Protective Effect of Brewer's yeast On Methimazole- Induced -Adrenal Atrophy (A Stereological Study)

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Induction of hypothyroidism by thioamide drugs will cause adrenal gland atrophy and decrease in its hormones. To prevent side effect on the adrenal gland, brewer's yeast, a natural product rich in vitamins and minerals was used. Streological techniques were applied to measure the volume of adrenal gland. For this purpose, 48 Sragueˆdawley rats were randomly divided into one control and three experimental groups. In group 1, methimazole was administered at the dose of 30 mg/kg/day days, in group 2, 120 mg/kg/day of, brewer's yeast, in group 3, 30 mg/kg/day of methimazole plus 120 mg/kg/day of brewer yeast, and for the control group, an equal volume of saline (0.5 ml/rat/day) was orally given. After 30 days, all the animals were anesthetized and their adrenal glands were removed, fixed, embedded and stained. The volume of different zones of the adrenal glands was estimated by Cavalieri principle and point counting methods. Statistical analysis was performed using Mann-withney test and $p < 0.05$ was considered as statistically significant. The results indicated that methimazole decreased the volume of fasciculata zone in the cortex of the adrenal gland and also decreased the blood cortisol level. Brewer's yeast reduced the methimazole side effects on this zone.

In conclusion, it seems that the use of brewer's yeast could prevent methimazole-induced atrophy of the adrenal gland.

Key words: antithyroid drugs, Cavalieri method, supplements

INTRODUCTION

The use of synthetic drug for treatment of diseases could have positive effects on the target tissues; however, it might cause adverse effects on other tissues and organs. Thioamides are antithyroid drugs such as methimazole, carbimazole and propylthiouracil which are used in hyperthyroidism. These drugs inhibit thyroid peroxidase enzyme and decrease the synthesis of thyroid hormones [1]. Thiouracil antithyroid activity resulted from its interference with the iodination of thyroxine precursors and inhibited the conversion of thyroxin (T4) to tri-iodo-thyrosin (T3) in extra thyroid tissue and induced adrenal atrophy in rats [2]. Carbimazole affects the adult rat adrenal gland by decreasing the total width of the cortex and the number of cells [3]. Therefore, a gradual change of adrenal structure and loss of function is widely recognized as a contributor to a decline in health and is considered to be both an indirect cause as well as a side effect of many acute and chronic illnesses [4]. To prevent this event, some alternative treatments should be applied. There are vitamins, minerals and a number of botanicals known to support adrenal function and may have therapeutic effect [5]. Vitamins especially vitamin B_5 and minerals such as Magnesium, Chromium and selenium can improve the adrenal gland function [6, 7, 8, 9]. Herbs and medicinal plant preparations such as ginseng and Licorice root may influence the adrenal hormone activity by contributing to adrenal function [10, 4]. Therefore, prescription of substances containing these agents can be useful. Brewer's yeast is a herbal product of Saccharomyces cervisid and is used in production of lager and additives. It is an important nutrient containing vitamins, proteins and minerals [11]. It seems that the component in this substance can play a role in improvement of structure and function of adrenal gland. The important issue in this regard is the estimation of atrophy in different zones of the adrenal glands due to the effect of methimazole and also the degree of compensational effect of Brewer's yeast by stereological methods to calculate these effects quantitatively. Stereology is one of the applied mathematics branches which enable us to do three-dimensional calculations by the use of two-dimensional microscopic studies. One of the measurable parameters in stereology is the calculation of organ and tissue volumes. These volumes are changed during many diseases; they therefore can be useful in evaluation of diseases. Cavalieri-principle is used as a standard method for determination of organ and tissue volumes [12].

Because methimazole causes atrophy and probably decreases the total volume of the adrenal glands, in this study, the volume of different zones of adrenal gland will be measured after the use of methimazole and Brewer's yeast. The hormones levels of the adrenal glands are also measured.

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Table 1 The comparison of means of total volume, cortex volume (zona glomerulosa, zona fasciculate, zona reticularis), medulla volume and coefficient of error in different groups in female rat

Variables / groups	VT (mm^3)	VC (mm^3)	VM (mm^3)	VZG (mm^3)	VZF (mm^3)	VZR (mm^3)
Control	8.8 + 1.46	7.92 + 1.34	0.88 + 0.12	1 + 0.20	6.43 + 1.13	0.78 + 0.13
CE	0.06	0.06	0.06	0.09	0.06	0.06
Methimazole	7.31 + 0.82 *×≠	6.59 + 0.73 *×≠	0.72 + 11	0.92 + 0.13	5 + 0.56 *×≠	0.65 + 0.08
CE	0.06	0.07	0.07	0.08	0.07	0.11
Brewer's yeast	10.58 + 1.32*	9.80 + 1.21 *	1.04 + 0.14	1.20 + 0.20 *	7.79 + 1.16 *	0.91 + 0.13
CE	0.07	0.07	0.07	0.07	0.08	0.08
Methimazole + Brewer's yeast	9.30 + 1.29 ×	8.31 + 1.10 ×	0.98 + 0.21 ×	1.08 + 0.15	6.40 + 0.82 ×	0.82 + 0.13
CE	0.06	0.06	0.07	0.06	0.08	0.07

* $p < 0.05$ relative to control
× $p < 0.05$ relative to brewer's yeast group
≠ $p < 0.05$ relative to methimazole plus brewer's yeast group

MATERIALS AND METHODS

Experimental protocol. Forty eight adult female Sprague-dawley rats weighing 160–190 g were obtained from the Laboratory Animals Center of Shiraz University of Medical Sciences, (Shiraz, Iran) and treated according to the standard directive. They were singly housed in cages with chopped wood bedding. Methimazole was prepared from Merk Company (Germany) and brewer's yeast was purchased from Zanza Company (England). Two weeks after arrival, the rats were randomly assigned to three experimental and one control groups, each group including 12 rats. The animals in the first and second groups were received methimazole (30 mg/kg/day) and brewer yeast (120 mg/kg/day), respectively and the third experimental group received a combination of methimazole (30 mg/kg/day) and brewer's yeast (120 mg/kg/day) for 30 days by gavages. The control group received 0.5 ml distilled water/day for thirty days. The animals were allowed *ad libidum* access to standard rat chow (produced by the Laboratory Animal Center of Shiraz University of Medical Sciences) and water throughout the experiment. They were maintained on a constant 12-hour light/dark cycle with a relative temperature of 22 ± 2°C and at 60–80% relative humidity. A blood sample was obtained from their tail vein at the day 30 for estimating aldosterone, cortisol and androgen. The blood samples were centrifuged at 3000 rpm for 10 minutes and plasma was used for measurement of hormones by Radioimmunoassay kit according to the manufacturer's instruction.

Histological procedure. At the end of the experiment, according to Iran Veterinary Organization Ethics, the animals were deeply anesthetized and the suprarenal glands were removed. The tissues were fixed in buffered formaldehyde for one week and embedded in paraffin; complete serial sections (5 micrometer thickness) were cut and stained with Heidenhain's azan.

Stereological study. The Cavalieri method [13] was used as an estimator of gland volume. Thus, ten to twelve sections were selected using a systematic sampling design and a random start for stereological estimations. Each sampled section was analyzed using a video-microscopy system made up of a microscope (E-200, Nikon, Japan) linked to a video camera (SONY, Japan, SSC Dc 18 P), a P4 PC computer, and a LG monitor (795 FT plus) to determine the parameters. By means of stereology software designed at our lab, the stereological probe (points) was superimposed upon the images of the tissue sections and viewed on the monitor. The gland volume was estimated using the following formula:

$V(total) = \Sigma P.(a/p).d$

Where the "V (total)" was the gland volume, "ΣP" was the sum of the points falling on the section profile, "a/p" was the area associated with each point at the level of tissue, and "d" was the distance between the sections sampled. "a/p" was calculated by the follwing formula:

$(a/p) = (\Delta x \times \Delta y)/M^2$

Where "Δx" and "Δy" were the distance between two adjacent points of the grid in x-axis or y-axis, respectively. "M" was the final linear magnification of the microscopic images.

The accuracy of the volume estimate was evaluated through the coefficient of error of the Cavalieri principle. The formula used to assess the error coefficient (CE) was $CE = 1/\Sigma A \times [1/12\ (3a + c - 4b)]^{1/2}$, where ΣA is the sum of all section area; a is sum of all the products a × a; b is the sum of all products a × (a + 1); and c is the sum of all products a × (a + 2) [14].

Statistical analysis: The results were analyzed using Mann-Whitney U-test. P-value less than 0.05 were considered statistically significant.

RESULTS

In this study, the effect of methimazole, brewer's yeast and the combination of both compounds on the total volume (VT), different parts of cortical volume (VC) and medulla volume (VM), and also levels of adrenal hormones were evaluated.

Stereological study:

According to Table l–1, methimazole decreases the VT by 17% and VC 17% of the adrenal gland

Table 2 The comparison of means of adrenal gland weight and the level of cortisol, aldosterone and androgen in different groups in female rat

variables / groups	Weight (mg)	Cortisol (µg/dl)	Aldosterone (pg/ml)	T3 (ng/ml)	T4 (µg/dl)
Control	23 ± 4.08	2.67 ± 0.90	596 ± 388	98.41 + 14	3.15 + 0.63
Methimazole	21 ± 2.41 ×	1.55 ± 0.83 *×≠	590 ± 298	92.83 + 11	2.89 + 0.5 ×
Brewer's yeast	25.5 ± 5.03	3.22 ± 0.78 *	672 ± 316	108 + 17	4.06 + 0.41
Methimazole + Brewer's yeast	23.6 ± 3.96	2.60 ± 0.56 ×	614 ± 324	106 + 23	3.15 + 0.5 ×

* $p < 0.05$ relative to control
× $p < 0.05$ relative to brewer's yeast group
≠ $p < 0.05$ relative to methimazole plus brewer's yeast group

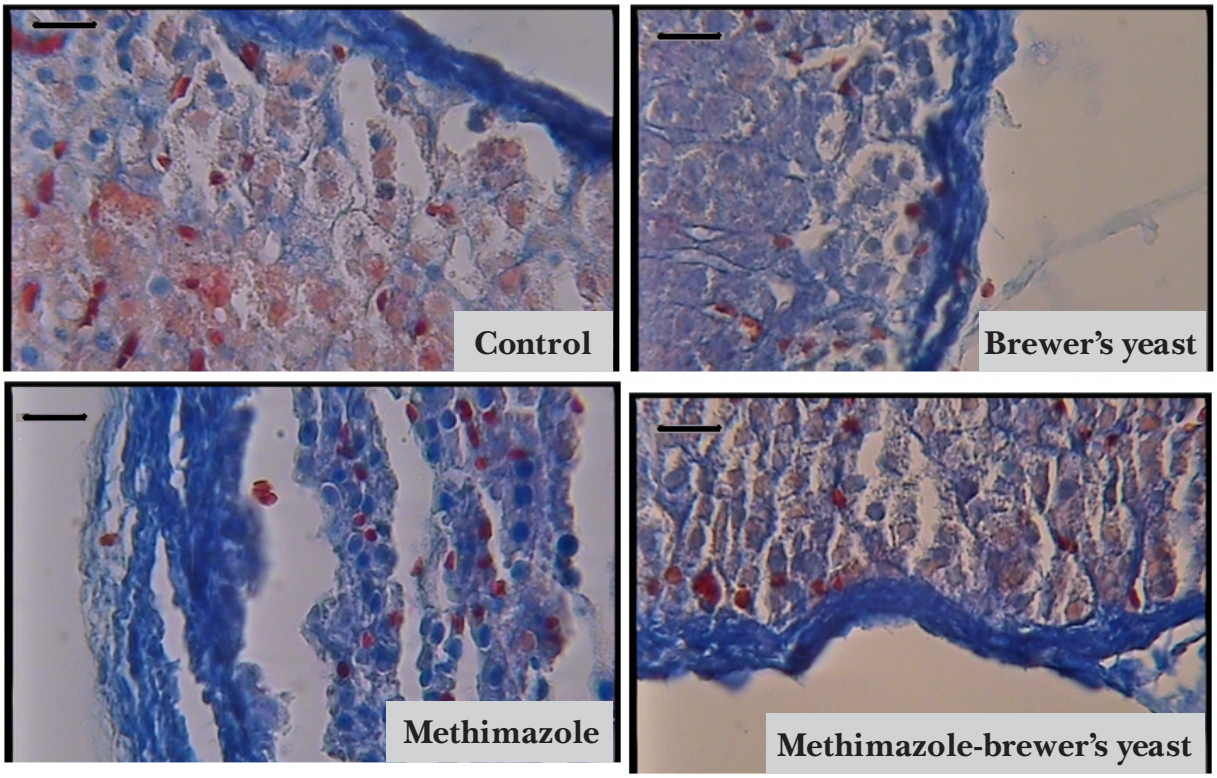

Fig. 1 GlumerolusaHistolojical section of different groups in zona glomerulosa with Heidenhain's azan stain (Scale bar = 10 µm)

as compared to the control group ($p < 0.05$). In the cortex, only the volume of zona fasciculata (ZF) was decreased 22% relative to the control ones ($p < 0.05$).

In the brewer's yeast group, the VT 20% and the VC 24% of the adrenal gland were significantly increased compared to other groups. In the cortex, the volume of zona glomerulosa (ZG) 20% and ZF 21% was increased relative to the control group ($p < 0.05$).

In the brewer's yeast plus methimazole group, the VT 27%, VC 26% in all zones and VM 36% were significantly increased as compared to the methimazole group ($p < 0.05$).

Hormonal study:

The results of hormonal analysis are shown in Table -2.

In methimazole group, the cortisol level was decreased 42% relative to the control group. In the methimazole and brewer's yeast group the level of cortisol (68%) was significantly increased as compared to the methimazole group. However, the level of cortisol in the brewer's yeast group (20%) was significantly increased compared to the control group ($p < 0.05$). In this study the level of aldosterone was the same, and showing no change in all groups. The level of androgen could not be measured because its concentration was lower than the limits.

Histological study:

Fig. 1 shows the histological finding of ZG in different groups. In this zone cellular arrangement of methimazole group was destroyed relative to control

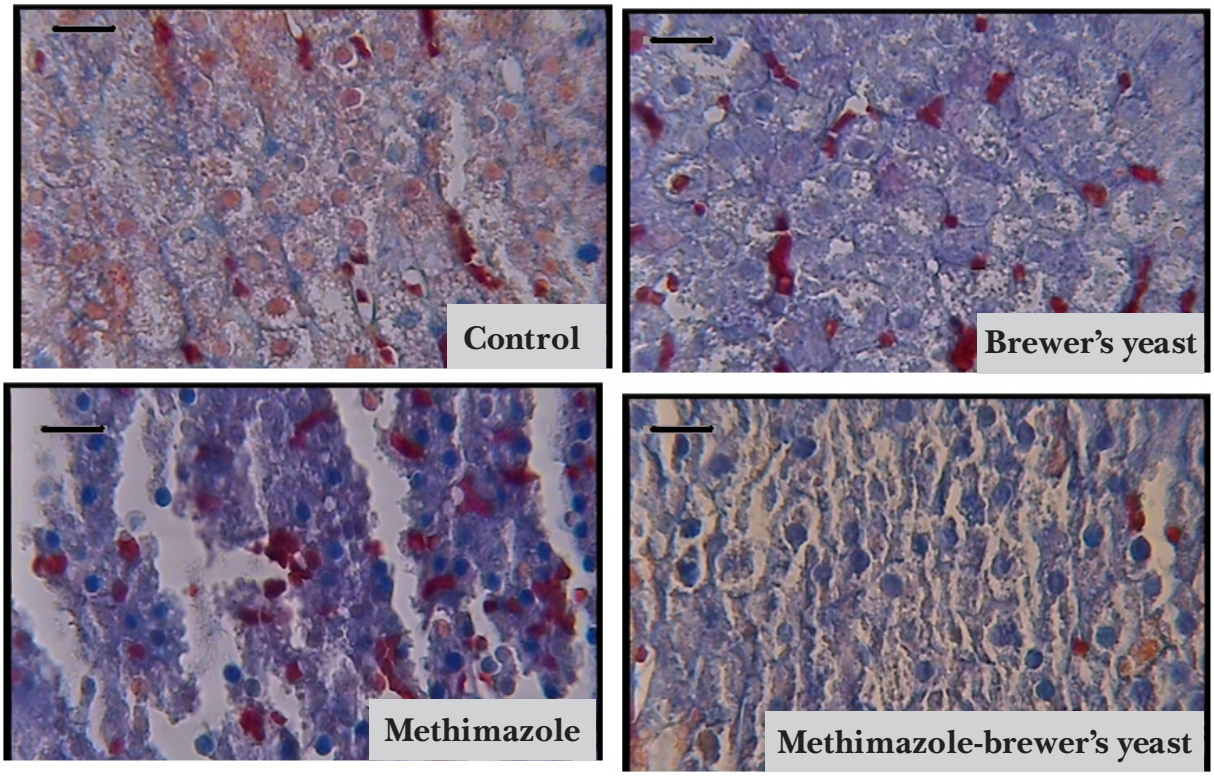

Fig. 2 Histological section of different groups in Zona Fasciculata with Heidenhain's azan stain (Scale bar = 10 µm)

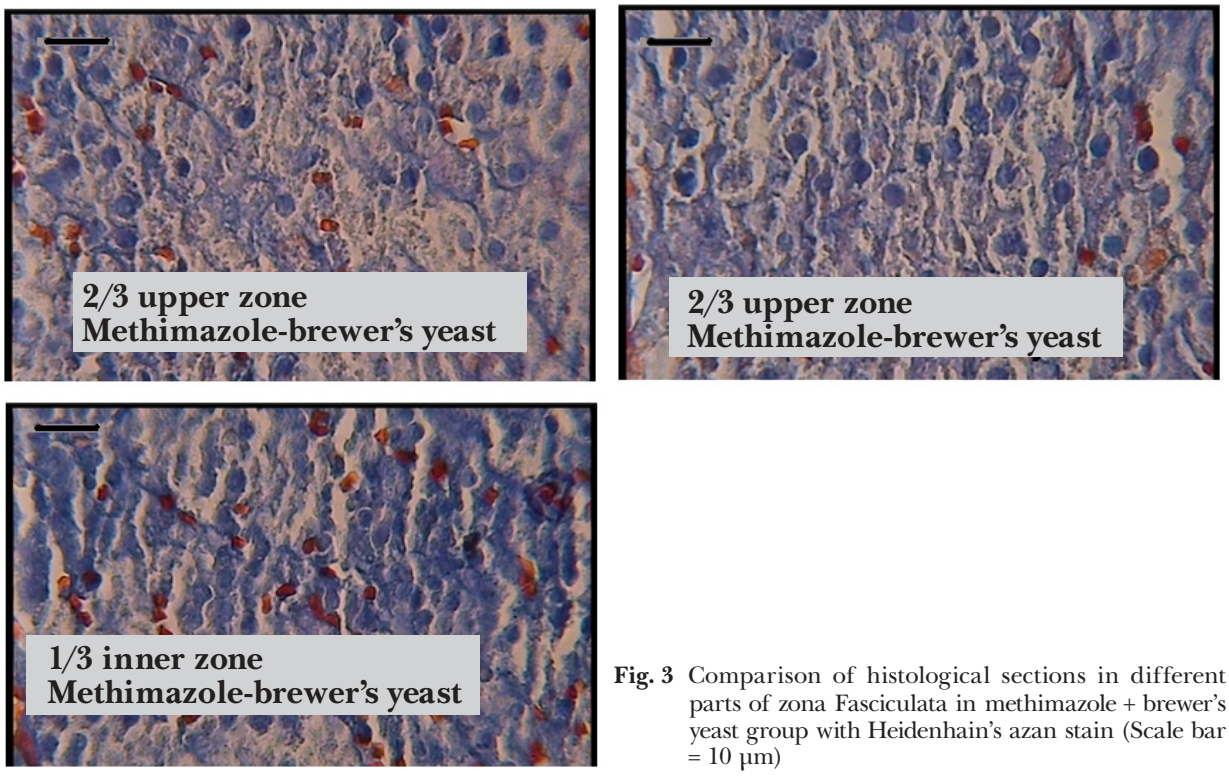

Fig. 3 Comparison of histological sections in different parts of zona Fasciculata in methimazole + brewer's yeast group with Heidenhain's azan stain (Scale bar = 10 µm)

group but in methimazole plus brewer's yeast group, the cellular arrangement of ZG return to normal. It is notable that the cellular arrangement in brewer's yeast was normal.

In Fig. 2, the histological finding of ZF was shown. In methimazole group, most cells were shrinkaged with dense nucleus, the amount of lipid droplet was decreased. In methimazole plus brewer's yeast group, the cellular structure of upper part of ZF contain normal cell with large amount of lipid and clear nucleus. In the inner part of ZF, the majority of cells were dense nucleus and shrinkaged cytoplasm (Fig. 2, 3). In the brewer's yeast group, the cells of ZF were normal, with large lipid droplet (Fig. 2).

In the ZR, the cellular structure and their arrangement in brewer's yeast group was normal but in methimazole group and in methimazole plus brewer's yeast, the most of cells were shrinkaged and lipofuscin pigment were more apparent.

DISCUSSION

Induction of hypothyroidism by thioamides such as methimazole, carbimazole and propylthiouracil, might cause adrenal atrophy and reduction of its hormones [1, 2]. The atrophy of different tissues usually reduces the tissue volume [15]. In this research, it has been shown that methimazole reduces the adrenal volume but the main question is that which part of adrenal gland has been affected by this drug. Some reports show that, during the treatment by thiamazole both the adrenal cortex and medulla were atrophied [16–18]. In this study, methimazole had no effect on the medulla but reduced the cortical volume and decreased the volumes of ZF relative to the control group ($p < 0.05$). Histological study in this group also revealed that the cells of ZF became shrinked and their nuclei became denser. It seems that the volume reduction of this zone is due to shrinkage or restriction of some parts or organelles of these cells. Rebuffat *et al*. (1982) also showed that in the atrophy of ZF, the number of smooth endoplasmic reticulum decreased significantly and the volume of mitochondria was reduced [19]. Researches showed that propylthiouracil increased the number of liposomes and lysosomes which subsequently reduce the lipid droplets and influenced the mitochondrial degeneration in the cells of the inner zone of the adrenal cortex [18–19]. Therefore, it seems that one of the reasons of volume reduction in ZF is due to decreased mitochondria and smooth endoplasmic reticulum. But what is the mechanism of anti-thyroid drugs on the atrophy of ZF? It is probably related to hormone regulation in combination with hypothalamus self – regulation, in such a way that anti-thyroid drugs such as methimazole decreases CRF and ACTH by interfering Hypothalamico – Hypohyseal axis which eventually leads to circulating corticosterone decrease and changes in structure and function of the adrenal gland [20]. Inhibition of ACTH release coincides with increase in autophagic vacuoles and lysosome function in the cytoplasm of the adrenal cortex cells [19, 21] that might be lead to reduction of ZF volume and corticosteroid level .In this study; methimazole reduced the corticosteroid level by affecting the adrenal gland. Lo MJ *et al* (1998) also showed that propylhtiouracil could decrease corticosteroids [22]. The question is that whether we can avoid the side effects of antithyroid drugs on the adrenal gland. The numbers of the supplements such as vitamins B-complex and minerals can improve the adrenal gland function. Reports show that pantothenic acid or vitamin B_5 enhances the adrenal cells and increases coticosterones secretion [6, 23]. Magnesium [7] Chromium [8, 24] and selenium [9] can increase corticosteroid secretion. In this research, brewer's yeast was used for enhancement of adrenal gland function instead of synthetic supplements. Brewer's yeast is a natural product which is derived from Saccharomyces cervisid. It contains amino acids, lipids, different vitamins B-Complex, minerals such as iron, calcium, germanium, zinc, selenium and specially chromium [25–27], which seems to improve the adrenal function. The results of this study showed that brewer's yeast could increase the adrenal volume. A stereological study showed that brewer's yeast increased VC and VM in the adrenal gland. A histological study also showed that brewer's yeast induced rearrangement of ZG and cell hypertophy of the upper part of ZF. The mechanism is not very clear; however, it seems that brewer's yeast increased ACTH production by influencing on hypothalamic – hypophyseal axis and inducing ACTH secretion. ACTH-stimulated production of corticosterone by the adrenocortical cells of the upper part of ZF showed a notable hypertrophy. This is probably associated with an increase in the volume of the mitochondrial compartment and proliferation of the smooth endoplasmic reticulum and the number of lipid droplets, which leads to the rise of the average volume of the upper part of ZF [28–29]. The histological study of this research showed that in the lower part of ZF and ZR there were a few lipid droplets and some cellular necrosis was observed in methimazole and brewers yeast group, Although volume of these zones were increased. It seems that brewer's yeast could not exert any effect on cellular hypertrophy in recent part; however; it increased the volume of this zone probably due to other factors such as congestion of blood capillary or cells diffusion and increased level of lipofuscin pigments.

In conclusion, it seems that the side effect of thioamides on adrenal gland are specific and that brewer's yeast can increase ACTH, which in turn enhances cotisol production by increasing the volume of the upper part of ZF. Therefore, it seems that brewer's yeast may decrease the methimazole – induced cell injuries in adrenal gland.

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