Summary & Conclusions
Isatin (2,3-dioxoindoline) is a naturally occurring metabolite with a distinct distribution in various rat tissues and has been detected in human urine also (Watkins et al., 1990). Isatin is linked with conditions of stress and anxiety in rat and man. It is assumed to play certain specific physiological functions in the brain (Glover et al., 1991). Pharmacologically, isatin exhibits a wide range of actions. Among these, its anticonvulsant action has been extensively studied in various types of experimentally induced seizures. Still, few studies have been carried out to delineate the mode of its pharmacological actions and to understand its specific physiological functions.

The objectives of this study were to investigate the effect of isatin on systems which are essential for normal functioning of CNS and are associated with conditions of stress, anxiety and convulsions. Therefore, in vitro effects of isatin and its derivatives were studied on neurotransmitter catabolizing enzymes, MAO and AChE. The effects of isatin and its derivatives were also studied on the activity of Na,K-ATPase, which controls the movement of ions across the cell membranes and is essential for nerve-conduction. The findings of in vitro studies were extended to in vivo experiments, to examine whether isatin exhibits effects similar to those observed under in vitro conditions. The levels of 5-HT and 5-HIAA and various brain lipids were
studied following oral administration of isatin at different doses. The findings of these studies are summarized below.

**In vitro Studies**

1. DAB was used as the substrate to assay MAO activity and it was found to be selective for MAO-B. Thus, MAO-B was assayed in these studies.

2. Isatin was a potent inhibitor of MAO activity *in vitro* with 47% inhibition at 10 μM concentration. 5-MeI showed 72% inhibition at the same concentration, while 5-BrI caused 74% inhibition at 5 μM concentration. HAO and HPO were less effective while 5-ISA was inert as an inhibitor of MAO.

3. The kinetics of inhibition of MAO activity by isatin was of mixed type and reversible in nature. At pH 7.6, 10 μM isatin increased the $K_m$ of MAO by 53%, while reducing $V_{\text{max}}$ by 54%. The values of $K_{ic}$ and $K_{iu}$ were 4.28 and 8.46 μM, respectively.

4. The effect of pH on $K_m$ and $V_{\text{max}}$ of MAO activity showed four pK values: 5.5, 6.2, 7.0, and 9.0. The degree of enzyme inhibition by isatin varied with pH of the assay system.

5. The degree of inhibition of MAO activity by isatin was strongly dependent upon temperature. The enzyme inhibition was reduced from 65% at 24°C to 44% at 37°C. At 40°C and above, isatin protected the enzyme from heat.
denaturation. 5-BrI and 5-MeI also showed heat protection of the enzyme activity. The degree of heat protection had direct relation with the degree of inhibition of the enzyme by the isatins. 5-ISA was inert, it neither inhibited the enzyme activity nor protected it from heat denaturation. From Arrhenius analysis, Ea of the enzyme was calculated to be 14.2 and 18.5 kcal/mole in absence and in presence of 10 μM isatin, respectively.

6. On the basis of these findings, it was concluded that isatin binds with sulfhydryl groups of the enzyme protein, which was responsible for its inhibitory action as well as protection at higher temperatures.

7. Isatin inhibited rat brain AChE activity by 41% at 1.0 mM and by 57% at 1.5 mM concentrations. 5-BrI and 5-MeI at 1.0 mM concentration showed 48% inhibition of the enzyme activity. 5-ISA, HAO and HPO were essentially inert towards AChE activity in rat brain.

8. Rat erythrocyte AChE activity was inhibited by 50 and 67% at 1.0 and 1.5 mM concentrations of isatin respectively. 5-BrI and 5-MeI showed only 37 and 39% inhibition respectively at 1.0 mM concentration. 5-ISA, HAO and HPO were again inert under these conditions.

9. The kinetics of inhibition of rat brain AChE activity by isatin was of mixed type and reversible in nature. The value of apparent K_m was enhanced by 31% and that of
$V_{\text{max}}$ was reduced by 42%. The values of $K_{i_c}$ and $K_{i_u}$ of enzyme inhibition by isatin were 0.833 and 1.167 mM, respectively.

10. Inhibition of AChE activity by isatin as a function of pH was maximum at pH’s 8.5 and 9.0. The enzyme inhibition was reduced with decrease in pH of the assay system.

11. Isatin inhibited the enzyme activity by 47% at 25°C and 29% at 37°C. Arrhenius plot showed biphasic curve with $T^\circ C$ of 31.6 and 30°C in absence and presence of 1 mM isatin, respectively. The activation energy (Ea) of the enzyme was 4.74 and 1.85 kcal/mole in absence of isatin and 12.2 and 4.2 kcal/mole in presence of isatin, above and below $T^\circ C$, respectively.

12. Effect of a few group specific reagents was also studied on AChE activity in absence and presence of 1mM isatin. The enzyme activity was inhibited to the tune of 42% by 0.25 mM iodine, which was enhanced to 50% on addition of 1 mM isatin. However, at 0.5 mM concentration of iodine, the enzyme was completely inhibited and addition of isatin reduced the inhibition to 82%. At 0.05 μM eserine concentration, addition of 1 mM isatin reduced the inhibition from 54 to 40%. At higher eserine concentrations, isatin did not show any effect on enzyme activity. In presence of 25 mM nitrous acid, 1 mM isatin increased the enzyme inhibition from 33 to 43%. However,
there was no effect in presence of higher nitrous acid concentrations. Addition of isatin together with formaldehyde showed an additive inhibition at all the concentrations of formaldehyde (100-400 mM) used.

13. Isatin inhibited the Na,K-ATPase activity by 70% at 10 mM concentration, while Mg-ATPase and total-ATPase activities were inhibited by 79 and 74% respectively. 5-MeI showed 47, 55 and 52% inhibition of Na,K-ATPase, Mg-ATPase and total-ATPase activities respectively, at 10 mM concentration. HPO and 5-ISA also produced a similar degree of enzyme inhibition. HAO was less effective producing 25, 41 and 29% inhibition of Na,K-ATPase, Mg-ATPase and total-ATPase respectively. Among these isatins, 5-BrI was the most potent inhibitor of the activities of brain ATPases. It inhibited the enzyme activities by 50% at 2mM concentration.

14. Isatin and its derivatives showed varying effects on alkaline phosphatase activity when it was assayed using different substrates. With disodium phenylphosphate as substrate, alkaline phosphatase activity was inhibited in brain, kidney, liver, intestine and serum of rats by 40, 49, 49, 34 and 26%, respectively. However, with disodium β-glycerophosphate as substrate, the enzyme activity was stimulated (18 and 38% in serum and intestine, respectively) by isatin. The inhibition of serum and intestinal alkaline phosphatase activities by
isatin and its derivatives was small compared to that of other tissues.

15. The results of in vitro studies showed that certain structural characteristics of the isatin molecule are essential for its inhibitory effects on enzymes. These include a free 3-oxo group and a substituent like -Br or -CH$_3$ group at C-5 position. Sulphonic acid group at C-5 renders isatin less effective. The structural resemblance of isatin with enzyme substrate and hydrophobicity of the enzyme active site are other favourable features of isatin-enzyme interactions.

in vivo STUDIES

Isatin was administered orally to rats at effective anticonvulsant doses of 150 and 300 mg/kg b. wt. for different periods. The results are summarized below.

1. Administration of isatin to rats at 300 mg/kg for acute studies (2h, 6h and 24h) did not change MAO activity in the brain. Chronic treatment at this dose level for 10 and 20 days also did not affect the enzyme activity. However, a significant increase (p<0.01) of 20% in enzyme activity was observed when isatin was given at a dose level of 150 mg/kg for 15 days.

2. There was no change in brain AChE activity in rats given either acute or chronic isatin treatments. Similarly, erythrocyte membrane AChE activity remained unaffected under these conditions.
3. Both chronic and acute isatin treatments of the rats did not affect the activities of Na,K-ATPase, Mg-ATPase or total-ATPase in the brain.

4. The levels of brain 5-HT were significantly increased by 27% (p<0.001) after 2 h and 15% (p<0.005) after 6 h of single dose of isatin (300 mg/kg) to the rats. The same dose when given for 10 and 20 days did not alter the 5-HT levels. This showed that isatin exerts a short-lived effect on brain 5-HT which corresponds to its peak action time as an anticonvulsant in the MES test. The chronic isatin treatment did not cause any long-lasting effect on the levels of 5-HT.

5. Brain 5-HIAA level was unaffected in rats fed 300 mg/kg isatin for 2 h, 6 h and 10 days. However, after 20 days, a significant decrease (p<0.001) of 25% was observed.

6. Administration of isatin at 150 mg/kg for 10 days did not alter the levels of total lipids, phospholipids, cholesterol, glycolipids, gangliosides and plasmalogens in rat brain. Continuation of the isatin treatment for 20 days also did not significantly induce changes in the lipid levels.

7. There was a 19% increase (p<0.01) in total lipids and phospholipids, 10% increase (p<0.01) in cholesterol and 17% increase (p<0.01) in glycolipids with no change in gangliosides when rats were fed with 300 mg/kg isatin for 10 days.
Oral administration of isatin (300 mg/kg) for 20 days had no significant effect on total lipids, phospholipids, cholesterol and gangliosides of the brain. However, the levels of glycolipids \( (p<0.01) \) and plasmalogens \( (p<0.001) \) were significantly increased under these conditions.

These findings indicate that while isatin is a potent inhibitor of MAO, AChE and ATPases \textit{in vitro}, it does not have similar effects under \textit{in vivo} conditions. Isatin has been shown earlier also to affect the activities of several other enzymes and transport systems for glucose and amino acids (Glover et al., 1991) \textit{in vitro}. Its opposite effects under \textit{in vivo} conditions were attributed to its possible metabolism in the body (Ichihara et al., 1956). Several other possibilities could also be responsible for the lack of isatin effects on enzymes \textit{in vivo}. These include its possible conversion into isatinic acid and its inability to reach its site of action in sufficient concentration due to limited solubility in aqueous cytoplasm. Because of its reversible binding to enzyme protein, it is also possible that inhibition is not detected since it gets diluted in the assay system (Yuwiler, 1990).

Under \textit{in vivo} conditions, isatin is very selective in its actions. Alterations in the levels of glycolipids and plasmalogens on chronic isatin treatment may be of significance since plasmalogens are characteristic lipids of nervous
system and glycolipids play a significant role in normal CNS functioning. Similar effects of isatin on brain serotonin levels have been observed by McIntyre and Norman (1990). It is possible that its effects on serotonergic system may be responsible for some of its CNS affecting properties.