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BIOLOGICAL STUDIES ON INDOLE DERIVATIVES-III
Effects of 2-Oxo-3-Indolyl Derivatives on Cardio-hepatic Enzymes and Blood Cells

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Isatin (I) and isatin-3-thiosemicarbazone (II) decreased the levels of SGOT, serum alkaline phosphatase, erythrocyte and leukocyte count, whereas isatin (I) also lowered the SGPT activity. These changes probably indicates a change in the function and physiology of liver and blockage of histogenesis.

Key words: Isatin (I)-isatin-3-thiosemicarbazone (II), Enzymological haematological effects, Rabbits.

Introduction

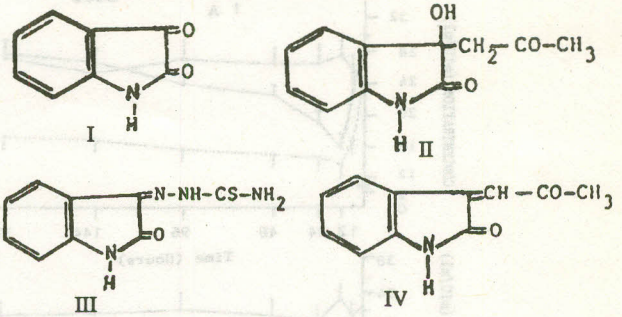
Isatin-3-thiosemicarbazone and its N-methyl derivatives have been used for many years for the treatment of various viral diseases [1,2]. These are presently being used in the prophylactic measures against small-pox, alastrim and eczema [3,4]. Mueller *et al.* [5] reported that indole-2, 3-dione (I) and indole-2-one are Monoamine oxidase (MAO) inhibitors in rats, while indole-2,3-dione also possessed epileptogenic activity [6]. Popp *et al.*[7] have reported that certain isatin derivatives exhibited a marked anticonvulsant activity. Recently we reported the effects of 3-hydroxy-3-acetonyloxindole (III) and 3-acetonylidene oxindole (IV) on transaminases[8]. Fisher *et al.* [9] found that indolenes possess serotonergic activity, while Kabayashi *et al.*[10] reported the use of isatin derivatives in treating ulcer diseases.

The objective of the present investigation is to evaluate and assess the toxic effects of isatin (I) and isatin-3-thiosemicarbazone (II) on the general physiology and cellular metabolism of mammals. Since the blood and liver are the two important tissues, which a drug confronts after entry into the body, major toxicological changes are lilkely to be expressed in these two tissues. Liver is a central organ of metabolism, where biotransformation or detoxication of all the drugs occurs. The various enzymatic reactions of microsomal enzymes in the process disturb the normal hepatic physiology and structure, which is relfected in various molecular and cytological abnormalities. The hepatic cell plasma membranes may rupture and thus allow leaching out of hepatic enzymes in the blood [11-13]. Blood, therefore, is a general indicator of various metabolic abnormalities.

The serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) which represent protein metabolism, and an energy yielding enzyme alkaline phosphatase are the liver function parameters. In the present study the levels of these enzymes have been estimated alongwith determination of blood cell count in rabbits under the influence of 2-oxo-3-indolyl derivatives to assess the toxicity of these compounds.

Experimental

Synthesis of isatin-3-thiosemicarbazone. Thiosemicarbazide (9.1 gm) was taken into the thimble of soxhlet extractor fitted on a 250 ml round bottom flask which contained isatin (14.7 gm) and ethanol (150 ml). Reaction mixture was refluxed for 8 hrs till all the thiosemicarbazide was consumed. On cooling to room temperature, bright yellow crystals of isatin-3-thiosemicarbazone (II) separated out were collected and washed with ice cold ethanol (3 x 25 ml). Recrystallization from ethanol furnished II (12.85 gm) m.p. 223° (Lit. 223°)[14].



Pharmacological studies. The present study was undertaken on healthy male domesticated rabbits *Oryctolagus cuniculus* with an everage body weight of 0.85–1.15 kg. The animals were kept for two weeks in the animal house of the faculty of Pharmacy, Punjab University , for acclimatization. During acclimatization period fresh green clover and tap water were provided *ad libitum*. The animals were divided into three groups having six animals in each. A zero hr. blood sample was taken from all the animals for analysis. Similarly total leukocyte count (TLC) and total erythrocyte count (TEC) were also done for all the rabbits before the administration of the drugs.

Isatin (I) in the dose of 250 mg/kg body weight was administered orally to the group-I for five consecutive days. Under similar conditions isatin-3-thiosemicarbazone (II) was

given to the group-II. After last dosing, the blood samples were taken 6,12,24,48,96,144 and 192 hrs after the last dose (5th dose) for enzymological studies and 2,4,6,12,24,48,96,144 and 192 hrs after the last dose for haematological studies. The blood samples were drawn from the marginal ear vein of the rabbits. The serum was separated for analysing the activity of SGOT, SGPT[15], serum alkaline phosphatase[16] and total protein[17]. TLC and TEC were also determined from the blood by standard techniques as described by Hall and Malia[18]. Group-III was placebo group and received empty hard gelatin capsule. The placebo readings and zero hr. readings of group I and II did not show any significant difference in all the biochemical parameters. So the effect of the drug was compared with the zero hr. reading of the same group.

Statistical analysis. Mean values of all the parameters were calculated. Analysis of variance were applied to check the significance[19].

Results and Discussion

Oral administration of isatin (I) and isatin-3-thiosemicarbazone (II) affected the activity of the important cardio-hepatic enzymes such as AP, SGOT and SGPT(Fig. 1). The mean zero hr. values for different enzymes from group-I were AP, 30.23±1.59 mIU/ml., SGOT 28.73±1.50mIU/ml., SGPT 27.65±2.00 mIU/ml, total protein 5.93±0.35 gm/100ml while in group-II the values were AP, 28.27±2.39 mIU/ml, SGOT 25.02±1.53 mIU/ml, SGPT 26.73±1.58 mIU/ml and total protein 4.78±0.92 gm/100ml. Both the drugs decreased the activity of AP and is lowest at 6 hrs after the last dosing. Then the values started to increase and returned to normal at the end of the experiment. Both the drugs decreased the activity of SGOT and was lowest at 12 hrs. In group-I the values returned near to normal at the completion of experiment but in group-II the SGOT values remained below normal. Isatin (I) significantly decreased the activity of SGPT, which was maximum at 12 hrs while isatin-3-thiosemicarbazone (II) did not alter the activity of SGPT. Both the drugs did not change the total protein contents significantly.

The mean zero hr values for TLC and TEC were 10.47±0.52 x 10^3/ml and 5.11±0.38 x 10^6/ml in the group-I and 9.98±0.31 x 10^3/ml and 4.67±0.15 x 10^6/ml with group-II. Both the drugs decreased the TLC and TEC. This decline was mximum at 2 hrs with isatin and at 4 hrs with isatin-3-thiosemicarbazone. Recovery in the TEC was noticed with both the drugs and the count returned to normal at the end of the experiment.

Analysis of variance revelealed that in case of isatin (I), the different time intervals, type of serum parameters and their interactions are significant for P = 0.05. Similarly for isatin-3-semicarbazone (II) the serum parameters and the interaction between time intervals and serum parameters are significant

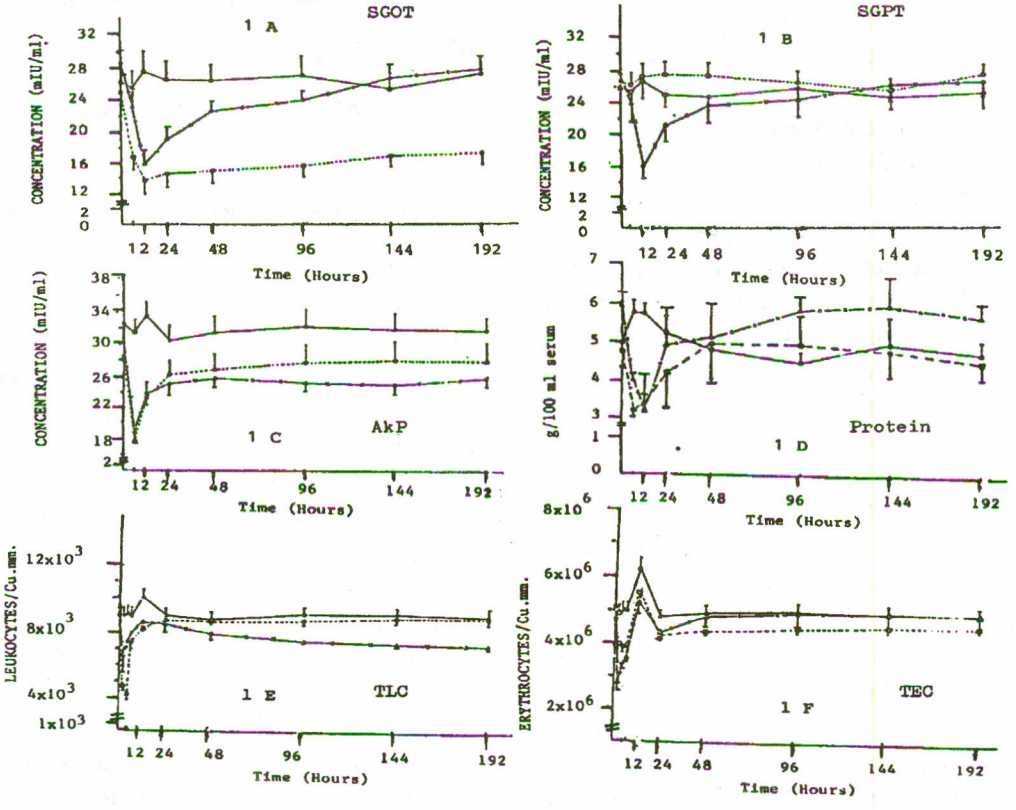


Fig. 1. Effect of (250 mg/kg body weight) isatin (Δ—x—Δ) and (250 mg/kg body weight) isatin-3-thiosemicarbazone (□---- -□) on SGOT (1A), SGPT (1B), serum alkaline phosphatase (1C), total protein (1D), total leucocyte count (1E) and total erythrocyte count (1F) of rabbits. (control o—o).

TABLE 1. EFFECT OF ORAL ADMINISTRATION OF ISATIN (250 mg/kg) ON DIFFERENT SERUM PARAMETERS AT VARIOUS TIME INTERVALS.

Serum parameters	Time									
	0 hr.	2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	144 hrs.	192 hrs.
SGOT level (mIU/ml)	28.73 ±1.50	–	–	23.13 ±2.37	15.93 ±1.77	19.10 ±1.54	22.67 ±1.30	24.02 ±1.20	26.75 ±1.63	27.82 ±1.56
SGPT level (mIU/ml)	27.65 ±2.00	–	–	23.90 ±2.10	16.23 ±1.85	21.20 ±2.00	23.60 ±2.13	24.43 ±2.26	26.12 ±1.98	26.57 ±1.87
AP (mIU/ml)	30.23 ±1.59	–	–	18.83 ±1.27	23.77 ±1.56	25.10 ±1.45	25.82 ±1.10	25.33 ±1.14	25.00 ±1.22	25.90 ±1.15
Total Protein (gm/100 ml)	5.93 ±0.35	–	–	4.01 ±0.19	3.16 ±1.01	4.93 ±0.975	5.15 ±0.84	5.89 ±0.35	6.01 ±0.75	5.75 ±0.39
TLC ($z \times 10^3$/ml)	10.47 ±0.52	7.52 ±0.76	8.47 ±0.40	8.96 ±0.40	9.56 ±0.40	9.45 ±0.44	8.97 ±0.31	8.58 ±0.18	8.44 ±0.17	8.34 ±0.16
TEC ($z \times 16^6$/ml)	5.11 ±0.38	2.80 ±0.26	3.40 ±0.19	3.55 ±0.11	5.22 ±0.32	4.35 ±0.20	4.86 ±0.32	4.98 ±0.36	5.00 ±0.36	5.00 ±0.36

SGOT = Serum glutamate oxaloacetate transaminase., SGPT= Serum glutamate pyruvate transaminase., AP = Serum alkaline phosphatase., TLC = Total leukocyte count., TEC = Total erythrocyte count.

± S.E. of the mean., $F_1 = 9.375^*$ $P<0.05$, $F_2 = 342.38^{**}$ $P<0.05$, $F_3 = 2.89^*$ $P<0.05$

* Significant, ** Highly significant

TABLE 2. EFFECT OF ORAL ADMINISTRATION OF ISATIN-3-THIOSEMICARBAZONE (250 mg/kg) ON DIFFERENT SERUM PARAMETERS AT VARIOUS TIME INTERVALS.

Serum parameters	Time									
	0 hr.	2 hrs.	4 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	96 hrs.	144 hrs.	192 hrs.
SGOT (mIU/ml)	25.02 ±1.53	–	–	16.85 ±1.69	13.70 ±1.81	14.55 ±1.79	15.02 ±1.72	15.68 ±1.46	17.03 ±1.43	17.32 ±1.34
SGPT (mIU/ml)	26.73 ±1.58	–	–	26.30 ±1.62	27.30 ±1.68	27.60 ±1.64	27.53 ±1.56	26.50 ±1.54	25.47 ±1.51	27.40 ±1.27
AP (mIU/ml)	28.97 ±2.39	–	–	17.63 ±1.45	23.33 ±1.93	26.25 ±1.87	26.90 ±2.03	27.83 ±2.17	28.13 ±2.23	27.97 ±2.28
Total Protein (gm/100 ml)	4.78 ±0.42	–	–	3.20 ±0.19	3.39 ±0.25	4.23 ±0.98	5.00 ±1.01	4.98 ±0.77	4.85 ±0.69	4.56 ±0.43
TLC ($z \times 10^3$/ml)	9.98 ±0.31	5.70 ±0.27	5.20 ±0.35	8.37 ±0.35	9.20 ±0.24	9.68 ±0.29	9.70 ±0.24	9.76 ±0.25	9.94 ±0.29	9.97 ±0.29
TEC ($z \times 10^6$/ml)	4.67 ±0.15	4.00 ±0.06	3.90 ±0.18	3.97 ±0.10	5.56 ±0.20	4.25 ±0.11	4.42 ±0.14	4.52 ±0.13	4.60 ±0.14	4.64 ±0.15

SGOT = Serum glutamate oxaloacetate transaminase., SGPT = Serum glutamate pyruvate transaminase., AP = Serum alkaline phosphatase., TLC = Total leukocyte count., TEC = Total Erythrocyte Count.

± S.E. of the mean, $F_1 = 2.07$ $P>0.05$, $F_2 = 202.93^{**}$ $P<0.05$, $F_3 = 1.27^*$ $P<0.05$

* Significant, ** Highly significant

for P = 0.05; whereas all the time intervals are equally effective.

Our results partly compiled with our earlier findings[8], we observed a significant decrease in SGPT activity and an increase in SGOT activity with 3-hydroxy-3-acetonyl oxindole (III). According to Cohen [20] pyridoxal phosphate is the co-enzyme for transaminases. He found that tissues of pyridoxine deficient rats have a low transaminase activity, so it may be possible that both the drugs effected this co-enzyme which could be responsible for lowering the activity of transaminases. But the other possibilities such as inhibition of the synthesis of the enzymes or its substrate cannot be ruled out. These changes in the enzymes activities were probably due to the occurrence of a change in the function and physiology of liver and muscles. Detailed studies are required to put more light on these effects. Amongst transaminases, the SGPT is quickly recovered (within 2 days) while it takes 4 days for the recovery of normal level of SGOT in the isatin treated group and no recovery of isatin-3-thiosemicarbazone treated group. Both the drugs perhaps block alternate source of energy, specially through glucoreogenesis by inhibiting the enzymes. The SGPT is recovered soon, because of rapid excretion/metabolism of drug, while SGOT is damaged enough to inhibit the recovery. Akp probably is the most sensitive indicator of toxicity. This is very broad based enzyme, which though suggests some damage, but not the exact site of action. Akp is reduced, which could be becuase of enzyme inhibition thus leading to lowering of metabolic activity.

The lowering effect of (I) on TLC was marked and the values remained significantly low even upto the end of the experiment while the TLC values became almost normal in group-II which was administered isatin-3-thiosemicarbazone. It may be possible that both the drugs sensitized the bone marrow on the basis of an allergy which resulted in low count of TEC. This mechanism of reduction in TEC by bone marrow depression was observed by Mark *et al.* [21] and Penny *et al.* [22] with one week treatment of chloramphenical. But the mechanism of lowering the TEC similar to penicilline as explained by Korlkovas and Burckhalter [23] cannot be ruled out. These authors have reported that the reduction of TEC was due to chelating property of penicilline with haemoglobin. It is possible that the metabolites of the drugs I and II form chelates with iron of haemoglobin and resulted in the reduction of TEC.

In conclusion the drug I and II probably indicates a change in the function and physiology of liver/muscle and blockage of histogenesis. Both the drugs were well tolerated and no untoward incident of restlessness, respiratory distress, convulsion or mortality took place among the animals during the experimental period.

During this investigation the biotransformation and biopharmaco-kinetic studies of isatin have been made and detailed biotransformation mechanism has been reported elsewhere [24].

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