

## EFFECT OF ADMINISTRATION OF ISATIN ON ALKALINE PHOSPHATASE OF VARIOUS ORGANS OF RAT

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**Summary:** *In vivo* administration of isatin (200 mg/kg) significantly lowered the activity of rat kidney alkaline phosphatase after 5 hr but enhanced the activity of rat duodenal and jejunal enzyme after 2 and 5 hr ( $P < 0.01$ ). The increased activity of the duodenal and jejunal alkaline phosphatase might be due to the induction of the enzyme by isatin.

**Key words:** isatin alkaline phosphatase rat organs induction

### INTRODUCTION

Isatin (2,3-dioxo-indoline) has been found to be a potent and non-toxic anticonvulsant agent against both supramaximal and hyponatraemic electroshock seizures in rats (4). Intravenous administration of the drug did not produce any effect on the blood pressure and respiration in dogs and cats (4). Isatin can cross the blood-brain barrier and its active 3-keto group can bind free ammonia which might possibly be one of the causative factors of epileptic seizures (6). It was also reported that isatin antagonized electrical and pentylenetetrazole tonic seizures in mice (5).

Isatin has also been found to be a modifier of monoamine oxidase (5) and acid and alkaline phosphatases (9,10). The present study describes the *in vivo* effect of isatin on alkaline phosphatase activity in various organs of rat. The mechanism of isatin action on duodenum and jejunum alkaline phosphatase has also been studied.

### MATERIALS AND METHODS

Eighteen male albino rats (150-200 g each) were divided into three groups of six animals each. The animals were fasted overnight and were provided with water *ad libitum*. Isatin was administered orally in a single dose of 200 mg/kg body weight. This dose was found to elicit anticonvulsant effect against electroshock seizures in rats (4). Rats of the first group were killed without the administration of isatin to serve as control, while those of groups II and III were killed after 2 (the peak period of isatin action as anticonvulsant) and 5 hr of isatin administration respectively. The various tissues were removed promptly and homogenized (liver 10%; heart 5%; kidney 0.5%; duodenum, jejunum and brain 2.5% w/y) in ice-cold distilled water. The homogenates were centrifuged at 3000 x g for 15 min and the clear supernatants were used for the assay of the enzyme activity.

Alkaline phosphatase was assayed by the method of Kind and King (3). The reaction mixture comprised of 1.0 ml carbonate-bicarbonate buffer (100 mM) of pH 9.4, 1.0 ml of 0.01 M disodium phenyl phosphate as the substrate and 0.1 ml of the supernatant. This mixture was incubated for 15 min at 37°C and the reaction was stopped by addition of 0.8 ml sodium hydroxide (0.5 N). The tubes were thoroughly shaken and 1.2 ml of sodium bicarbonate (0.5 N), 1.0 ml of 4-amino antipyrine (0.6 %) and 1.0 ml of potassium ferricyanide (2.4%) were added and the colour was immediately read at 520 nM.

To delineate the mechanism of isatin activation of the duodenal and jejunal alkaline phosphatase, rats were divided into four groups of six animals each and fasted overnight. The control group was injected 1.0 ml of 0.15 M NaCl. The second group was orally fed with isatin (200 mg/kg body weight) in 1.0 ml of 0.15 M NaCl. The third group was injected 300 µg cycloheximide (in 1.0 ml of 0.15 M NaCl) and the fourth group was injected 300 µg cycloheximide (in 1.0 ml of 0.15 M NaCl) as well as fed orally with isatin (200 mg/kg). The animals were sacrificed after 2 hr of the treatment, the duodenum and jejunum removed, and alkaline phosphatase activity was determined as described above.

## RESULTS

Table I shows the effect of isatin on alkaline phosphatase activity of various organs. The maximum and minimum enzyme activities were noted in the duodenum and liver respectively,

TABLE I: Alkaline phosphatase activity of rat tissues after 2 and 5 hr of isatin (200 mg/kg body weight) administration.

Organ	Enzyme activity*			% variation	
	0 hr.	2 hr	5 hr	2 hr	5 hr
Liver	0.002711 ±0.000040	0.003318 ±0.000070	0.003260 ±0.000055	+22.38	+20.25
Kidney	0.2873 ±0.0062	0.2670 ±0.0071	0.1851** ±0.0096	-7.06	-35.57
Heart	0.02740 ±0.0053	0.02259 ±0.0031	0.02661 ±0.0048	-17.50	-2.70
Duodenum	0.3890 ±0.0055	0.0693** ±0.0032	0.8422** ±0.0050	+56.63	+116.50
Jejunum	0.1172 ±0.0058	0.2331** ±0.0061	0.2758** ±0.0082	+98.89	+135.32
Brain	0.009224 ±0.0028	0.01025 ±0.0036	0.01033 ±0.0051	+11.12	+11.99

\* µmoles of phenol liberated/min/mg protein.

\*\* (P < 0.01)

while the overall order was duodenum > kidney > jejunum > heart > brain > liver. The data show that isatin had no uniform pattern of action on alkaline phosphatase activity of different organs. The activities of brain, heart and liver alkaline phosphatase showed insignificant change whereas the kidney enzyme was significantly lowered after 5 hr and those of duodenum and jejunum showed significant increases after 2 and 5 hr of isatin administration.

Cycloheximide, when injected alone, was found to lower duodenal and jejunal alkaline phosphatase activities by 55.2 and 61.7% respectively (Table II). Further, when isatin was administered along with cycloheximide, the enzyme activity was still half of that of the control values.

TABLE II: Effect of cycloheximide and isatin administration on duodenal and jejunal alkaline phosphatase.

Group	Compound injected	Enzyme activity*	
		duodenum	jejunum
I (control)	Saline	0.5140	0.1584
		±0.0081	±0.0092
II	+ isatin	0.8615**	0.3202**
		±0.0111	±0.0043
III	+ cycloheximide	0.2301**	0.06057**
		±0.0016	±0.0036
IV	+ cycloheximide + isatin	0.2990**	0.07891**
		±0.0012	±0.0041

\*  $\mu$ moles of phenol liberated/min/mg protein.

\*\* ( $P < 0.001$ ).

## DISCUSSION

The *in vivo* effect of isatin on alkaline phosphatase activity of various organs of rat suggests its organ-specific action. These results confirm the well established heterogeneity of alkaline phosphatase (1). Further, the results obtained with intestinal alkaline phosphatase are in contrast to the *in vitro* results which have shown that this enzyme is inhibited by isatin (7). Similar differences in chick brain acid and alkaline phosphatases have been reported by Singh *et al.* (8). This difference in behaviour might possibly be attributed to the decomposition of isatin in the body (2).

The observation that only the activity of intestinal alkaline phosphatase exhibited an increase also suggests that this enzyme may be different from those of other organs. This has

also been demonstrated by Fishman *et al.* (1) who showed that L-phenylalanine inhibited only the intestinal and the placental enzyme in the *in vivo* experiments.

The effect of cycloheximide on duodenal and jejunal alkaline phosphatase activities suggests that the increased activities of these enzymes by isatin might be due to induction of the enzymes.

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