# IMMUNOPHARMACOLOGICAL STUDIES ON PICRORHIZA KURROA ROYLE-EX-BENTH PART III : ADRENERGIC MECHANISMS OF ANTI-INFLAMMATORY ACTION

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**Summary :** Nature of adrenergic mechanisms contributing to anti-inflammatory effect of Picrorhiza kurroa suggested from earlier studies was explored in Wistar albino rats. Water soluble fraction of alcoholic extract of rhizomes (PK) potentiated castor oil-catharsis on oral administration but direct subplanter PK-injections failed to exibit any local irritancy and oedema. Propranolol pretreatment counteracted while phentolamine enhanced anti-inflammatory effect of PK in carrageenin-induced inflammation. 6-hydroxy-dopamine pretreatment antagonised the said PK-effect and in such animals both ephedrine and isoprenaline augmented anti-inflammatory effect of PK, the former interaction being more conspicuous. PK-treatment of rats did not influence adrenaline uptake by lung slices *in vitro*. The results suggest that a non-neural augmentation of β-adrenoceptor function or consequent cellular events mediates the anti-inflammatory effect of PK.

**Key words :** picrorhiza kurroa anti-inflammatory drug adrenergic drug

## INTRODUCTION

Picrorhiza kurroa (KUTKI)-rhizome is a reputed Ayurvedic remedy with confirmed therapeutic potential in immune disorders (3, 4, 9). Earlier, a consistent but somewhat delayed anti-inflammatory effect of alcoholic extract in several models of immune and nonimmune inflammation was reported (7). This study indicated involvement of an adrenergic mechanism in the anti-inflammatory effect, alongwith an interference with release or lytic activity of the lysozomal enzymes. There appeared no likelihood of any direct or

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indirect glucocorticoid effect. Experiments conducted to determine above-indicated adrenergic and other mechanisms underlying the anti-inflammatory effect of the P. kurroa extract are reported here.

## MATERIAL AND METHODS

Extraction procedure adopted to prepare watersoluble fraction of alcoholic extract of Picrorhiza kurroa rhizomes was described earlier (7). Final concentration of the extract was so adjusted with distilled water that 1 *ml* represented extractives from 100 *mg* of the parent alcoholic extract (dry weight). This extract (PK) was always administered orally to the rats. Control animals received equivalent volumes of distilled water (DW).

*Animal experiments :* Male Wistar rats (100 to 200 *g*) put under standard laboratory conditions and fed with Hindustan lever pellets for at least one week were used. PK or DW was always given orally at 9 a.m. Pharmacologic agents used as tools to explore adrenergic mechanisms were given i p. as aqueous solutions as specified. Pedal inflammation in rats was produced by injecting 0.1 *ml* of 1% aqueous solution of carrageenin in the left plantar aponeurosis as described by Winter *et al.* (12). Paw volume measurements were done by mercury displacement in a manometric assembly. Oedema was assessed at 3 hr following carrageenin insult.

*Local irritancy test (10) :* PK was concentrated by freeze drying to give 1 *mg/ml* and 10 *mg/ml* solutions. 0.1 *ml* of such solutions was injected in plantar aponeurosis of rats. Control animals received DW similarly. Changes in paw volumes were determined at 1, 2 and 3 hr post-injection.

*Involvement of adrenergic receptors :* Rats, both control (DW) and PK treated (100 *mg/kg* x 3 days, oral) were administered either phentolamine mesylate (100 *mg/kg*) or propranolol HCl (25 *mg/kg*) 1 hr after last dose of PK (or DW) on third day 1 hr later the animals were subjected to carrageenin insult. For studies involving adrenergic denervation, a day after injection of 6-hydroxy-dopamine (6-OHDA, 100 *mg/kg*, ip), the animals were given either PK (100 *mg/kg*) or DW for 3 days. On 3rd day (2 hr after the last dose of PK or DW) the animals were given either (dl) - isoprenaline HCl (0.5 *mg/kg*, i.p ) or ephedrine sulphate (100 *mg/kg*, i.p.). Half an hr after these treatments, they were subjected to carrageenin pedal oedema test.

The technique to assess any extraneuronal uptake blocking action of PK was based on principle described by Alfonso and O'Brien(1). The rats were given DW or PK (100 *mg/kg*, daily, for 3 days). Two hours after the last dose the animals were decapitated. Three slices

about equal in size were taken from lungs of each rat and were rinsed with 100 *ml* of oxygenated mammalian Ringer solution for 2 min. 5 *ml* of Adrenaline HCl solution (100 *μg/ml*) was taken in a series of cuvettes. Weighing the cuvetts before and after addition gave the weight of the added tissue. Sharply at 5 min, the lung tissue was removed. The fluid samples were bioassayed (with concurrent reference adrenaline solution) by examining heart rate-increments in isolated frog heart preparation caused by 0.2 *ml* of each solution. Differences in tachycardia were calculated as a function of incubation with 100 *mg* of lung tissue. Relative reductions in adrenaline concentrations exhibited by lung tissue from control and PK-treated rats were determined to indicate extraneuronal tissue uptake.

*Castor oil diarrhoea (6) :* Groups of animals received either DW or PK for 3 days. On third day (2 hr after the last dose) they were administered 1 *ml*/100 *g* of castor oil orally. Rats were then kept individually in cages with a wiremesh floor covered by white paper. At 1, 2, 3 and 4 hr the papers were changed. Number of characteristic droppings on the papers were noted for each animal.

Student t-test was used to analyse the results.

## RESULTS

*Local irritant effect of PK :* No inflammatory swelling was seen after plantar injections of either concentrations of PK.

*Adrenergic manipulations :* Phentolamine tended to inhibit and propranolol potentiated the inflammatory response to carrageenin in control animals; the changes were not, however, statistically significant. Propranolol but not phentolamine treatment significantly antagonised the anti-inflammatory effect of PK treatment (Table I-groups I and III).

In 6-OHDA treated rats PK treatment failed to produce any significant anti-inflammatory effect. Both isoprenaline and ephedrine tended to reduce the inflammatory oedema in such rats; the reduction was statistically significant with ephedrine only. In 6-OHDA treated rats that received PK treatment, isoprenaline or ephedrine administration was associated with greater anti-inflammatory effect than that seen in the DW controls. In the doses used in the study, ephedrine effect was more prominent than that of isoprenaline (Table I-groups II and IV).

Incubation with lung tissue led to significant loss of potency of adrenaline solution as deduced from rate increment in isolated frog heart. The loss in such potency of samples incubated with lung of PK treated rats was comparable to that due to the DW controls (Table II).

TABLE I : Effect of pharmacologic manipulations of adrenergic function on carrageenin-induced paw oedema in control and PK (water soluble fraction of alcoholic extract of P. Kurroa) treated albino rats.

| *Group* | | *n* | *Pretreatment* A | *Pretreatment* B | *% increase in paw volume (3 hr) Mean±SEM* | *% difference (over control)* |
|---|---|---|---|---|---|---|
| *CONTROL* (Distilled water) | | | | | | |
| I | a | 5 | None | None | 33.4±3.2 | |
| | b | 8 | ,, | Phentolamine 100 *mg/kg* | 28.9±2.9 | 13.5% (Ia) |
| | c | 9 | ,, | Propranolol 25 *mg/kg* | 42.0±4.3 | 26.5% (Ia) |
| II | a | 6 | 6-OHDA 100 *mg/kg*, ip | None | 40.24±4.2 | |
| | b | 9 | ,, | Isoprenaline | 29.4±4.6 | 27% (IIa) |
| | c | 7 | ,, | Ephedrine | 27.32±3.9 | 32% (IIa)* |
| *PK TREATED* | | | | | | |
| III | a | 6 | None | None | 17.6±3.9 | |
| | b | 7 | ,, | Phentolamine 100 *mg/kg* | 15.6±4.1 | |
| | c | 7 | ,, | Propranolol 25 *mg/kg* | 32.6±4.3 | (IIIa)** |
| IV | a | 5 | 6-OHDA 100 *mg/kg*, ip | None | 38.21±3.6 | |
| | b | 6 | | Isoprenaline | 25.3±2.9 | 30% (IVa) |
| | c | 6 | | Ephedrine | 21.62±5.8 | 43% (IVa)* |

A indicates treatment given one day prior to institution of 3 day oral regimen with DW or PK.

B indicates drug treatments given 1 hr after completion of 3 day oral regimen with DW or PK in groups I and III and 2 hr after in groups II and IV.

*$P < 0.05$; **$P < 0.025$

TABLE II : Increment in rate of isolated frog heart with adrenaline solution, and effect of incubation with rat lung-slices.

| *Group* | *n* | *Increments in Heart Beats/Min. Mean±SEM* | *P Value with Reference Group* |
|---|---|---|---|
| I Reference Solution | 10 | 14.4±1.3 | |
| II Solution incubated with lung slices | | | |
| A. DW treated | 5 | 11.2±0.7 | <0.05 |
| B. PK treated | 5 | 10.6±1.1 | <0.05 |

*Castor oil diorrhea :* Castor oil effect was maximal at 2 hr. The diarrhoeal droppings in PK treated rats were more frequent (difference 16 4% $P<0.05$) than the DW control at this time. The maximal effect was also prolonged by PK to 3 hr. Thus both the intensity and the duration of diarrhoea was increased by PK treatment.

## DISCUSSION

Earlier studies revealed that the delayed anti-inflammatory effect of PK was partially mediated through a catecholaminergic mechanism (7). Lack of irritant effect negates possibility of anti-inflammatory effect through counter irritant action of PK. The same observation also rules out any major direct cytotoxicity and mediator depleting action in PK as speculated by others (5), e.g. clinical anti-asthmatic effect of rhizome powder was preceded by initial aggravation of symptoms and prolonged treatment reduced pulmonary histamine content in guinea pigs (5, 9). On the other hand, the observation is in agreement with the reported lysozomal stabilizing and mast cell stabilizing effect of PK (7, 8). The apparent controversy may be due to the fact that crude rhizome powder was used by others while we used extracted PK in this study. A small inhibition of inflammatory responses by an α-adrenoceptor antagonist, phentolamine and potentiation by a β-adrenoceptor blocking agent propranolol, is in agreement with the literature (11). The demonstration that propranolol significantly reduced while phentolamine tended to increase the anti-inflammatory effect of PK may suggest a role of endogenous anti-inflammatory mechanism primarily involving β-adrenoceptors and/or augmentation of subsequent cellular biochemical events.

That the anti-inflammatory effect of PK depends upon functional adrenergic neurones was evident in 6-OHDA pretreatment experiments. Similar observation is made in reserpinized rats (7). That isoprenaline and ephedrine had some inti-inflammatory effect in control and PK treated rats agrees with the concept that sympathetic activation (more precisely, β-receptor activation) is concerned with anti-inflammatory effect. A better effect of ephedrine seen here could just be due to difference in bio-equivalence of doses used. PK treatment and ephedrine were synergistic in-anti-inflammatory effect after 6-OHDA treatment; this localises the site of action of PK in target cell rather than in prejunctional nerve elements. Lack of similar synergism between PK and isoprenaline was intriguing but it could be due to extensive catabolism of the latter by COMT. PK itself does not seem to alter catecholamine metabolism since isoprenaline had a comparable inti-inflammatory effect in control and PK treated groups (Table I). No proof also was obtained to suggest that PK blocks extraneuronal uptake of catecholamines.

PK treatment did not reduce castor oil diarrhoea, which is reduced by inhibitors of prostaglandin spnthesis/action (2). This rules out mediation of PK effects through alteration of arachidonate metabolism.