hyperpyretic state).

RESULTS

TA produced a significant decrease in the temperature of rats in which pyrexia (fever) was produced by yeast. The anti-pyretic effect of TA was dose dependent. In the dose of 60 mg/Kg, it started its effect ($p<0.01$) earlier (at 30 min) than in the dose of 30 mg/Kg; but the effect of 30 mg/Kg was longer i.e. at 120 min it was more significant ($p<0.01$) than the effect of 60 mg/Kg dose. When compared to aspirin, initially its action was similar to aspirin but its effect reduced (decreased) earlier than aspirin (Table 1).

DISCUSSION

The yeast lipopolysaccharides (LPS) when administered to the animals circulate in the blood and reach the blood vessels of the hypothalamus. Here in the endothelial cells of the blood vessels (that constitute the blood brain barrier), LPS stimulate the expression of two enzymes i.e., COX-2 and PGE synthase[14]. The induction of these two enzymes leads to the production of PGE_2. The inflammatory mediator IL-1 and the bacterial LPS from the infectious agents also cause induction of the

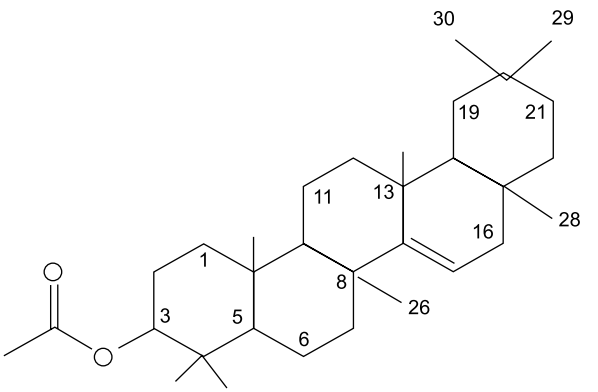

Figure 1: Chemical structure of taraxerol acetate

Table 1: Effect of TA on Yeast induced pyrexia in Albino rats (n = 5)

Time in minutes	Temperature (° F)					
	0 hr	30min	60min	90min	120min	180min
Normal saline	98.29 ±0.25	100.2 ±0.23	100.3 ±0.43	100.2 ±0.37	100.2 ±0.22	98.41 ±0.18
Aspirin (150) mg/kg	98.37 ±0.06	95.49 ±0.33**	96.4 ±0.13**	96.55 ±0.14**	97.55 ±0.16**	97.87 ±0.09**
TA (30 mg/kg)	98.62 ±0.12	97.87 ±0.09	97.28 ±0.03**	97.41 ±0.13**	98.62 ±0.21**	98 ±0.31*
TA (60 mg/kg)	98.47 ±0.08	97.65 ±0.13**	97.36 ±0.14**	97.21 ±0.11**	98.21 ±0.19*	98.21 ±0.03*

above mentioned two enzymes.

The PGE_2 formed in the endothelial cells diffuses out from these cells in the organum vasoculosum lamina terminalis (OVLT) region of the hypothalamus. This region of the hypothalamus controls fever and has EP_3 receptors. PGE_2 binds to these receptors and cause fever. The mutant mice having no EP_3 receptor will not develop fever after the administration of LPS, IL-1 or PGE_2[15]. Similarly LPS will not cause fever in COX-2 –/– (Knock out) mice[16]. PGD_2 is also found in humans, with limited distribution found in large amounts only in brain and mast cells but practically nowhere else. The receptor for PGD_2 i.e., DP receptors are found in brain and are coupled to the Gs protein with the formation of cAMP. The body temperature is lowered when PGD_2 is administered.

CONCLUSION

In conclusion, this study has shown that Taraxerol acetate has antipyretic effects in addition to the various physiological effects earlier reported by other authors. The gastroprotective effects and antiulcer activities of TA are quite encouraging because most commonly prescribed Non Steroid Anti-Inflammatory drugs (NSAIDs) produces gastrointestinal side effects. Therefore that consumption of TA has additional benefits in the form of its soothing effects on the gastric mucosa.

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