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BIOLOGICAL AND HISTOPATHOLOGICAL CHANGES OF RATS AS AFFECTED BY FEEDING ON CHOLESTEROL ENRICHED DIET CONTAINING PROBIOTIC SOYA FETA LIKE-CHEESE

BY

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ABSTRACT

This study was aimed to explore some of the health promoting effect of probiotic UF Feta - like cheese with regard to their potential role in lowering the cholesterol level and evaluation of the histopathological changes of rats (liver, kidney, heart) by feeding the rats on cholesterol-enriched diet containing probiotic UF soy Feta - like cheese The beginning period of feeding showed a significant differences between negative group and other groups in total cholesterol level, triglycerides, HDL cholesterol and LDL + VLDL - cholesterol levels The groups of rats which fed on T3 and T6 recorded the highest lowering in total cholesterol level The triglycerides level showed a significant difference of decrease in all groups of UF Feta- like cheese and the lowest level recorded in T3 .HDL - cholesterol level decreased up to the end of the forth week all the groups fed on UF Feta like cheese treatment, but at the end of 6 weeks the level of HDL. cholesterol increased, while the (LDL+ VLDL) level gradually increased up to 4 weeks then it decreased at the end of 6 weeks as it recorded the lowest level The histopathological analysis of rats (liver, kidney and heart) showed some degenerative changes in the determined tissues. While the organs in negative group (1) showed non significant changes .Group 3 – 8 (treated groups) recorded decrease in these histopathological changes.

INTRODUCTION

Several studies have tended to demonstrate a relationship between the presence of soymilk fermented by lactic microflora and reduction in serum cholesterol. The mechanisms behind the serum cholesterol lowering action include the effect of the fermentation of product on the cholesterol metabolizing enzyme system in liver, the promotion of excretion of cholesterol through faeces, the inhibition of cholesterol absorption by binding of cholesterol to lactic acid bacterial cells, the promotion of excretion by the binding of bile acid to lactic acid bacterial cells, and the assimilation of cholesterol by lactic acid bacteria (Kawase *et al.* 2000)

The potential role of dietary soy in the prevention and treatment of chronic diseases has been recognized for a long time, the amino acid profile of protein and other non-protein components present in soy may be partially as possible for hypercholesterolemic effect (Anthony *et al.* 1996 and Balmir *et al.*, 1996).

So, this research was planned to explain the effect of the probiotic and soy Feta-like cheese on biological analysis and histopathological changes of rats fed on a cholesterol enriched diet.

MATERIALS AND METHODS

Materials

Bile acids, these acids (cholic acid, chenodeoxycholic acid and lithocholic acid) standard were supplied by Sigma chemical company (St. Louis, Mo., U.S.A).

The diagnostic kits used in this study were obtained from Sentinel CH. Millan, Italy.

Rats: Sixty Adult albino rats of average weight between 120-150 g were obtained from Crops Tech. Department,Food Technology Institute

Basal diet: The composition of the basal diet, minerals and vitamins mixture were according to AOAC, (1998) and Ibrahim, (2002)

Cheese treatments

The retentate of milk mixture (milk + soymilk 2: 1) of about 28%TS was heated at 75°C for 2 min., then cooled to 40°C and divided into two parts. The first one was treated with 1, 2 and 3% mixed starter containing 50% *Bifidobacterium Bb12* + 50% lactic acid bacteria including (*Lb. casei sub spp casei*+ *L.lactis sub spp lactis* + *L. lactis sub spp cremoris* 1: 1. 1 (treatments 1,2and 3 respectively) .The second part was treated with 1,2and3% mixed starter containing 25% *Bifidobacterium Bb12* + 75% lactic acid bacteria (treatments 4,5and 6 respectivcly).The control cheese of whole milk retentate(treatment 7) and soymilk retentate (treatment 8) were treated with 1% lactic acid bacteria without Bifidobacterium..Potssium sorbate and calcium chloride were added to all treatments at a rate of 0.01 and 0.02%, respectively with exception of treatment 8 whereas, calcium chloride was 0.5%. Then 1 ml of 0.2 normal standard rennet solution per kg of retentate was added. All treatments were filled in 500g plastic containers and left to complete coagulation. All produced cheeses were stored refrigerated (~5°C) up to 45 days.

Methods

. Biological analysis:

1-Body weight: The body weight was calculated as the following:

- Gain in body weight (%) = $\frac{\text{final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$
- Daily body weight gained = $\frac{\text{final body weight} - \text{initial body weight}}{\text{Period (45 days)}}$

2-Biochemical parameters of blood serum:

All biochemical parameters of serum were determined using kits. CH. Millour, Italy

A- Determination of serum total cholesterol:

Cholesterol was determined according to method of Richmond (1973) at wave length 546 nm.

B- Determination of serum triglycerides:

Triglycerides were determined according to the method of Fossati and Principle (1982) at wave length 546 nm.

C- Determination of serum High density lipoprotein cholesterol (HDL-cholesterol):

HDL-cholesterol was determined according to the method of Gordon (1977) at wave length 546 nm.

D. Determination of low density lipoprotein cholesterol (LDL-cholesterol) and very low density lipoprotein cholesterol (VLDL):

LDLandVLDL-cholesterol was calculated according to the method of Hatch and Lees (1968) as follows:

LDLand VLDL-cholesterol (mg/dL) = Total cholesterol – HDL-cholesterol

Experimental

Sixty adult albino rats (120-150 gm) were fed on basal diet for one week (adaptation period). After the adaptation period, they divided randomly into 10 groups (6 rats/each) and designated as follows: The first group was fed on basal diet (cholesterol free diet) throughout the experimental period of 7 weeks and was considered to be as negative control. The second group was fed on basal diet contained 0.5% cholesterol (cholesterol-enriched diet) and considered as positive control. The other eight groups were fed on a basal diet contained 0.5% cholesterol (cholesterol-enriched diet) to create hypercholesterolaemic rate for one week, then they were fed for six weeks on a cholesterol–enriched diet supplemented with different cheese treatments from T1 to T8 as described in Table (1). The weight of each rat was recorded weekly and blood samples were collected by withdrawing every two weeks from vein plexus eye, centrifuged at 3000 rpm to obtain the blood serum, which stored at (-20°C) for biochemical analysis. On the other hand, at the end of the experiment (9 weeks), rats were sacrificed and the blood was collected in clean test tubes and centrifuged to obtain the serum. The organs (heart, liver, kidneys) were excised from each rat, and were kept in formalin solution (10%, v/v) for the histopathological examination.

Table (1): The experimental rat groups and their diets

	Group	Experimental diets
Cholesterol free diet	(1)Negative control	100g basal diet + 47.1 ml water
Cholesterol-enriched diet	(2) Positive control	99.5g basal diet + 0.5g cholesterol + 47.1ml water
	(3) Treatment 1	52.4g basal diet* + 0.5g cholesterol + 47.1g (cheese T1)
	(4) Treatment 2	52.4g basal diet*+ 0.5g cholesterol + 47.1g (cheese T2)
	(5) Treatment 3	52.4g basal diet* + 0.5g cholesterol + 47.1g (cheese T3)
	(6) Treatment 4	52.4g basal diet* + 0.5g cholesterol + 47.1g (cheese T4)
	(7) Treatment 5	52.4g basal diet*+ 0.5g cholesterol + 47.1g (cheese T5)
	(8) Treatment 6	52.4g basal diet*+ 0.5g cholesterol + 47.1g (cheese T6)
	(9) Treatment 7	52.4g basal diet*+ 0.5g cholesterol + 47.1g (cheese T7)
	(10) Treatment 8	52.4g basal diet*+ 0.5g cholesterol + 47.1g (cheese T8)

Treatment 1 (T1): Teatment 8 (T8) as mentioned above. *basal diet for T1 –T8are free from casein and fat

3-Histopathological examination

For histopatholiogical examination each sample of kidney, liver and heart of all slaughtered rats was examined histopathologically. Specimens of kidney, liver and heart immediately fixed in 10% neutral buffered formalin solution, processed routinely for paraffin embedding. Sectioned at 4um and stained with routine hematoxylin and eosin (H&E) stain for general histopathological finding (Druy & Waling 1976). The photographs of the histopathological lesions were done at Dept. Hist & Cytol. Fac. Vet. Med. Zagazig Unvi. Benha Branch.

4- Statistical analysis:

The results were statistically analyzed according to the method described by Clarke and Kenpson (1997).

RESULTS AND DISCUSSION

Body weight

Table (2) cleared that the body weight at commencement has no pronounced differences in most groups fed on cholesterol-enriched diet containing probiotic soy-cheese and the two control groups (negative and positive). The body weight of rats of all groups increased by advancing in the experimental feeding period. The gained body weight was at its maximum for positive control, while the negative control recorded the lower gained body weight at the end of the experimental feeding period. Similar results were reported by Ibrahim (2002).

Blood serum analysis:

Fig (1) shows the changes in serum total cholesterol level,triglycerides level,HDL-cholesterol and serum VLDL + LDL cholesterol (mg/dl) of rats as affected by feeding on cholesterol-enriched diet containing probiotic soy UF Feta-like cheese

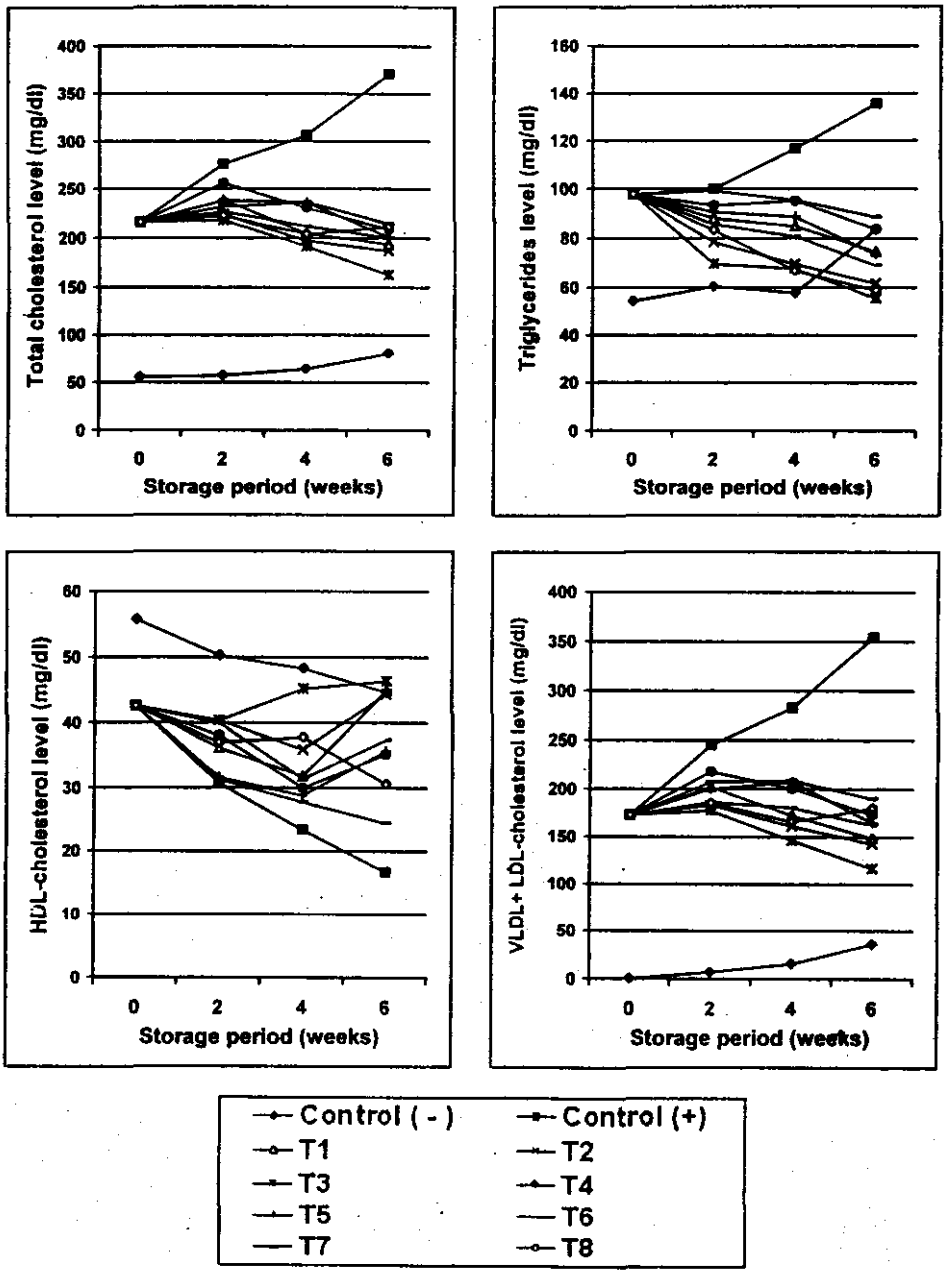


Fig. (1): Blood serum analysis of rats as affected by feeding on cholesterol-enriched diets containing probiotic and UF soy-Feta like cheese treatments (mg/dl).

Table (2): The changes in body weight of rats as a result of feeding on cholesterol – enriched diet supplemented with probiotic UF soy Feta -like cheese.

Animal Group diet	Initial body weight	Final body weight	Gain in body weight	Daily body weight gained
	(gm)	(gm)	(%)	gm
Negative control	141.50	192.50	36.04	1.13
Positive control	141.00	225.17	59.70	1.87
T1	141.00	217.30	54.11	1.70
T2	141.00	213.00	51.06	1.60
T3	141.00	220.33	56.26	1.76
T4	141.00	222.50	57.80	1.81
T5	141.00	215.33	52.72	1.65
T6	140.00	217.00	55.00	1.71
T7	141.00	220.83	56.62	1.77
T8	141.00	207.00	46.81	1.47

- Negative control = basal diet without cholesterol
- Positive control = basal diet with 0.5% cholesterol.

A- Total cholesterol level (mg/dl):

. At the commencement no differences appeared in the total cholesterol level between the positive control group and all other groups. The serum total cholesterol concentration for positive control was increasing all over the feeding period .In contrast, the serum total cholesterol reduced in the other groups of rats fed on cholesterol-enriched diet containing supplementation of cheese treatment T1 to T8. Also, it could be noticed that the probiotic and soy-cheese supplementation were more efficient for lowering serum total cholesterol level. Treatment 3 (UF-Feta like cheese containing soymilk and 3% starter culture of 50% bifidobacterium + 50% lactic culture) recorded the maximum lowering effect on total cholesterol

The more pronounced hypercholesterolemic effect in this study was due to both the probiotic and soymilk of the UF-Feta like cheese. These results are in agreement with Kikuchi-Hayakawa *et al.*, (2000) who demonstrated that bifidobacterium fermented soymilk had a hypolipidemic effect in overiectomized hamsters. Also, the obtained results are in accordance with that obtained by Homma (1988), Imaizumi *et al.*, (1992) and Ibrahim (2002).

Significant differences ($p < 0.05$) were detected for total cholesterol as a result of applying different treatments.Different feeding period caused significant difference in total cholesterol level. Applying T3 at the fourth period lowered the cholesterol level in the experimental rats significantly compared with negative control.

B- Serum triglycerides level (mg/dl):

At the beginning of the experimental feeding period there was a pronounced difference between negative control, positive control and the supplementary groups. At the same time, the positive control and all the other treatments recorded the same level of serum triglycerides .

Serum triglycerides level for both the negative and positive control increased allover the experimental feeding period . On the other hand, the serum triglycerides level decreased for all other treatments. It could be noticed that the lowest triglycerides value was recorded for treatment 3 followed by treatment 8, this confirm the effect of probiotic together with the soymilk diet of treatment 3 and the effect of soymilk alone of the diet of the treatment 8. The obtained results are in agreement with those obtained by Kikuchi-Hayakawa *et al.* (2000), who reported that feeding overiectomized hamsters on fermented soymilk decreased the triglycerides from 232 mg/dl in control to 180 mg/dl after four weeks. Similar results were also recorded by Ibrahim (2002) who found that soymilk decreased serum triglycerides but bifidobacteria fermented soymilk was more effective to reduce the serum triglycerides. The obtained results of the present study also confirmed that obtained by Schaarmann *et al.* (2001).

The statistical analysis showed that there were significant($p < 0.05$) differences among studied treatments for triglycerides. Also, feeding period caused significant differences for this trial. Maintime, the interaction between both factors expressed significant differences and the best treatment was obtained by applying T3 at the fourth period of storage (55.67 mg/dl).

C- Serum HDL cholesterol (mg/dl):

There were no differences in serum HDL-cholesterol between the positive control and all other treated groups. At the same time, the negative control was pronounced higher than all other groups as it was 55.75 mg/dl. During the first two weeks of the feeding experiment, the serum HDL-cholesterol level decreased for the positive control, negative control and all other experimental groups (T1-T8). It could be noticed that HDL-cholesterol level of treatment 3 was slightly higher than all other groups. These results agree with Kikuchi-Hayakawa *et al.* (1998) who reported that both soymilk and bifidobacterium fermented soymilk increased the HDL-cholesterol level in hamsters fed on a cholesterol enriched diet. This reflect the effect of bifidobacterium and soymilk of UF-Feta cheese of treatment 3 in the present study as it contains the highest starter percent (3%) and included 50% bifidobacteria culture+ 50% lactic culture. Similar results also were obtained by Ibrahim (2002).

Significant differences($p < 0.05$) were recorded for HDL – Cholesterol level as a result of applying different treatments. Also feeding period led to significant differences for this trial. The interaction between both factors exhibited significant difference. T3 reflects the highest significant ($p < 0.05$) value for HDL – cholesterol level being 46.35-mg/ dl at the end of feeding period.

D- Serum VLDL + LDL cholesterol (mg/dl):

It could be noticed that the negative control was lower in VLDL + LDL cholesterol than the positive control and the other supplemented dietary groups at the beginning of the experimental feeding period. The positive control and other supplementary groups recorded the same level of VLDL + LDL cholesterol. The serum VLDL + LDL cholesterol level of the positive and negative control increased during the feeding period. The VLDL + LDL cholesterol of the supplemented dietary groups (treatment 1 to treatment 8) recorded lower level than the initial values and than the positive control. Similar results were recorded by Kikuchi-Hayakawa *et al.* (1998) who found that the hamsters fed on cholesterol enriched diet plus soymilk serum VLDL + LDL cholesterol was decreased compared with hamsters fed on cholesterol-enriched diets without soymilk. The results also are in agreement with those obtained by Schaarmann *et al.* (2001) and Ibrahim (2002) who found that all the groups given probiotic and non-probiotic diets had lower plasma VLDL + LDL cholesterol than those receiving cholesterol-enriched diet (positive control), whereas the rats receiving probiotic yoghurt or soy-yoghurt were more effective in lowering serum VLDL + LDL cholesterol levels than given non-probiotic (soymilk and yoghurt) diets at the end of the experimental period.

Histopathological analysis

The results cleared the effect on kidney, liver, and heart of rats as follows:

1-Kidney

Group 1 (Negative)

The gland showed normal appearance of glomurli, proximal convoluted tubules, distal convoluted tubules, loop of heneli and collecting ducts. (Plate 1,A).

Group 2 (Positive)

Degenerative changes in the renal tissues. Cloudy swelling in the tubular epithelium which showed intracytoplasmic acidophilic albumin granules .

Focal areas of dystrophic calcification. Some glomurli showed fragmentation of the tuft and replaced by basophilic calcified mass (Plate 1,B).

Group 3-8 (Treated groups)

Some histopathological changes were recorded in the renal tissue include; cloudy swelling of the renal epithelium, with intracytoplasmic eosinophilic granules with star shape lumen.Focal areas of calcification in the renal tissues especially renal tubules (Plate 1,C).

Group 9 (Control UF- Feta like cheese)

Mild degenerative changes in the form eosinophilic substance in the lumen of the tubules.Some of the glomurli showed destruction and others showed shrinkage (Plate 1,D).

Group 10 (control UF soy Feta- like cheese)

The histopathological changes were mild, only represented by desquamation of the lining epithelium of the renal tubules with some destruction of few glomurli (Plate 1,E).

2-Liver

Group 1 (Negative)

The gland showed normal central vein, hepatic veins and bile canaliculi. The hepatic cord consists of hepatocytes with one or two nuclei and acidophilic cytoplasm (Plate 2,A).

Group 2 (Positive)

Degenerative changes in the hepatocytes, include vacular & hydrobic degeneration with pale acidophilic cytoplasm (Plate 2,B).Focal areas of necrosis distributed between the hepatocytes which appear as fine nuclear eosinophilic materials infiltrated with mononuclear leucocytes. Fatty changes were also recorded in the cytoplasm of some hepatocytes.

Group 3-8 (Treated groups)

Mild degenerative changes in the parenchyma of liver in the form of hydrobic degeneration with pyknotic nuclei (Plate 2,C).Congestion in the portal blood vessels with mild mononuclear cellular infiltration.

Group 9 (Control UF- Feta like cheese)

The hepatocytes showed vacuolation & centeral vein showed dilatation (Plate 2,D).

Group 10 (control UF soy Feta- like cheese)

The degenerative changes were present as that of previous groups. Only a new finding represented by severe congestion of the blood vessels. (Plate 2,E).

3-Heart

Group 1 (Negative)

The heart showed normal appearance of cardiac muscle with multinucleated muscle fibers (Plate 3,A) and acidophilic cytoplasm.

Group 2 (Positive)

Hyaline degeneration in the cardiac muscles, intermuscular hemorrhage were recorded in the blood vessels of the heart (Plate. 3,B)..

Group 3-8 (Treated groups)

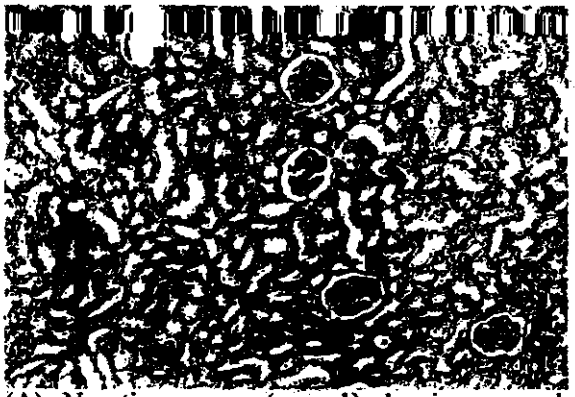
The cardic muscle showed non significant histopathological changes (Plate 3,C)

Group 9 (Control UF- Feta like cheese)

Non significant changes were recorded but some mild intermuscular edema were reported in Plate 3,D.

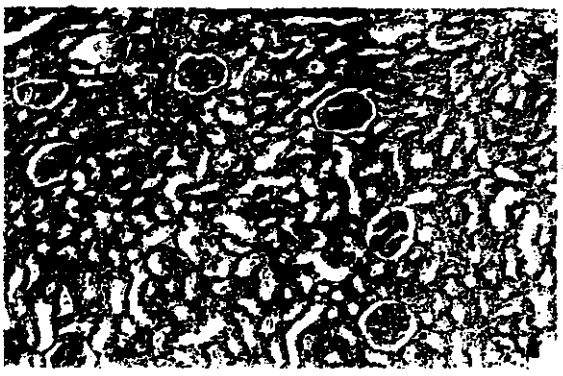
Group 10 (control UF soy Feta- like cheese)

Mild intermuscular edema (Plate.3, E). Also intermuscular congestion of some blood vessels was recorded in some microscopic observations.

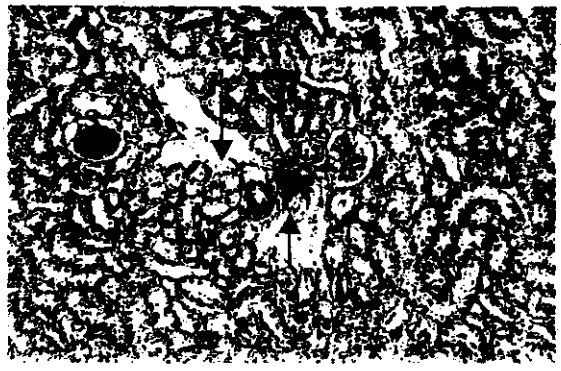


(A) Negative group (group1) showing, normal renal corpuscle (r), proximal convoluted Tubules (p), distal convoluted tubules (d) and collecting duct (c). H & E × 250

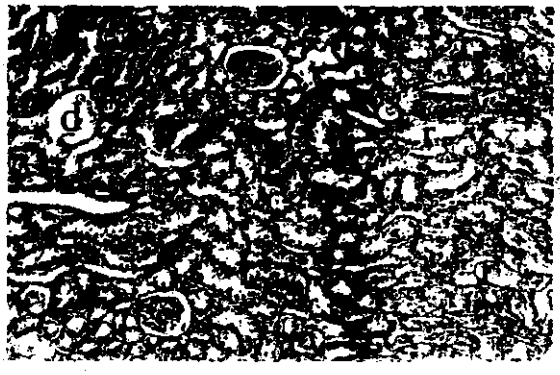
(B) Positive group (group2) showing ,calcified masses
(c) , destruction and shrinkage of the renal corpuscle .



(C) Treated group (Group 3-8) showing, cloudy swelling (c) in renal tubules. H & E .....× 250

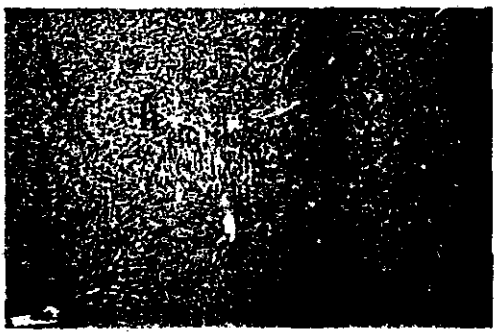


(D) Feta like- cheese group (Group 9) showing, cloudy swelling (c), shrinkage of renal corpuscle (S) and destruction in the epithelium (arrow). H & E .× 250

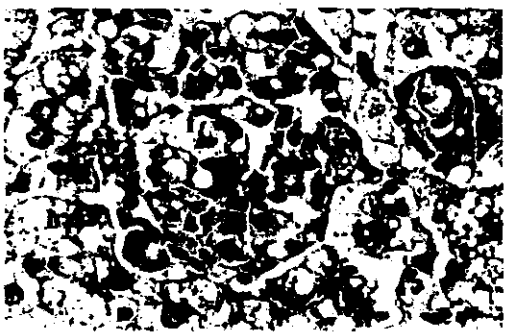


(E) Soy Feta like cheese group (Group 10) showing, destruction of some renal corpuscle (d) and desqumation of renal epithelium (r). H & E .× 300

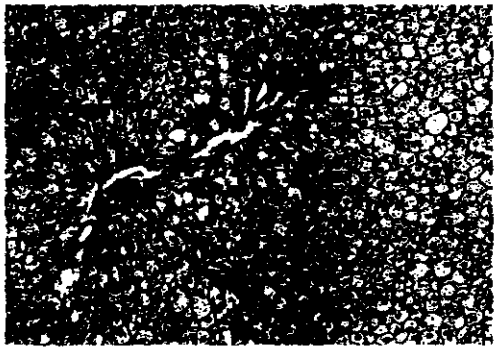
Plate (1): Cross section of kidney.



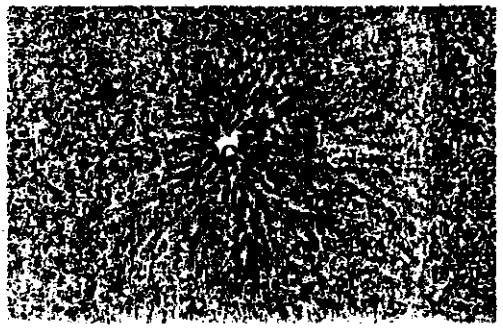
(A)Negative group (group1) showing, hepatic cords (h) and central Vein (c) H & E .....× 250



(B)Positive group (group2) showing the hydropic degeneration(h)and fatty changes in the hepato cytes (f) H & E .× 1000



(C) Treated group (Group 3-8) showing, congestion in the portal blood vessels (p) and hydropic degeneration in the hepatic cords (h) H & E.× 300



(D) Feta cheese group (Group 9) showing, vacular degeneration in the hepatocyte (v) and diltation of the central vein (c).H & E.× 250

E)Soy Feta like-cheese group (Group10) showing, degenerative changes and severe congestion in central vein (c) and portal blood vessels (p).H & E .× 300

Plate (2): Cross section in liver

(A) Negative group (group1) showing, normal appearance of cardiac muscles (m)H & E ..× 250

(B)Positive group (group2)showing, intermuscular haemorrhage (h) H & E .....× 250

(C) Treated group (Group 3-8) showing, mild inter muscular edema (e) H & E ..× 300

(D)Feta like - cheese group (Group 9) showing, mild inter muscular edema (e) H & E ..× 300

(E) Soy Feta like-cheese group(Group 10) showing, mild inter muscular edema (e) H &E.× 300

Plate (3): Cross section in heart.

The server histopathological finding were present in the internal organs of the positive group and gradually decreased from severe effect to mild effect after long runtime of treating with soy UF Feta-like cheese plus probiotic bacteria.

CONCLUSION

From the previous study it could be concluded that the feeding on UF Feta-like cheese containing soymilk and 3% starter culture containing 50% *Bifidobacterium Bb12* + 50% lactic acid bacteria including (*Lb. casei sub spp casei+ L.lactis sub spp lactis + L. lactis sub spp cremoris* lowerd total cholesterol level in blood serum and improve the histopathological changes in kidney,liver and heart of rats which fed on cholesterol-enriched diet

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التغيرات البيولوجية والهستوباثولوجية للفئران نتيجة التغذية على جبن شبية الجبن الفيتا المحتوى على لبن فول الصويا والبكتريا العلاجية

محمد بدير الالفى، عبد العاطى محمد عبدالعاطى، محمود فوزى يونس، شعراوى جمال عثمان*، وليد عبدالمتعال جافور*

قسم علوم الاغذية كلية الزرا عة بمشتهر جامعة الزقازيق فرع بنها

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أجريت هذة الدراسة بهدف تقييم التغيرات البيولوجية والبيوكيمائية والهستوباثولوجية علي الفئران المغذاة علي عليقة تحتوي علي ٠,٥٪ كوليسترول والتي تحتوي علي جبن فيتا بة لبن فول صويا وبكتيريا علاجية من البيفدوبكتيريا وقد قسمت الفئران إلي عشر مجموعات وذلك لمعرفة التأثير الخافض لبروتينات فول الصويا والبكتيريا العلاجية علي مستوي كوليسترول الدم واللبيدات والجليسريدات الثلاثية وكذلك اهم التغيرات الهستوباثولوجية التى تحدث على الكلى والكبد والقلب وكانت أهم النتائج المتحصل عليها في هذه التجربة هي:

١- في بداية التجربة توجد فروق معنوية بين مجموعة الكنترول السالبة وباقي القياسات متمثلة في الكوليسترول الكلي، الجليسريدات

الثلاثية، الليبوبرتينات عالية الكثافة والليبوبرتينات منخفضة وشديدة إنخفاض الكثافة.

٢- سجلت المجموعات التي تم تغذيتها علي جبن الفيتا المحتوية علي لبن الصويا و٣ ٪ باديء منه ٥٠٪ أو ٢٥٪ بكتيريا *Bifidobacteria Bb12* أعلي انخفاضا في كل من مستوي الكوليسترول الكلي في الدم والجليسريدات الثلاثيةوالليبوبروتينات عالية الكثافة بينما ارتفعت الليبوبروتينات منخفضة وشديدة إنخفاض الكثافة (VLDL + LDL) حتي نهاية الأسبوع الرابع، تلي ذلك إنخفاض في نهاية الأسبوع السادس إلي مستوي أقل مما كانت عليه في بداية الأسبوع الأول .

٣- التغيرات الهستوباثولوجية:فقد ادت التغذية على الجبن الفيتا المحتوى على لبن فول الصويا والبكتريا العلاجية الى تحسين الخواص الهستولوجية لكل من الكلى والكبد والقلب حيث قللت بدرجة ملحوظة التخثر والتلف فى كل من النسيج الكلوى والكبدى وعضلات القلب التى كانت موجودة بشكل واضح فى المجموعة الموجبة (مجموعة ٢) لدرجة ان الخواص الهستولوجية للقلب والاوعية الدموية كانت سليمة وقريبة جدا فى خواصها من المجموعة السالبة (مجموعة ١)

مما سبق يتضح لنا ان تناول الجبن شبية الفيتا المصنع من خليط اللبن الطبيعى ولبن فول الصويا ٢: ١ والمدعم بالبيفيدوبكتريا بنسبة ٣٪ باديء من٥٠٪ بيفيدوبكتريا + ٥٠٪ بكتريا حامض اللاكتيك أدى إلى تحسين صورة الدم وحماية الجسم من تصلب الشرايين.