Evaluation of Potential Effect of Enriched-Chestnut Yoghurt on Rats with Hyperthyroidism Induced by L-Thyroxine

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Abstract

With growing interest in using alternative and complementary medicine, especially medicinal plants, because of low cost and less adverse effects. This study was performed to evaluate the anti-hyperthyroidism effects of medicinal herb, chestnut *(Castanea sativa mill)* that used to enrich yoghurt with different premixes on levothyroxine (LT4)-induced hyperthyroidism rats. Male wistar rats were divided into seven groups five each, namely, normal group (-ve), hyperthyroidism control group (+ve), hyperthyroidism plus PTU as treated with standard drug, hyperthyroidism plus enriched-chestnut yoghurt prepared with chestnut by cow milk (T_1) , hyperthyroidism plus enriched-chestnut yoghurt prepared with chestnut by blending cow milk [1:1] (T_2), hyperthyroidism plus enriched-chestnut yoghurt prepared with chestnut by blending cow milk at [3:1] (T_3) and hyperthyroidism plus enriched-chestnut yoghurt prepared with chestnut by blending cow milk at [6:1] (T_4), rats in all groups except normal were injected with LT4 for 2 weeks to induce hyperthyroidism and then were administrated each treatment for 6 weeks. Biomarkers linked to hyperthyroidism were determined. Results, showed that, serum TC, TG, LDL-c, VLDL-c, Urea Nitrogen, AST, ALT, Triiodothyronine and Thyroxine , meanwhile, were significantly reduced in T_3, T_4 and PTU groups compared with the hyperthyroidism (+ve) group . Meanwhile, Serum HDL-c, uric acid, creatinine, and TSH were significantly increased in the enriched-chestnut yoghurt with different premixes and PTU groups. Results suggest that chestnut might suppress different biomarkers, Triiodothyronine, Thyroxine and TSH activities by modulating their concentrations and therefore, enriched yoghurt with chestnut could be an alternative therapy for hyperthyroidism.

Key Words: chestnut, Hyperthyroidism, Liver functions, PH, Thyroid hormones

Introduction

Plants and their corresponding potential preparations have been used from old civilizations owing to their remarkable botanical in both prevention and treatment of oxidative stress and its complications. These plants attracted the attention owe to the inherent antioxidant activity of the bioactive that decrease the free radical induced oxidative damage. Consumption of various plants parts that have high amounts of phenolic compounds is vital to protect from oxidative damage **(Salehi *et al.,* 2020).** Sweet Chestnut *(Castanea sativa mill)* belongs to the family Fagaceae, and generally cultivate all through the clement countries (**Pandey *et al.,* 2018).** There is growing evidence showing that the consumption of chestnut has become more vital for human nutrition and prompting health benefits. The chestnut considered as rich source of fiber, unsaturated fatty acids, protein, starch, vitamins, minerals and different bioactive constituents that promoting health (**Muzaffar and Maqbool 2016 and Oniszczuk *et al.,* 2019).** Chestnut has been popularly used in folk medicine for treatment of different respiratory disorders and recent literatures have displayed that the Castanea sativa possesses cardioprotective **(Chiarini *et al.,* 2013)**, antimicrobial **(Jukic and Trutic 2014)**, neuroprotective (**Santulli *et al.,* 2017),** anti-obesity complication **(Budriesi et al., 2018)** and antioxidant activities **(Pandey *et al.,* 2018)** as well as the ability to prevent DNA damage **(Lenzi *et al.,* 2017).**

Since, yoghurt is commercially obtainable in various kinds and is highly valued for ease of consumption as fermented dairy food and its sensory properties **(Gao *et al.,* 2018).** Even though yogurt contains different health promoting constituents, it stills a poor source of phytochemical components. Nowadays, there is a popular approach to enhance phenolic components of the yoghurt and likewise improve antioxidant profile by adding fresh or processed fruits in yogurt and dairy products. At the same time, using supplementation of natural antioxidant sources in yogurt also meets the consumer demands for “clean label” foods **(Granato *et al.,* 2017 and Raikos *et al.,* 2019).**

On the other hand, thyroid hormones have a key role in metabolism, development, growth and thermoregulation such as triiodothyronine (T3) and thyroxine (T4) are tyrosine based hormones and thyroid glands are responsible for production and metabolism regulation **(Kim and Lee 2019)**. Hyperthyroidism is a non-balanced of thyrotoxicosis and considered as metabolic condition caused by overproduction of thyroid hormone in the thyroid gland **(De Leo *et al.,* 2016).** The main purpose of this work was to investigate the potential effect of chestnut enriched-yoghurt with different premixes on thyroid function in L-thyroxin receiving rats.

Materials and methods

Materials:

Chestnut Edulis (*Castanea Sativa Mill*) were obtained from local market and cut in small pieces. Commercially yoghurt cultures were purchased from Chr. Hansen, Laborotories , Capenhage , Denmark. Cow milk and fresh sweet water: cow milk [1:1] were obtained from Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

Drugs: Thiouracil © (4-Hydroxy-2-mercapto-6-methylpyrimidine) and L-thyroxin© Thiouracil were products of Sanofi- Synthelabo Company, Paris, France.

Methods:

Enrichment Combination of yoghurt: four treatment of yoghurt formulated to contain:

YC_1: Yoghurt prepared by cow milk
EC_2: Yoghurt prepared by blending cow milk: cow milk [1:1]
EC_3: Yoghurt prepared by blending cow milk: cow milk [3:1]
EC_4: Yoghurt prepared by blending cow milk: cow milk [6:1]

Each premix also contained 3% sucrose and 0.5 % gelatin, all premixes were homogenized at 85 °C for 10 min and then cooled to 45 °C as described by (**Collins *et al.*, 1991**). Commercial yoghurt culture (1%) Direct Vat Set (DVS) of *streptococcus thermophillus* and *Lactobacillus delbrueckii sub sp. Bulgaricus* of the milk volume was added (**Lee *et al.*, 1990**). The milk mixture was placed in 250 ml cups and kept in an incubator at 45 °C for 3-6 h, and then the cups were transferred to refrigerator.

Proximate composition:

Yoghurt enrichment were analyzed for moisture, protein, lipid and ash content using standard methods (**A.A.C.C., 2000**). Total carbohydrate content was determined by difference. The pH was determined using a pH meter while acidity was measured as describe by (**Osundahunsi *et al.*, 2007**).

Animals and diets:

The study was performed on male albino rat's weight between 120-140 g. The animals were obtained from Laboratory of Animal Colony, Helwan, Egypt. Rats were fed the basal diet for 7 days before the beginning of the experiment for adaptation. The standard diet comprised of prepared according to **(Reeves *et al.*, 1993),** diet and water were provided ad libitum.

Experimental design:
After adaptation period the animals were randomly divided into 7 groups of 5 rats each and one of them was kept as a normal (-ve) control group. Rats were injected intraperitoneally with L-thyroxin drug at a dose of 0.5 mg/kg for 15 days to induce hyperthyroidism by the method used by **(Davidson *et al.,* 1978)**. LT4 group was kept as a positive (+ve) control group and rats in group (G4-G7) were orally administrated at a dose of 2 ml yoghurt/rat/day by gavage for six weeks. The composition of these groups shows as follows:

Group (3) LT4 + PTU: Fed on basal diet and administrated with 6-n-propyl-2-thiouracil (PTU, 10 mg/kg) drug according to the previous established method (**Saxena *et al.,* 2012**).

Group (4) LT4 + T_1: Fed on basal diet and **YC** $_1$ yoghurt

Group (5) LT4 + T_2: Fed on basal diet and EC $_2$ yoghurt

Group (6) LT4 + T_3: Fed on basal diet and yoghurt with premix EC $_3$: cow milk [3:1]

Group (7) LT4 + T_4: Fed on basal diet and yoghurt with premix EC $_4$: cow milk [6:1]

Biochemical evaluation:
Serum aspartate aminotranalansferase (AST) and alanine amino transferase (ALT) concentrations were determined according to (**Reitman and frankel 1957**). Serum total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein were determined by the methods of (**Roeschlau *et al.,* 1974 and Fossati and Prencipel 1982),** respectively. Serum creatinine, uric acid and urea levels were determined according to (**Bartles *et al.,* 1972**, **Caraway, 1955 and Han *et al.,* 1984),** respectively. Serum levels of Triiodothyronine, Thyroxine and thyroid stimulating hormone (TSH) were analyzed by colorimetric competitive enzyme immunoassay using individual ELISA kit according to (**Larsen, 1972**, **Schuurs and Van Weeman, 1997 and Bhowmich *et al.,* 2007**), respectively.

Statistical analysis:
Results are expressed as the mean standard deviation SD. Data were statistically analyzed of variance "ANOVA" test at ($P \leq 0.05$) according to **(Vandallen, 1997),** using SPSS statistical software, version 13.0 was used for these calculations.

Results and Discussion

Physio-chemical composition of enriched-chestnut yoghurt

The changes in physiochemical composition of enriched-chestnut with yoghurt are shown in Table 1. The moisture contents range from 86.82 to 88.61 .It can observed that the highest value of protein and fat were in EC_4 (3.90 and 3.79) containing yoghurt prepared by chestnut with water: cow milk at percent of [6:1], and the lowest values of protein and fat were found in YC $_1$ (2.36 and 1.58) containing yoghurt prepared by cow milk this may be owing to the addition of ingredients which are rich source of protein which eventually increased the level of protein in EC $_4$. Meanwhile, highest value of carbohydrate was observed in **YC** $_1$ (8.59) contains yoghurt prepared by just chestnut with water and lowest value was observed in EC $_4$ (3.66) contains yoghurt prepared by chestnut with water: cow milk at percent of [6:1]. Chestnut regarded as gluten free and its protein considered as a high quality protein comparable with eggs **(Yadav *et al.*, 2014).** Furthermore, the elevation in protein and fat levels of enriched-chestnut yoghurts linked with addition of chestnut to yoghurt treatments (**Singh *et al.*, 2017).**

PH values of enriched-chestnut yogurts were ranged from 2.34 to 4.79. The **YC** $_1$ yoghurt cow milk had lower pH value than those of the cow milk addition. Using starter bacteria such as *delbrueckii ssp. bulgaricus* in a combination for production of yoghurt, it caused low pH and these low values of pH considered a harsh environment for the survival of probiotic organisms **(Mohamed *et al.*, 2017).** The pH of yoghurts gradually increased, showing value of 4.79 in T_4 and this is considered as a suitable pH degree for the yoghurt. **Ozcan *et al.*, (2017)** reported that the addition chestnut in fermented dairy products stimulate the growth of probiotic bacteria and enhance its functionality**.** The total acidity of enriched-chestnut yoghurts ranged from 0.18 to 0.36. The total acidity steadily increased because of the accumulation of lactic acid produced by lactic acid bacteria **(Sengul *et al.*, 2012).** In addition, **Oniszczuk *et al.*, (2019)** reported that chestnut considered as one of powerful plants with distinct taste and health-promoting properties.

Table 1: Physio-chemical composition of enriched-chestnut with yoghurt treated.

Parameters	YC_1	EC $_2$	EC $_3$	EC $_4$
Moisture %	86.82	87.47	88.61	88.21
Protein %	2.36	2.85	3.96	3.90
Fat %	1.58	2.29	2.53	3.79
Ash%	0.69	0.67	0.86	0.66
carbohydrate	8.59	6.79	4.47	3.66
PH	2.34	3.08	3.54	4.79
Total acidity	0.18	0.24	0.25	0.36

Data are expressed as mean, n = 3. **YC** $_1$**:** Yoghurt prepared by cow milk, **EC** $_2$**:** Yoghurt prepared by blending cow milk: cow milk [1:1], **EC** $_3$**:** Yoghurt prepared by blending cow milk: cow milk [3:1], **EC** $_4$**:** Yoghurt prepared by blending cow milk: cow milk [6:1]

Changes on weight gain, feed intake and FER of control and Hyperthyroidism Rats.

The influence of continuously consuming enriched-chestnut yoghurt treatments with different premixes on health of hyperthyroidism rats was examined by measuring body weights each week (Table 2). There was a gradual decrease in body weight gain and feed intake of positive control group (+ve) by 79.10% and 22.30% when comparing with normal control group (-ve). However after consumption of enriched-chestnut yoghurts with different premixes (T_1, T_2, T_3 and T_4) for 4 weeks, feed intake in normal control (-ve) and different treated groups (PTU, T_1, T_2, T_3 and + T_4) did not show differences in comparison with hyperthyroidism group (+ve). Furthermore, weight gain of T_1 and T_2 groups was no different compared to the PTU which treated by anti-thyroid drug (Table 2). Comparing with the control group (-ve), there was a significant decrease in weight gain of the hyperthyroidism control group (+ve) and T_3 and T_4 group ($P < 0.05$). These results supported by other finding of **Khaled and Abul-Fadle (2017)** who found a reduction in body weight with hyperthyroidism condition. Furthermore, **Ríos-Prego *et al.*, (2019)** as they reported that thyroid dysfunctions associated by weight loss (gain) of rats. Hyperthyroidism has been linked with underweight and weight loss. Hyperthyroidism Subjects have an adrenergic hyper-stimulation with rises in thermogenesis and metabolism so, a greater overall energy expenditure which causing a tendency on weight loss. In addition, thyroid hormones dysfunction considered as major target which involved in the regulation of body weight **(Gionfra *et al.*, 2019).**

Table 2: Changes on weight gain, feed intake and FER on hyperthyroidism rats groups in comparison to normal control group (-ve).

Groups	Weight gain (g)	%	Feed intake (g/d)	%	FER	%
Contr.(-ve)	96.76±8.11 a	-	17.54± 2.20 a	-	0. 187±0.03a	-
Contr. (+ve)	27.64±8.11d	- 79.10	14.34± 2.20d	- 22.30	0.67±0.06e	+ 272.22
PTU	96.59± 6.11 c	- 1.18	17.90± 2.03 a	- 0.78	0.182±0.02 b	- 2.67
T_1	86.13±9.13 c	- 13.80	16.46±2.32 a	- 7.13	0.185±0.04ab	- 1.07
T_2	87.27±9.17 c	- 10.11	16.35±2.21 a	- 7.19	0.183±0.03b	- 2.14
T_3	88.04±9.17 b	- 9.44	16.08±2.92 a	- 7.02	0.185±0.04 ab	- 1.07
T_4	93.44±9.17 b	- 6.40	16.66±2.32 a	- 7.13	0.180±0.04 c	- 3.74

Data represented as mean ± SD, n = 5., Mean values in each column having different superscript (a, b, c, d ,e) are significantly different at ($p \leq 0.05$) , **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1] .

Changes of lipid profile concentrations of control and Hyperthyroidism Rats.

In (Table 3) studying changes of lipid profile among control and hyperthyroidism rats, a highly significant ($p < 0.05$) increase in total cholesterol level was recorded in hyperthyroidism (+ve) group, when comparing with normal control group (-ve). Conversely, a high significant decrease in total cholesterol level was observed in treated groups feeding with enriched-chestnut yoghurts, when compared with untreated (+ve) group. Serum triglycerides levels were also elevated in the hyperthyroidism (+ve) group by (67.03%) when compared to normal control rats group . There was a significant decrease on TC, TG, LDL-c, total lipids and VLDL-c levels of treated groups that feeding on enriched-chestnut yoghurts with different premixes (T_1, T_2, T_3 and T_4) when compared with hyperthyroidism rats (+ve) ($p<0.05$). Meanwhile, there was a slightly increase on TC, TG, LDL-c and VLDL-c concentrations of treated groups that feeding on enriched-chestnut yoghurts especially premixes (T_3 and T_4) by (2.61, 2.14%; 5.76, 4.49%; 6.97, 6.32% and 5.98, 4.65%), respectively in comparison to normal control (-ve) group. these results confirmed with other finding of **Jovanovic *et al.*, (2017)** who reported that chestnut extract reversed the alleviations of dyslipidemia in diabetic rats which reduced cholesterol and triglycerides values to control level. In addition, chestnut extract considered as rich source of tannins (**Yıldız *et al.*, 2017**), and tannins acted to hamper the development of hyperlipidemia by stopping the activity of pancreatic lipase and inhibiting energy intake in obese mice (**Budriesi *et al.*, 2018).** In addition, Thyroid hormones dysfunction considered as major factors affected on lipogenesis and lipid metabolism **(Gionfra *et al.*, 2019).**

Table 3: Changes of lipid profile concentration on hyperthyroidism rats groups in comparison to normal control group (-ve).

Groups	TC mg/dl	%	TG mg/dl	%	LDL-c mg/dl	%	HDL-c mg/dl	%	VLDL-c mg/dl	%
Contr.(-ve)	75.49± 3.86 e	-	45.16± 3.28 c	-	38.30± 5. 25 d	-	28.35± 4.71 a	-	9.03± 1.457 e	-
Contr. (+ve)	121.46± 3.84 a	+ 60.89	75.43± 8.15 a	+ 67.03	86.31± 8.83 a	+ 125.35	19.47± 1.18 e	- 31.32	15.85± 3.83 a	+ 75.52
PTU	76.48± 4. 78 c	+ 1.31	45.34 ± 3.03 c	+ 0.62	39.52± 3.76 e	+ 3.185	28.38 ± 1.76 a	+ 0.10	9.09± 1.61 bc	+ 0.66
T_1	81.27± 5. 52 b	+ 7.65	51.19± 6.46 b	+ 13.35	46.98± 5.75 b	+ 22.66	24.03± 0. 37 c	- 15.24	10.29± 1.62 d	+ 13.95
T_2	80.14± 3.65 b	+ 6.16	49.18±5.795 b	+ 8.90	42.65± 1.74 c	+ 11.36	27. 71 ±2.36 b	- 2.26	9.83± 1.59 b	+ 8.86
T_3	77.46± 4.65 c	+ 2.61	47.76± 4.75 b	+ 5.76	40.97± 4.74 c	+ 6.97	26.92 ± 3.36 b	- 5.04	9.57± 1.59 b	+ 5.98
T_4	77.11± 3.66 c	+ 2.14	47.19± 4.75 b	+ 4.49	40.72± 4.74 c	+ 6.32	26. 96 ± 2.36 b	- 4. 90	9.45± 1.59 b	+ 4.65

Values are expressed as mean ± SD; n = 5, Mean values in each column having different superscript (a, b, c, d ,e) are significantly different at ($p\leq0.05$) , **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1]

Changes of renal functions activities on normal and hyperthyroidism rats groups

The hypothyroidism effects of enriched-chestnut yoghurts premixes on LT4-induced hyperthyroidism rats are shown in Table 4. In normal control (-ve) rats, serum uric acid levels were 1.51 ± 0.42 mg/dl. However in positive group (+ve), serum uric acid levels was decreased significantly to 0.99 ±0.62 mg/dl ($p < 0.05$). The serum uric acid levels of hyperthyroidism rats treated with two premixes of yoghurts which prepared with chestnut by cow milk at percent 3:1 and 6:1 were slightly increased by 19.20% and 15,89% compared with normal group (-ve). During the 4 week post treatment period with the different premixes of enriched yoghurts with chestnut, renal function gradually improved, but not uniformly (Table 4). These results similar to investigation of **Jovanovic *et al.,* (2017)** who reported that chestnut extract reversed the alleviations of urea nitrogen concentration in diabetic rats to control group level. Thyroid hormones are necessary for growth and development of renal and for the maintenance of water and electrolyte homeostasis which affected by hyperthyroidism condition. On the other hand, the kidney has a key role in the synthesis, metabolism, elimination and secretion of thyroid hormones **(Kannan *et al.,* 2017).** Meanwhile, **Srivastava *et al.,* (2018)** reported that renal dysfunction is associated with hypothyroidism.

Table 4: Changes of renal functions activities on hyperthyroidism rats groups in comparison to normal control group (-ve).

Groups	Uric acid mg/dl	%	Creatinine mg/dl	%	Urea Nitrogen mg/dl	%
Contr.(-ve)	1.51±0. 42 d	-	1.34±0. 54 d	-	38.99± 2.91d	-
Contr. (+ve)	0.99±0. 62 a	- 34.44	3.91±0.47 a	+ 191.79	52.96±3.57 a	+ 35.83
LT4 + PTU	1.62±0.14 c	+ 7.28	1.55±0.13 c	+ 15.67	36.70±2.39 c	- 5.87
T_1	1.91±0.16 b	+ 26.49	2.07±0.17 b	+ 54.48	40.39±4. 48 b	+ 3.59
T_2	1.84±0. 80 b	+ 21.85	2.10±0.25 b	+ 56.72	40.21±3.38 b	+ 3.13
T_3	1.80±0. 70 b	+ 19.20	1.74±0.25 c	+ 29.85	37.76±3.38 c	- 3.15
T_4	1.75±0. 60 b	+ 15.89	1.64±0.25c	+ 22.39	37.21±3.38 c	- 4.56

Data represented as mean ± SD, n = 5., Mean values in each column having different superscript (a, b, c, d ,e) are significantly different at ($p \leq 0.05$), . **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1].

Changes of AST and ALT activities on normal and hyperthyroidism rats groups

With regard to liver function , LT4 led to a remarkable increase in serum AST and ALT levels in positive control (+ve) group as comparing with normal control group (-ve) ($p < 0.05$). Feeding on enriched-chestnut yoghurt with different premixes decreased ($p < 0.05$) activities of AST and ALT levels of hyperthyroidism rats near to the level of the control (-ve). Though, the significant elevation ($p < 0.05$) witnessed in AST and ALT levels of hyperthyroidism rats (Table 5 and Figure 1), which reduced by consumption of enriched-chestnut yoghurt with different premixes (T_2 and T_4 followed T_3 and T_1) that near to the normal control that recorded (46.82±3.02, 46.82±3.12, 47.08±4.13 and 50.08±4.28 vr 84.07±5.58 U/L). The liver damages accompanied by thyroid hormones dysfunction regardless of hyperthyroidism and alleviations in activities liver enzymes associated with oxidative stress induced by hyperthyroidism **(Kim *et al.,* 2012).** Furthermore, hyperthyroidism has a major role on liver functions **(Gionfra *et al.,* 2019).** In addition,

Jovanovic *et al.*, (2017) reported that administration of chestnut extract to diabetic rats caused a reduction on ALT concentration equally to value of normal control group and they linked its action to the systemic antioxidant effects of chestnut extract.

Table 5: Changes of AST and ALT activities on hyperthyroidism rats groups in comparison to normal control group (-ve).

Groups	AST (U/L)	%	ALT (U/L)	%
Contr.(-ve)	43.82±2.11 d	-	22. 51±1. 78 d	-
Contr. (+ve)	84.07±5.58 a	+ 91.85	46.36±3. 27 a	+ 105.95
LT4 + PTU	42.86±2.75 d	- 2.19	22.46±2. 13 d	- 0.22
T_1	50.08±4.28 b	+ 14.28	27. 99±3.43 b	+ 24.34
T_2	46.82±3.02 c	+ 6.84	26. 73±2.14 b	+ 18.75
T_3	47.08±4.13 c	+ 7.44	25. 13±3.43 c	+ 11.64
T_4	46.82±3.12 c	+ 6.84	24. 53±2.14 c	+ 8.97

Data represented as mean ± SD, n = 5., Mean values in each column having different superscript (a, b, c, d ,e) are significantly different at (p≤0.05), . **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1] .

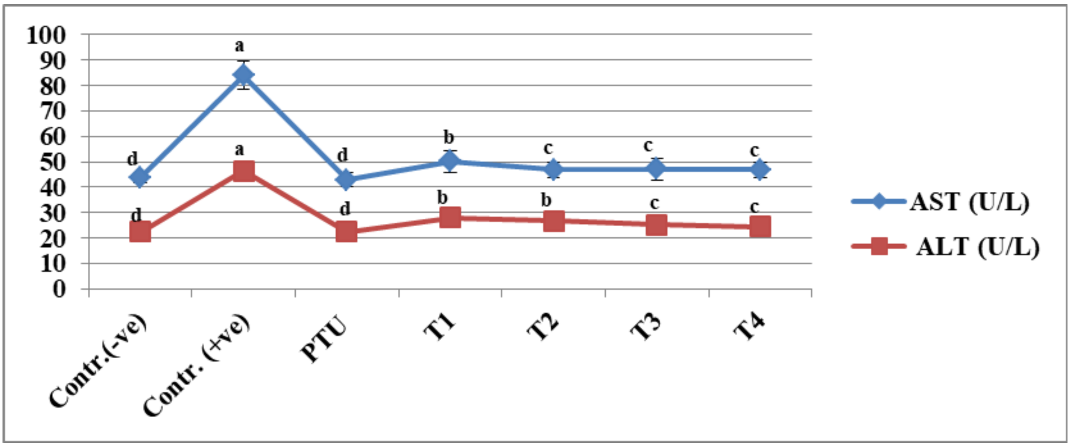

Figure 1: Changes of AST and ALT activities on normal and hyperthyroidism rats groups, Data represented as mean ± SD, n = 5., Mean values in each bar having different superscript (a, b, c, d ,e) are significantly different at (p≤0.05), . **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1]

Changes of Triiodothyronine, Thyroxine and TSH activities on normal and hyperthyroidism rats groups

Table (6) showed changes in serum levels of Triiodothyronine, Thyroxine and TSH among different groups. Hyperthyroidism simply defined as increases of serum Triiodothyronine and Thyroxine with decrease of serum

TSH, a pituitary hormone that regulated thyroid functions **(Kim *et al.,* 2012).** As expectation, in the positive control group (LT4), there was a significant increase in serum level of Triiodothyronine (6.82±2.01, $p<0.05$) and Thyroxine (17.33±4.55, $p< 0.05$), but, a significant decrease in serum level of TSH (2.84±0.16, $p<0.05$), in comparison to that in the normal control group (3.02±1.7), (10.17±6.75) and (5.03±0.32) respectively. Feeding on enriched-chestnut yoghurt treatment T_3 and T_4 that containing premixes chestnut on yoghurt by cow milk at percent (3:1 and 1:1 respectively) significantly reduced Triiodothyronine level and Thyroxine in the T_4 group (3.30 ±1.2 and 10.43±0.70 ng/dl, $P < 0.05$) and in the T_3 group (4.41± 0.12 and 10.59±0.70 ng/dl, $P < 0.05$) compared to hyperthyroidism control group (+ve), also comparing these groups with Control group, there are an increase in the activities of T3 and T4 by 46.03 and 9.27% respectively. Serum TSH levels in the hyperthyroidism control group (2.84 ±0.16 µIU /ml) were significantly lowered compared to the normal group (5.03± 0.32 µIU/ml, $P < 0.05$). Thyroid stimulating hormone (TSH) considered as the main hormone that activated thyroid gland to produce thyroxine T3 and T4 which trigger the metabolism of various every tissue in the body **(Kim and Lee 2019)**. Administration of enriched-chestnut yoghurt premixes with cow milk at percent (3:1 and 1:1) significantly improved TSH levels in the T_3 and T_4 groups (4.98 ± 0.21 and 5.12±0.32 µIU/ml, $P < 0.05$) compared to the normal control group. Meanwhile, there was nonsignificant of PTU treated group and T_3 and T_4 groups at levels of Thyroxine and TSH. These results were confirmed by **Park *et al.,* (2016)** who found that PTU administration ameliorated hyperthyroidism reducing T4 and T3, and increasing both TSH (Table 6 and Figure 2) .

In this study serum Triiodothyronine and Thyroxine levels increased in LT4- receiving rats, and according to literature of **Salehi *et al.,* (2020)** as they reported that herbal plants enhance thyroid function. So, consumption of enriched-chestnut yoghurt treatments T_3 and T_4 withstand against this increase in Triiodothyronine and Thyroxine concentrations. At the same time, these results are considered as direct evidences that enriched-chestnut yoghurt with different premixes have favorable ameliorating effect on the hyperthyroidisms and related complications induced by LT4 through systemic antioxidant effects **(Pandey *et al.,* 2018)** , such as and tannins that regulate thyroid function by way of their general characters of anti-oxidation and inflammatory inhibition **(Yıldız *et al.,* 2017).**

Table 6: Changes of Triiodothyronine, Thyroxine and TSH concentration on hyperthyroidism rats groups in comparison to normal control group (-ve).

Groups	Triiodothyronine T3 (ng/dl)	%	Thyroxine T4 (ng/dl)	%	TSH (uIU/ml)	%
Contr.(-ve)	3.02±1.7 c	-	10.17±6.75 c	-	5.03±0.32 a	-
Contr. (+ve)	6.82±2.01 a	+ 125.83	17.33±4.55 a	+ 70.40	2.84±0.16 d	- 43.54
PTU	3.32±0.23 c	+ 9.93	10.02±3.11 c	- 1.47	4.98± 0.21 b	- 0.99
T1	5.96±0.03 a	+ 97.35	11.15±3.78 b	+ 9.63	3.65± 0.16 c	- 27.43
T2	4.81±0.11b	+ 44.88	10.89±3.00 c	+ 7.08	4.47± 0.11 b	- 11.13
T3	4.41± 0.12b	+ 46.03	10.59±3.11 c	+ 4.13	4.98± 0.21 b	- 0.99
T4	3.30±1.2 c	+ 9.27	10.43±0.70 c	+ 2.55	5.12± 0.32 a	+ 1.79

Values are expressed as mean ± SD; n = 5, Mean values in each column having different superscript (a, b, c, d ,e) are significantly different at ($p \leq 0.05$), **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1]

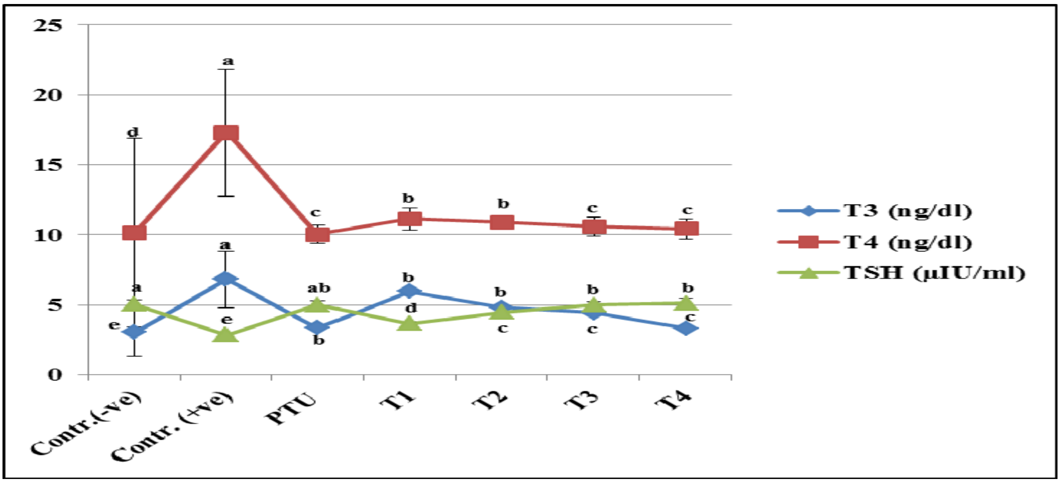

Figure 2: Changes in T3, T4 and TSH concentration on control and hyperthyroidism rats groups, Values are expressed as mean ± SD; n = 5, Mean values in each bars having different superscript (a, b, c, d ,e) are significantly different at ($p \leq 0.05$), **T_1:** Yoghurt prepared by cow milk, **T_2:** Yoghurt prepared by blending cow milk: cow milk [1:1], **T_3:** Yoghurt prepared by blending cow milk: cow milk [3:1], **T_4:** Yoghurt prepared by blending cow milk: cow milk [6:1].

Conclusion

Based on above results, it concluded that LT4 induced hypothyroidism and related biomarkers of lipid profile, renal and liver activities inhibited by oral treatment of enriched-chestnut yoghurt with different premixes especially treatment T_3 and T_4 . In addition, these premixes enhanced thyroid hormones as direct evidences that blending chestnut on yoghurt with different premixes improved and have favorable amelioration influence on the hyperthyroidism and related damages in organs in LT4-induced hyperthyroidism on rats. Further studies on mechanism and other linked factors are needed.

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الملخص

تقييم التأثير المحتمل للزبادي المدعم بأبو فروة على الفئران المصابة بزيادة نشاط الغدة الدرقية الناتج عن حقنها بهرمون الغدة الدرقية

أن تزايد الاهتمام بالطب البديل والتكميلي ادي الى استخدام النباتات الطبية بسبب التكلفة المنخفضة وقلة الآثار السلبية له. أجريت هذه الدراسة لتقييم التأثير المضاد لزيادة نشاط الغدة الدرقية بانتاج زبادي وظيفي مدعم بأبو فروة على الفئران التى تم حقنها بجرعة ليفوثيروكسين (LT4). وتم تقسيم ذكور فئران البيضاء إلى سبع مجموعات خمس في كل منها ، وهي المجموعة الكنترول السالبة (ve−) ، ومجموعة الكنترول الموجبة (المصابة بزيادة نشاط الغدة الدرقية) (+ ve) ، والمجموعة المعالجة بدواء PTU ، والمجموعة المعالجة (زيادة نشاط الغدة الدرقية) تناولت الزبادي المدعم بأبو فروة (حليب البقر) (T1) ، والمجموعة المعالجة زيادة نشاط الغدة الدرقية تناولت زبادي المدعم بأبو فروة (المحضر بابو فروة : حليب البقر بنسبة [١: ١] (T2) ، ،ثم المجموعة المعالجة زيادة نشاط الغدة الدرقية تناولت زبادي المدعم بأبو فروة المحضر بأبو فروة وحليب البقر بنسبة [٣: ١] (T3)، والمجموعة المعالجة زيادة نشاط الغدة الدرقية تناولت زبادي المدعم بأبو فروة المحضر بأبو فروة و حليب البقر بنسبة [٦: ١] (T4)، وتم حقن الفئران في جميع المجموعات باستثناء المجموعة الكنترول السالبة بـ LT4 لمدة أسبوعين للحث على فرط نشاط الغدة الدرقية . ثم تم إعطاء كل علاج لمدة ٦ أسابيع، وتم تحديد المؤشرات الحيوية المرتبطة بفرط نشاط الغدة الدرقية. أظهرت النتائج أن مصل TC و TG و LDL−c و VLDL−c و Urea Nitrogen و AST و ALT و Thyroxine, Triiodothyronine , قد انخفض بشكل كبير في مجموعات (T3) و (T4) و الدواء مقارنة بمجموعة فرط نشاط الغدة الدرقية (+ ve) . وفي الوقت نفسه ، تمت زيادة مستويات HDL−c ، وحمض البوليك ، والكرياتينين ، و TSH بشكل ملحوظ في الزبادى المدعم بابو فروة مع خلطات مختلفة ومجموعات والدواء(PTU). وتشير النتائج إلى أن ابو فروة قد يحسن العلامات الحيوية المختلفة ، وأنشطة Thyroxine, Triiodothyronineو TSH عن طريق تعديل تركيزاتها ، وبالتالي ، يمكن أن يكون الزبادي المدعم مع ابو فروة علاجًا بديلاً لفرط نشاط الغدة الدرقية.