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# Changes in enzyme levels in male albino rats due to effect of novel heterocyclic compound

**M. ANIL KUMAR**, M.KRISHNA REDDY AND M.SARANGAPANI

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See end of the article for authors' affiliations

Correspondence to :
**M. ANIL KUMAR**
Reproductive Physiology Unit, Department of Zoology, Kakatiya University, WARANGAL (A.P.) INDIA

## ABSTRACT

The present study is designed to study the effect of a novel heterocyclic compound on LDH, SDH and GDH activities in different tissues to understand the enzyme levels and their role in male albino rats. The treatment with ethyl - 1 - (2 - oxoindolin - 3 – ylideneamino) - 1,2,3,6 – tetrahydro - 4 – methyl - 2 – oxo - 6 - phenyl pyrimidine - 5 - carboxylate compound at dose level of 50 mg/kg/b.wt. for 21 days did cause a mild change in enzyme levels in reproductive and somatic tissues of treated rats. In order to assess physiological changes in testis, brain, heart, liver and kidney of rats, estimation of LDH, SDH and GDH activities has been under taken. The result showed a mild increase in LDH and SDH levels but in contrast, same treatment caused a mild decrease in GDH levels.

**Key words :** Heterocyclic compound, Physiological changes, Reproductive tissue, Somatic tissues, LDH, SDH, GDH

The development of new drug from heterocyclic compounds is an attractive proposition because heterocyclics are widely utilized compounds in both pharmaceutical and agricultural fields (Lang and Lin, 1984). In the present study, an attempt has been made to evaluate the effect of ethyl -1 - (2 - oxoindolin - 3 – ylideneamino) – 1,2,3,6 – tetrahydro - 4 – methyl - 2 – oxo - 6 -phenyl pyrimidine - 5 - carboxylate compound on LDH, SDH and GDH enzyme levels of rat. In previous work this novel heterocyclic compound has resulted reduction in sperm count (Anil Kumar, 2009) and also it showed cytotoxic activity (Ajitha, 2009). Further, it kas also been tried same compound weather it has any adverse effects on enzyme levels in somatic and reproductive tissues of rats such as testis, liver, kidney, heart and brain.

The novel heterocyclic compound developed in the medicinal chemistry laboratories, University College of Pharmaceutical Sciences, Kakatiya University, Warangal was selected for the present study. This compound is prepared adopting the appropriate methods available in literature and is characterized by spectral data. The new compound possessing pyrimidine moiety because of structural similarities with nucleic acid bases exhibits various biological activities. Literature revealed that indole derivative exhibits aldose reductase inhibition activity along with other biological activities keeping in view of biological significance of indole moiety and pyrimidine moiety present in the new compound so it has been planed to study the effect of this new compound on LDH, SDH and GDH enzyme activities adopting standard protocols available in literature.

## MATERIALS AND METHODS

### *Animals :*

Wistar strain male albino rats weighing about 180-230 g of age group of 16-18 weeks old, were housed in polypropylene cages under controlled conditions (25°± 2°C and 12hr photoperiod) and were provided standard pellet food (Agro Corporation Pvt. Ltd., Bangalore, India) and tap water *ad libitum*. Experimental procedures were adopted as approved by the animal experimentation ethics committee and maintained in accordance with the guidelines of the National Institute of Nutrition (NIN), Tarnaka, Hyderabad, India. The animals were used for the study after fifteen days of acclimatization.

### *Experimental design :*

Sixteen proven fertile male albino rats were divided into two groups (8 rats in each) and treated as follows.

*Group I:*

Rats served as control and they were intraperitoneally administered with 0.3 ml of vehicle (1% sodium carboxy methyl cellulose) for 21 days.

*Group II:*

Rats were administered intraperitoneally novel

heterocyclic compound (50mg/kg b.wt./day) suspended in 1% sodium carboxy methyl cellulose for 21 days.

The biochemical investigations were performed after due standardization and were based on calorimetric method. On the 22nd day, the treated rats were sacrificed and the testis, liver, kidney, heart and brain were removed, rinsed and blotted from each sub group. They weighed and stored at -20°C for further study. Statistical analyses were carried out using students "t" test.

***Estimation of enzyme levels:***

The foregoing enzyme assays were performed after due standardization. Aliquots of homogenates were selected such that the initial rates were approximated as nearly as possible, yet providing sufficient products to fall in a convenient range for the calorimetric measurements. All the assays were made under substrate conditions following zero order kinetics. Enzyme activities were expressed as m moles of product formed or substrate cleaved/g wet weight of the tissue/hr.

Lactate dehydrogenase activity and succinate dehydrogenase activity were estimated according to Nachlas *et al.,* (1960), as modified by Prameelamma *et al.* (1975). Glutamate dehydrogenase activity was assayed according to Lee and Lardy (1965).

## RESULTS AND DISCUSSION

As antifertility activity compound selected novel heterocyclic compound investigated for its physiological activity in male albino rats. As the acute intraperitoneal $LD_{50}$ value of compound was 500 mg/kg/b.wt. determined, ten-fold lower ($1/10^{th}$) concentration was selected as sub lethal to study the effect of the compound (50mg/kg/b.wt.). It is randam for the treatment to observe the effect of the compound because different phases of spermatogenesis are completed in 3 weeks of time.

The studies biochemical parameters help to learn more about the changes that take place in the body and to identify the potential problems at an early stage itself. Hence, an attempt has been made to study the change in activity levels of LDH, SDH and GDH enzymes to understand the impact of this compound in male albino rats. In addition to changes in sperm count, mild changes were also noticed in enzyme activity levels under the effect of this compound. It is seen that relative to control, the LDH and SDH contents increased but GDH content decreased in reproductive and somatic tissues of treated rats.

The LDH levels in the testis, brain, heart, kidney and liver increased by 8.97%, 5.63%, 4.78%, 4.56% and 5.56%, respectively and SDH levels increased by 3.86%, 5.12%, 5.34%, 3.59% and 5.83%, respectively in those tissues of treated rats as compared to control animals, where as the levels of GDH decreased by 9.31%, 12.02%, 7.58%, 6.61% and 9.48%, respectively to that of control rats (Table 1, 2 and 3).

Lactate dehydrogenase (LDH) forms the centre for a delicately balanced equilibrium between catabolism and anabolism of carbohydrates (Everse and Kaplan, 1973). LDH is a bi-directional cytoplasmic enzyme capable of reversible formation of pyruvate and lactate in all eukaryotic and prokaryotic cells (Harold, 1975). This catalytic activity utilized $NAD^+$ and NADH coenzyme forms dependent upon the nature of substrate being converted to product (Palmer, 1995).

The LDH activity level in the testis, liver, kidney, heart and brain of control rats was in the order of testis> liver> kidney> heart> brain. The results obtained on the effect of the compound on LDH activity levels in testis, liver, kidney, heart and brain are shown in Table 1. Stimulation of LDH observed in the present study indicate that the end product of glycolysis *i.e.* pyruvate was not routed through Krebs cycle but through the lactic acid cycle under hypoxic conditions leading to the accumulation of lactic acid (Tripathi *et al.,* 2003). Increase of LDH level was mainly due to the break down of glycoprotein in to lactic acid formation (Subramanian, 2009). This may be also due to increased lactate oxidation to pyruvate by the enzyme LDH, which is indicated by high level of pyruvate with a decreased lactate level. The rise in LDH level helps in oxidation of lactate.

Succinate dehydrogenase (SDH) is an important enzyme of TCA cycle which catalyzes the reversible oxidation of succinate to fumarate and it is also associated with the electron transport chain due to its ability to transfer electrons to respiratory chain. The mitochondrial

**Table 1: Effect of novel heterocyclic compound on LDH levels (µ moles of formazon formed /g of wet weight of tissue /hr) in different tissues of male albino rats**

| Group | Testis | Brain | Heart | Kidney | Liver |
|---|---|---|---|---|---|
| Control SD | 489.51 ± 12.45 | 421.39 ± 15.84 | 652.11 ± 18.62 | 535.16 ± 18.26 | 762.24 ± 24.51 |
| Treated SD | 533.45 ± 11.78 | 445.13 ± 7.39 | 683.29 ± 17.55 | 559.61 ± 12.44 | 804.66 ± 18.82 |
| PC | + 8.97 | + 5.63 | + 4.78 | + 4.56 | + 5.56 |

All the values are mean ± SD (n = 8). SD – Standard deviation PC – Per cent change over control

**Table 2 : Effect of novel heterocyclic compound on SDH levels (µ moles of formazon formed /gm of wet weight of tissue /hr.) in different tissues of male albino rats**

| Group | Testis | Brain | Heart | Kidney | Liver |
|---|---|---|---|---|---|
| Control SD | 545.22 ± 13.43 | 408.13 ± 12.44 | 502.47 ± 13.25 | 522.12 ± 16.54 | 601.39 ± 12.12 |
| Treated SD | 566.29 ± 14.08 | 429.05 ± 11.71 | 529.33 ± 14.07 | 540.87 ± 13.59 | 636.47 ± 11.55 |
| PC | + 3.86 | + 5.12 | + 5.34 | + 3.59 | + 5.83 |

All the values are mean ± SD (n = 8). SD – Standard deviation PC – Per cent change over control.

**Table 3 : Effect of novel heterocyclic compound on GDH levels (µ moles of formazon formed /gm of wet weight of tissue /hr.) in different tissues of male albino rats**

| Group | Testis | Brain | Heart | Kidney | Liver |
|---|---|---|---|---|---|
| Control SD | 198.29 ± 14.59 | 429.36 ± 18.62 | 121.47 ± 17.94 | 256.74 ± 13.35 | 315.15 ± 8.56 |
| Treated SD | 179.82 ± 7.54 | 377.75 ± 16.96 | 112.26 ± 15.24 | 239.76 ± 12.25 | 285.25 ± 12.02 |
| PC | - 9.31 | - 12.02 | - 7.58 | - 6.61 | - 9.48 |

All the values are mean ± SD (n = 8). SD – Standard deviation PC – Per cent change over control.

respiratory enzyme SDH is a primary enzyme in the oxidative catabolism of sugars (Lehninger, 1993) and as such is used effectively as a marker of mitochondrial abundence and activity.

The SDH level in testis, liver, kidney, heart and brain of control rats was in the order of testis >liver >kidney >heart >brain. Table 2 demonstrates a mild increased in SDH activity levels in treated rats over control rats. The rise in SDH activity level indicates a rapid breakdown of metabolites and their utilization for the maintenance of life activities (Mehler, 1957). Increased activation of SDH may be due to increased feeding of ketoacids for citric acid cycle operation and increased metabolization of pyruvate towards citric acid cycle (Harper *et al.,* 1977).

Glutamate dehydrogenase (GDH) is a useful biochemical indicator of injury to the mitochondria (Henley *et al.,* 1966). GDH plays an important role in the elimination of nitrogenous excretory products. The released ammonia is either utilized for amino acid synthesis or detoxified and eliminated. GDH is also known to play a crucial role in protein metabolism in the cells affected by a variety of effectors (Ramanadikshithulu *et al.,* 1976). The regulatory role of this enzyme as observed in mammalian models in checking the deamination process has been reported earlier (Nagendra Reddy, 1991).

The GDH levels in testis, liver, kidney, heart and brain of control rats was in the order of testis >liver >kidney >heart >brain. The Table 3 represents that the GDH contents decreased in these tissues of treated rats as compared to control rats. The decrease in GDH activity was due to a decrease in enzyme with substrate and co-enzyme (Rama Devi, 1982).

## *Conclusion:*

An attempt has been made to assess the effect of novel heterocyclic compound on LDH and SDH levels as well as the level of GDH in testis, liver, kidney, heart and brain of rats. The results of this study demonstrated that the administration of the compound caused a mild increase in LDH and SDH contents where as the same compound caused a mild decrease in GDH content in tissues of treated rats and it indicates that the compound caused less effect on enzyme levels. It could be recommended that this compound can be used safely at lower doses as antifertility compound.

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**Authors' affiliations**

**M. KRISHNA REDDY,** Reproductive Physiology Unit, Department of Zoology, Kakatiya University, WARANGAL (A.P.) INDIA

**M. SARANGAPANI,** University College of Pharmaceutical Sciences, Kakatiya University, WARANGAL (A.P.) INDIA

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