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EFFICACY OF PHENOBARBITONE: STUDY OF HISTOLOGICAL PROFILE OF THYROID AND ADRENAL GLAND IN FEMALE ALBINO RATS.

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ABSTRACT: To assess the efficacy of phenobarbitone administration on gravimetrical changes, histological profile and their histometric analysis has been studied in thyroid and adrenal gland in female albino rats. Three groups of healthy adult female albino rats having six rats in each group were taken. The rats of groups II and III were administered phenobarbitone at the dose level 5.00mg and 7.5mg/100g body weight respectively intraperitoneally/daily for 30 days. However, the rats of group I (Control) were given saline alone. Completions of the experimental duration, the rats were sacrificed and the histological profile of thyroid and adrenal gland were carried out. The gravimetric analyses of thyroid glands were decreased and adrenal glands were increased significantly due to the chronic effect of phenobarbitone administration. Histometric changes of endocrine glands are parallel to the gravimetrical changes, the diameter of adrenal cortex and adrenal medulla are increased due to the administration of phenobarbitone.
Key words: Phenobarbitone, Thyroid, Adrenal, Endocrine, Rat

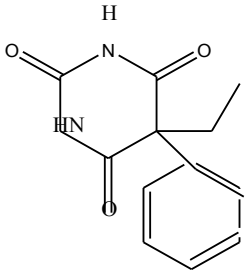
INTRODUCTION

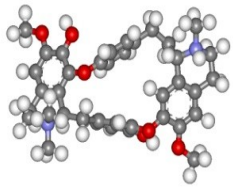
Barbiturates are generally used as sedatives, anticonvulsant and anaesthetic drugs, act through the central nervous system. They are known to inhibit pituitary hormonal secretion and reduce the adrenocortical secretion during the period of sedation (Everett, 1961, Sindgi and Rao, 1973a &b). Therefore, the structure and biological activities of some CNS influencing drugs, the actions of which modify the reproductive and endocrine activities is briefly summarized below. Hence, in our current study, the efficacy of the phenobarbitone administration on female endocrine organs was carried out.

PHENOBARBITONE

Phenobarbitone is a barbiturate, marketed between 1934-1945 under the brand name Luminal by Friedr Bayer *et al* The first barbiturate drug, barbital, was synthesized in 1902 by German chemists Emil Fischer and Joseph Von Mering at Bayer. By 1904 several related drugs, including phenobarbitone, had been synthesized by Fischer. Phenobarbitone was brought to market in 1912 by the drug company Bayer using the brand Luminal. It remained a commonly prescribed sedative and hypnotic until the introduction of benzodiazepines in the 1950s. Phenobarbitone is soporific, sedative and hypnotic properties were well known in 1912, but nobody knew it was also an effective anticonvulsant. It is the most widely used anticonvulsant worldwide and the oldest still commonly used. It also has sedative and hypnotic properties but, as with other benzodiazepines for these indications.

Barbiturates are the derivatives of barbituric acid obtained by replacing the two hydrogen of the carbon atom in position 5 of aromatic ring by alkyl group. An increase in the potency and decrease in the duration of action can be produced by increasing the length of alkyl group. Barbiturates are classified according to their duration of action, this being used on animal experiments.





Systematic (IUPAC) name 5-ethyl-5-phenylpyrimidine-2,4,6 (1H, 3H, 5H)-trione)

3d-Structure of Phenobarbitone

MATERIALS METHODS

Rat is used as experimental animal model since 1856. Since its domestication, the rat has probably contributed more substantially to the advancement of the biological sciences than any other laboratory species. Therefore, in the present study the rat in used as experimental model.

Animals:

Healthy, sexually matured, regularly cycling, colony bred virgin female rats of Wistar strain (*Rattus norvegicus*) aged three months and weighing 150 -200 gm were purchased from National Institute of Nutrition, Hyderabad. The rats were housed in polypropylene cages measuring 12"x10"8", under well ventilated animal house conditions (temperature: 28-31°C, photoperiod: 12 hours natural light and 12 hours natural dark, humidity: 50-55%). The rats were fed with balanced diet as per CFTRI formula and tap water ad *libitum*. They were maintained as per the principles of laboratory animals care (NIH Publication No. 85-88, 1985).

The animals were divided into three groups, each consisting of six rats in each group and treated as follows.

Group-I: Control, received 0.2 ml saline/ 100gm body weight.

Group-II: Group- IV: Received 5.0 mg phenobarbitone / 100gm body weight.

Group- III: Received 7.5 mg phenobarbitone / 100gm body weight.

The treatment was given for 30 days intraperitoneally between 10:00 to 11:00AM to cover 6 regular estrous cycles. The body weight of each rat was recorded every day before the treatment. The behavior of animals after receiving drug treatment was observed and compared with that of control.
On day 31st, 24h after last treatment, all the experimental rats were sacrificed by cervical dislocation. The thyroid and adrenal were dissected out immediately and separated free from the adherent tissue and weighed to the nearest mg on an electronic balance. Organs of each animal were fixed in Bouin's fluid, embedded in paraffin wax, sectioned as 5 µm, stained with haematoxylen–eosin for study histological profile. The micrometric measurements of like diameter of cortex and medulla of adrenal gland were carried out as described by Deb et al., 1965.

RESULTS

General observations:

Behaviour: The rats which received the chronic treatment of phenobarbitone were as active as the control rats. The phenobarbitone treatment caused sedation for 3 to 6 hours. This sedation caused is dose dependent. After period of sedation the animals show normal activities. However, the total intake of feed/day is not much altered due to drug administration.

Mortality: No mortality is observed in either control group or in the experimental group that received phenobarbitone.

Changes in the body weight: (Table -1)

The body weight of the rats which received low or high doses of phenobarbitone have shown non-significant reduction in the body weight for treatment of 30 days.

Table 1: Effect of Phenobarbitone on the Body Weight of Albino Rats

Sl. No.	Treatment / 100gm Body Weight	Initial Body Weight	Final Body Weight	Percent Change
1	Control	150.00 ± 3.66	160.00 ± 3.32	6.66
2	Phenobarbitone (5.00mg)	160.00 ± 3.65	168.60 ± 3.01	5.73
3	Phenobarbitone (7.5mg)	180.00 ± 3.65	186.16 ± 3.85	3.42

M ± SE = Mean ± Standard error.

*p<0.05, **p<0.01, ***p<0.001, when compared to saline treated rats.

Changes in the Thyroid and Adrenal Glands: (Table –2)

Thyroid: The gravimetrical observations of the thyroid glands have decreased in their weight significantly (p<0.01) and highly significantly (p<0.001) with the treatment of low and high doses of phenobarbitone. The histological observations of the thyroid glands of the follicles of these phenobarbitone treated shown decrease in size and slight degeneration is observed in some of the follicles.

Table 2: Effect of Phenobarbitone on Gravimetric Changes of Thyroid and Adrenal in Albino Rats

Sl. No.	Treatment	Thyroid		Adrenal	
		mg/100g body Wt.	Percent change	mg/100g body Wt.	Percent change
1	Control (0.2ml Saline)	10.68 ± 0.52	3.08	26.88 ± 0.57	4.50
2	Phenobarbitone (5.00mg)	8.64** ± 0.27	5.32	32.19** ± 1.03	8.15
3	Phenobarbitone (7.5mg)	6.23*** ± 0.55	6.01	39.63*** ± 1.25	9.18

M ± SE = Mean ± Standard error.

*p<0.05, **p<0.01, ***p<0.001, when compared to saline treated rats.

Adrenal: Phenobarbitone treatment has shown much effect in the increasing weight of the adrenal gland significantly (p<0.01) in lower dose and highly significant (p<0.001) in higher dose of the administration. The diameter of the adrenal cortex is increased and medulla shows hypertrophic cell with high secretion, as a result small sinusoid appear in the medulla with the treatment of phenobarbitone.

Histometrical changes in the Thyroid and Adrenal glands: (Figure I-IV)

Thyroid: The histological observations of the thyroid glands of the follicles of these phenobarbitone treated rats shown decrease in size and slightly degeneration were observed in some of the follicles.

Adrenal: The diameter of the adrenal cortex is increased and medulla shows hypertrophied with high secretion, as a result of small sinusoid appears in the medulla in treated of phenobarbitone. Histometric changes of adrenal glands are parallel to the gravimetrical changes, the diameter of adrenal cortex and adrenal medulla are increased due to the administration of phenobarbitone

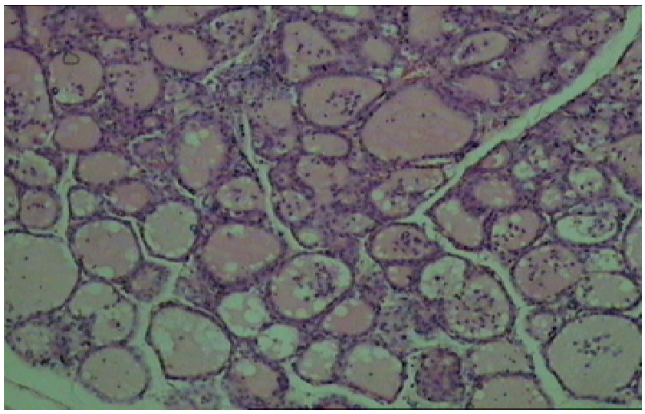


Figure: 1. Cross section of thyroid of control rat showing normal follicles (200X)

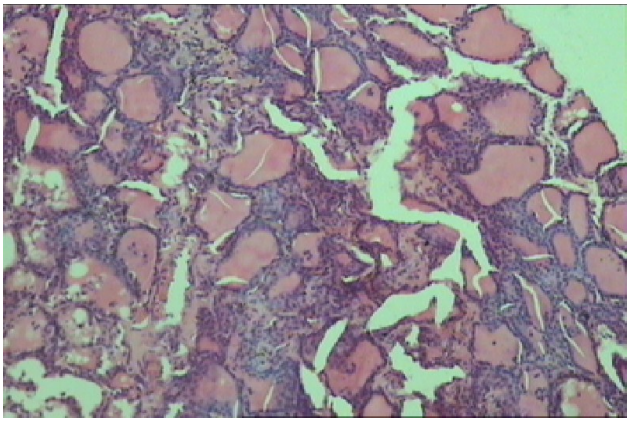


Figure: 2. Cross section of thyroid of phenobarbitone treated rat showing decrease in follicular size and secretion (200X)

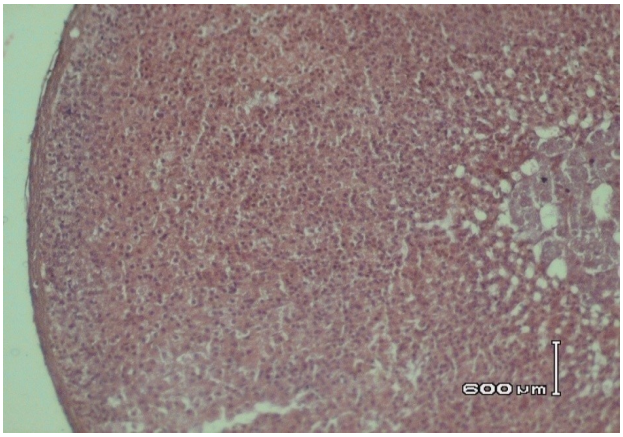


Figure: 3. Cross section of adrenal of phenobarbitone treated rat showing hyptertrophied cortex and medulla (200x)

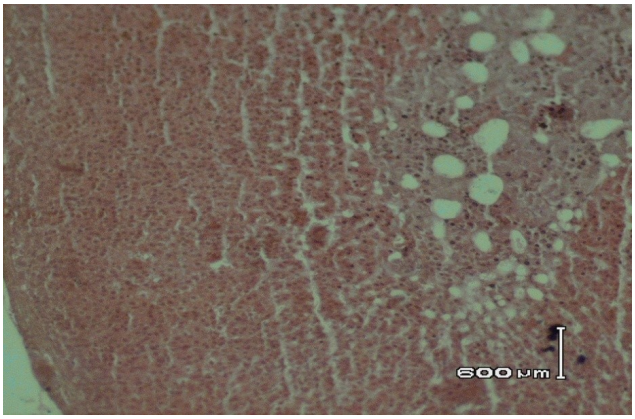


Figure: 4. Cross section of adrenal of control rat showing normal cortex and medulla (200X)

DISCUSSION

Though extensive work has been done on the mechanism of thyroid and adrenal gland and their hyper/hypotrophy, the reasons regarding the output of pituitary gonadotrophins may due to the administration of these controversial barbiturate drugs. As barbiturates are known to inhibit the secretion and release of pituitary gonadotrophins, reduce the adreno-cortical secretion, and alter the metabolism of steroids during sedation (Harwood and Mason, 1957, Purshottom et al., 1961, Meyer et al., 1971) it will be of interest to study their effect on endocrine organs of phenobarbitone administered rats. Well known toxic effects of Phenobarbital on the liver and thyroid were observed in a dose dependent manner, along with altered lipid, glucose, and electrolyte metabolism in male and female rats (Kojima et al., 2009). In our findings, the adrenals are heavier in the phenobarbitone treated rats with hypertrophied cortex and vacuolated medulla. The similar findings of Peeney and Averill, 1980, Phenobarbital appears to stimulate corticosteroidogenesis due in large part to enhanced hepatic corticoid metabolizing enzymes in male rats. The reduction in the weight and follicular dynamics of thyroid may be due to altered thyroid metabolism and inhibition of TSH hormone and increasing the weight and histometric measurements of adrenal may be due to the increased output of ACTH after sedation, similar findings of Sindagi and Rao, 1973 was described with graded doses of barbital sodium, and recently Li et al., 2011, proposed their findings with the treatment of Phenobarbital effect on oestrous cycle shown irregularity in cyclic rats and reduced the level of T3 and T4 hormone and resulted hypothyroidism. Phenobarbitone is known to alter the neuroendocrine signal to pituitary through hypothalamus during the period of sedation followed by adrenocortical hyper secretion. With, this context, conclusion of the study reveals that, the histological profile shows the atrophy of thyroid and adrenal gland and their hormone deficiency during the phenobarbitone administration and these glands are indirectly responsible for suppression of reproductive activities to regulation in hormone secretion in female albino rats. Further approach of molecular study will gives the clear findings of the efficacy of chronic administration of phenobarbitone.

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