Effects of chitosan versus curcumin on dorsal surface of tongue mucosa of albino rats fed on high-fat diet: histological and ultrastructural study

Amr Ahmed El- Sayed[1], Elham Fathy Mahmoud[2], Rasha Mohammed Taha[3]

1- Head of Dental Department at the Egyptian Ministry of Health One Day Surgery Hospital, Cairo, Egypt. Email: amoradentist@gmail.com
2- Professor, Head of Oral Biology Department, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Email: drelham_fathy@yahoo.com
3- Professor, Department of Oral Biology, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Email: rm.taha@hotmail.com

Corresponding author: Amr Ahmed El- Sayed, email: amoradentist@gmail.com

KEYWORDS

High fat diet, Curcumin, Chitosan, Tongue.

ABSTRACT

Aim: This work aimed to evaluate the effects of Curcumin versus Chitosan as a natural product on the dorsal surface of tongue mucosa of albino rats fed on high fat diet.

Materials and Methods: 40 rats were equally divided into 2 main groups (1- control group: received normal diet and distilled water , 2- experimental groups: divided into 3 subgroups: 2-A received high fat diet, 2-B: received High Fat diet + Curcumin, 2-C: received High Fat diet + Chitosan (curcumin and chitosan were administered to rats via gastric tube). Cholesterol level in blood was measured at day 0, 90 and 120. Rats euthanized at the end of 4 months and tongue specimens were processed for histological and immunohistochemical evaluation. All data were statistically analysed using one-way ANOVA (F) test with post hoc test (Tukey).

Results: A high-fat diet caused significant alterations to the tongue's dorsal surface, treatment with curcumin showed a slight improvement. On the other hand, chitosan demonstrated notable improvements to the tongue mucosa.

Conclusions: A high-fat diet has a significant cytotoxic effect on the dorsal surface of the tongue. Curcumin and chitosan may have a protective impact on hypercholesterolemia-induced tongue cytotoxicity.

Introduction:

A high fat diet means consuming an excessive amount of fat, particularly saturated fat. Consuming too much saturated fat can raise the harmful cholesterol levels in the body, which lead in increasing the chance of developing heart disease and other health problems. Furthermore, HFDs are frequently associated with an excessive consumption of calories, which can contribute to weight gain and obesity.[1]

Chronic exposure to a high-fat diet might cause cholesterol precipitation in the blood (hypercholesterolaemia). Elevated plasma total cholesterol (TC) levels are associated with coronary heart disease, atherosclerosis, and strokes.[2]

Hypercholesterolemia is a condition characterized by elevated cholesterol levels that can be managed with a variety of medications, including 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase inhibitors, bile acid sequestrants, activated lipoprotein lipase inhibitors, and cholesterol absorption inhibitors.[3]

The most common treatment for hypercholesterolemia is to reduce the use of pharmaceuticals such as statins and fibrates, which can have a variety of side effects. As a result, investigating alternative medicine, such as natural products, for managing hypercholesterolemia has recently become an acceptable plane against hypercholesterolemia.[4]

The majority of lipid-lowering variables are accompanied with significant treatment problems and serious adverse effects. In contrast, dietary fibers provide a safer option for lipid-lowering medication. Chitosan (CS), a dietary fiber, is biodegradable, biocompatible, and has numerous health benefits. These advantages include wound healing, anti-inflammatory and anti-cancer qualities, immunological modulation, haemostatic effects, lipid-lowering capacities, and antioxidant activity.[5]

The addition of chitosan to an animal feed reduces low density lipoprotein (LDL) cholesterol levels. In general, high-density lipoprotein (HDL) cholesterol may help prevent cardiovascular disease by converting cholesterol precipitated on peripheral tissues or blood vessel walls into an ester molecule. The ester molecule is subsequently carried to the liver, expelled via bile salt, and the blood cholesterol level is reduced. In contrast, LDL-cholesterol, the most common kind of blood cholesterol, precipitates easily on arterial walls, leading to arteriosclerosis. As a result, it is recognized as the primary risk factor for arteriosclerosis and cardiovascular disease.[6]

Curcuma longa, a traditional medicinal plant, is widely distributed in China and other Asian nations. Turmeric, the rhizome of Curcuma longa, is used as a spice to increase flavor and as medicine because of its therapeutic effects.[7]

Curcuminoids, the principal bioactive compounds derived from the rhizome of Curcuma longa L, are responsible for the major biological effects of turmeric.[8]

Experimental and clinical investigations have discussed the positive effects of curcumin supplementation on lipid profile and glycemic status.[9]

Curcumin, the most common form of curcuminoids, has numerous pharmacological actions, including antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, and anticancer activities.[10]

Statins and fibrates are common pharmacological treatments for hypercholesterolemia, but they can cause a variety of side effects. As a result, discovering an alternative drug, such as curcumin and chitosan, to manage hypercholesterolemia has proven to be an effective strategy.[11]

Materials and Methods

40 adult male albino rats with body weight 160- 180 gm each at the beginning of the experiment. Rats were acclimated 7 days before the experimentation. Rats were housed under good ventilation in separate cage (5 rats each) at the animal house of Faculty of Dentistry, Suez Canal University. The present research was conducted after the approval of the Research Ethics Committee (REC) of the Faculty of Dentistry, Suez Canal University in approval number 420/2021.

Grouping:

Rats were equally divided into two main groups as follows:

1-Control group (- ve control):

This group consisted of 10 rats received normal diet and distilled water through a gastric tube for 4 months and they served as control for other groups.

2- Experimental Groups:

Were subdivided into 3 subgroups as follows:

2- A High Fat Diet Group (+ve control):

This group consisted of 10 rats that received 1% cholesterol in their diet for 4 months. Cholesterol rich diet was prepared by a mixture of , cholesterol (10gr) (Fig.1), casein (120gr), salt mixture (50gr), vitamin mixture (10gr), soybean oil (250gr), choline (0.4gr), cellulose (130gr) and corn starch (429.6gr), bile salt mixture (2.5gr) necessary for intestinal absorption of cholesterol.[12]

2- B High Fat diet + Curcumin group:

This group consisted of 10 rats and fed a high fat diet for 4 months. Curcumin was delivered via gastric tube at the beginning of the fourth month in a daily dose of 1.5 mg/ Kg. BW. Dried powder was obtained from (Harraz) local market in Cairo for herbs.[13]

2- C High Fat diet + Chitosan group:

This group consisted of 10 rats and fed a high fat diet for 4 months. Chitosan tablets were crushed by mortar and pistol and dissolved in distilled water in a concentration of 10% and delivered by gastric tube at the beginning of the fourth month in a daily dose of 500 mg/ Kg. BW .[14]

Evaluation Methods:

1- Biochemical Analysis:

Cholesterol level was measured before induction of hypercholesterolemia (day 0), after induction of hypercholesterolemia (day 90) and at the end of the experimental period (day 120) after rat's treatment with Chitosan, and Curcumin.[15] Blood samples were collected from the orbital sinus of the rat.

2- Histological and immunohistochemical Evaluation

Rats euthanized at the end of 4 months. They euthanized by overdose inhalation of ether. After euthanization tongue specimens were collected to accommodate histological and immunohistochemical evaluation.

Samples preparation for H&E examination:

At the end of four months, each group's tongue was collected, and the samples were immediately fixed in a 10% buffered formalin solution for at least 48 hours. The specimens were prepared and stained with haematoxylin and eosin. The cover slipping technique began by bonding the cover slip glass to the tissue portion of the microscope slide glass. The mounting media is typically not soluble in water. As a result, the tissue should be dehydrated again using solutions with increasing percentages of alcohol and xylene.[16]

Samples preparation for immunohistochemical Examination:

All tongue specimens retrieved for immunohistochemistry were fixed in freshly prepared 4% (w/v) paraformaldehyde in 0.1 mol PBS l–1 and processed overnight in paraffin wax. Formalin-fixed

paraffin-embedded sections were dewaxed in xylene and rehydrated through graded alcohol to distilled water. The sections were subjected to antigen retrieval by boiling in a microwave for 20 min in 0.01 M sodium citrate buffer (pH 6.0). The primary antibody to caspase-3 (Transduction Laboratories, Lexington, KY) was applied at a dilution of 1:1000 and incubated overnight at 4°C. After incubation, the slides were treated with biotinylated rabbit ant mouse immunoglobulin (1:600 for 30 min; Dako Ltd., Ely, UK) washed as before, and then treated with streptavidin and biotinylated alkaline phosphatase according to the manufacturer's instructions (Dako). Caspase III expression appeared as brownish nuclear and cytoplasmic staining in tongue surface epithelium and underlying lamina propria. Additional tissue sections were stained in parallel, but with omission of the primary antibody as negative controls.[17]

Statistical Analysis:

Statistical analysis was done by statistical package for the social sciences (SPSS) version 28 (IBM Co., Armonk, NY, USA) commercially available software program for windows. Quantitative data and numerical data were presented as the mean, standard deviation (SD) and range, analysed across time-points using repeated measures ANOVA while analysed across different groups using one-way ANOVA (F) test with post hoc test (Tukey). A two-tailed P value < 0.05 was considered statistically significant. Mean lipid profile level (biochemical analysis) comparison between all groups were statistically examined.

Results

I- Histological Results (H&E): Histological sections of **control group** rats showed the filiform papillae were distributed evenly over the dorsal surface of the anterior two thirds of the tongue (Fig. 1A) Single fungiform papilla was seen scattered between the numerous filiform papillae with broader surface and highly vascular connective tissue core (Fig. 1B). lingual salivary gland between the muscles of the tongue (Fig. 1C). well organized muscle bundles (Fig. 1 D). **High Fat Diet Group** revealed atrophic and degenerative alterations. The filiform papillae appeared atrophic with signs of hyperkeratosis and a lot of cytoplasmic vacuolization in the epithelial cells (Fig. 1 E). The fungiform papillae showed degenerated taste bud with cytoplasmic vacuolization and a keratin layer on the surface of the papilla (Fig. 1 F). Lingual glands appeared with marked degenerative changes in the form of cytoplasmic vacuolization (Fig. 1 G). Lingual muscles showed disorganized and degenerated muscle fibers with multiple spaces in between and inflammatory cells infiltrations. Blood vessel engorged with blood was noticed (Fig. 1 H). **Hyperlipidemic rats treated with Curcumin** showed little improvement in their histological picture. Filiform papillae showed partial regain of their shape and architecture (Fig. 2 A). Fungiform papillae with vacuolated taste bud (Fig. 2 B). Lingual glands showed little improvement with cytoplasmic vacuolization. The mucous acinar cells showed cystic transformation of some cells (Fig. 2 C), Widened blood vessel engorged with blood and the connective tissue in between muscle bundles with some vacuoles were noticed (Fig. 2 D). **Hyperlipidemic rats treated with Chitosan** showed obvious improvement in their histological picture. The filiform and fungiform papillae showed almost normal histological appearance unless for some vacuolization (Fig 2 E, F). Lingual glands showed almost normal histological picture unless for some cytoplasmic vacuolization (Fig. 2 G). The muscles of the tongue were partially regenerated with some vacuoles in between muscle fibers. Blood vessels engorged with blood. Fat droplets spaces were also recognized (Fig. 2 H).

II- Scanning Electron Microscope Results: Scanning electron microscopic examination of the dorsal surface of the tongue from the **control group** showed filiform papillae covering the apex and body of the tongue, they were elongated, conical in shape with tapering end, had an intact covering and regularly arranged in one direction (Fig. 3 A). The fungiform papillae were scattered

in between filiform one (Fig. 3 B). **Rats fed with high fat diet** showed marked changes represented in the filiform papillae which appeared shortened, thinned and arranged in different orientation with a desquamation of the covering epithelium (Fig. 3 C). Fungiform papillae appeared wrinkled and keratinized with degenerated taste bud with elevated irregular and ill-defined taste pore. Fungal infection was noticed scattered on the surface of the papillae (Fig. 3 D). **Rats treated with curcumin** revealed almost normal filiform papillae while other areas appeared shortened and disorganized with rough keratin accumulation on its surface (Fig. 3 E). The fungiform papillae were normal and showed characteristic taste pore on their upper flat surface (Fig. 3 F). **The chitosan treated group** exhibited restoration of almost regular shape and arrangement of tongue papillae. Filiform papillae appeared slender regularly arranged with scattered fungiform papillae in between (Fig. 3 G). The fungiform papillae: appeared with its characteristic mushroom shape and regular surface with obvious taste pore (Fig. 3 H).

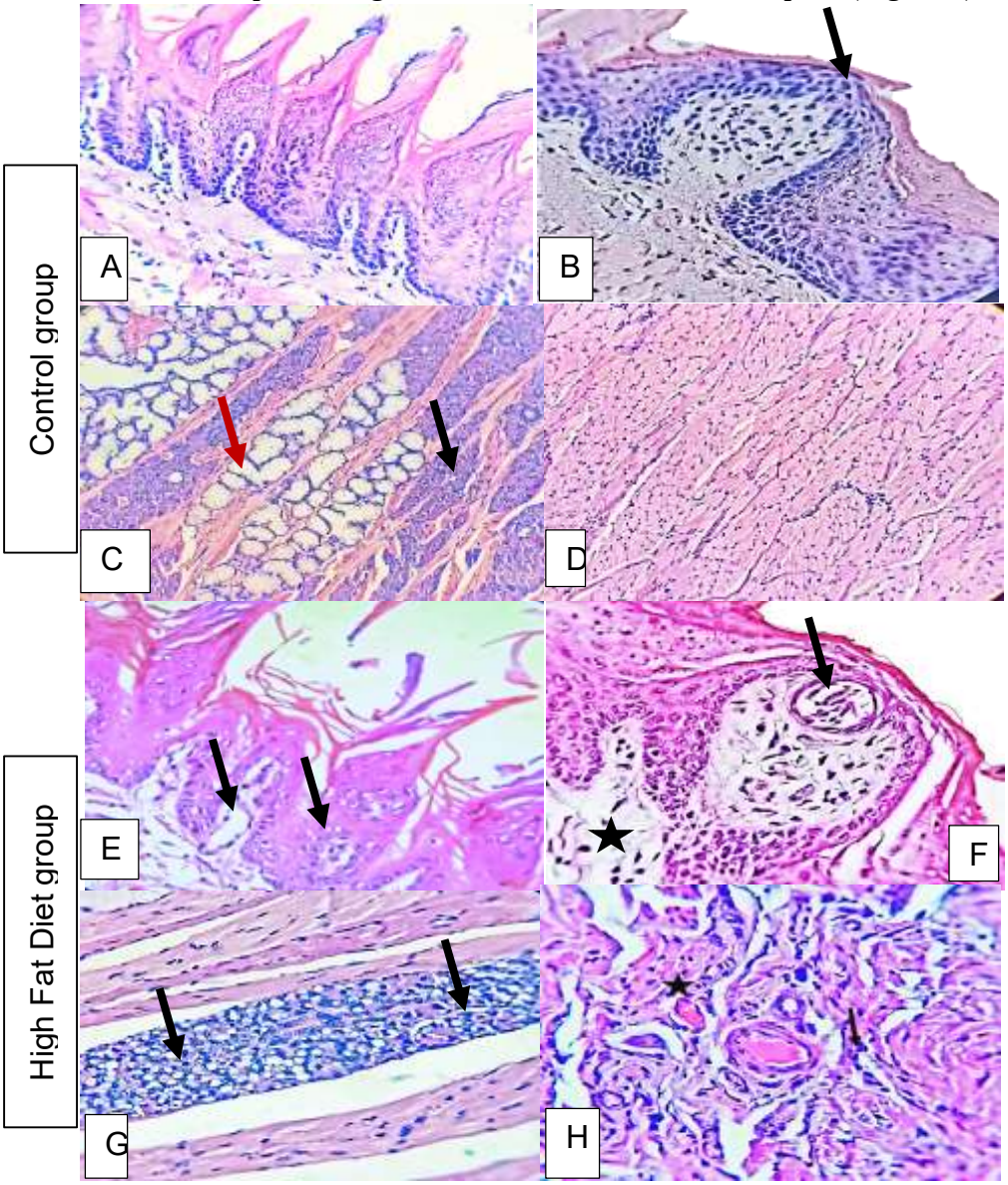

Figure (1): Photomicrograph showing: control group: **A, B, C, D.** High fat diet group: **E, F, G, H. A:** Sharp conical projections of filiform papillae with keratinized stratified squamous epithelial coverage. **B:** a Fungiform papilla (arrow) between filiform papillae. **C:** Pure mucous acini of Weber salivary glands (black arrow) and pure serous acini of Von Ebner salivary gland (red arrow) in between muscle bundles. **D:** Normal distribution of muscles bundles. **E**: Atrophied filiform papillae with cytoplasmic vacuolization of epithelium (arrows) and hyperkeratenization. **F:** fungiform papilla with degenerated taste bud (Arrow) and dissociation of collagen fibers (Star). **G:** Serous acinar cells with cytoplasmic vacuolations (black arrows). **H:** Blood vessel engorged with blood (Star) disorganized and degenerated muscle fibers with multiple spaces in between and inflammatory cells infiltrations (Arrow) **(H&E. C, D: x160. G:x 200. A, B, E, F: x400.)**

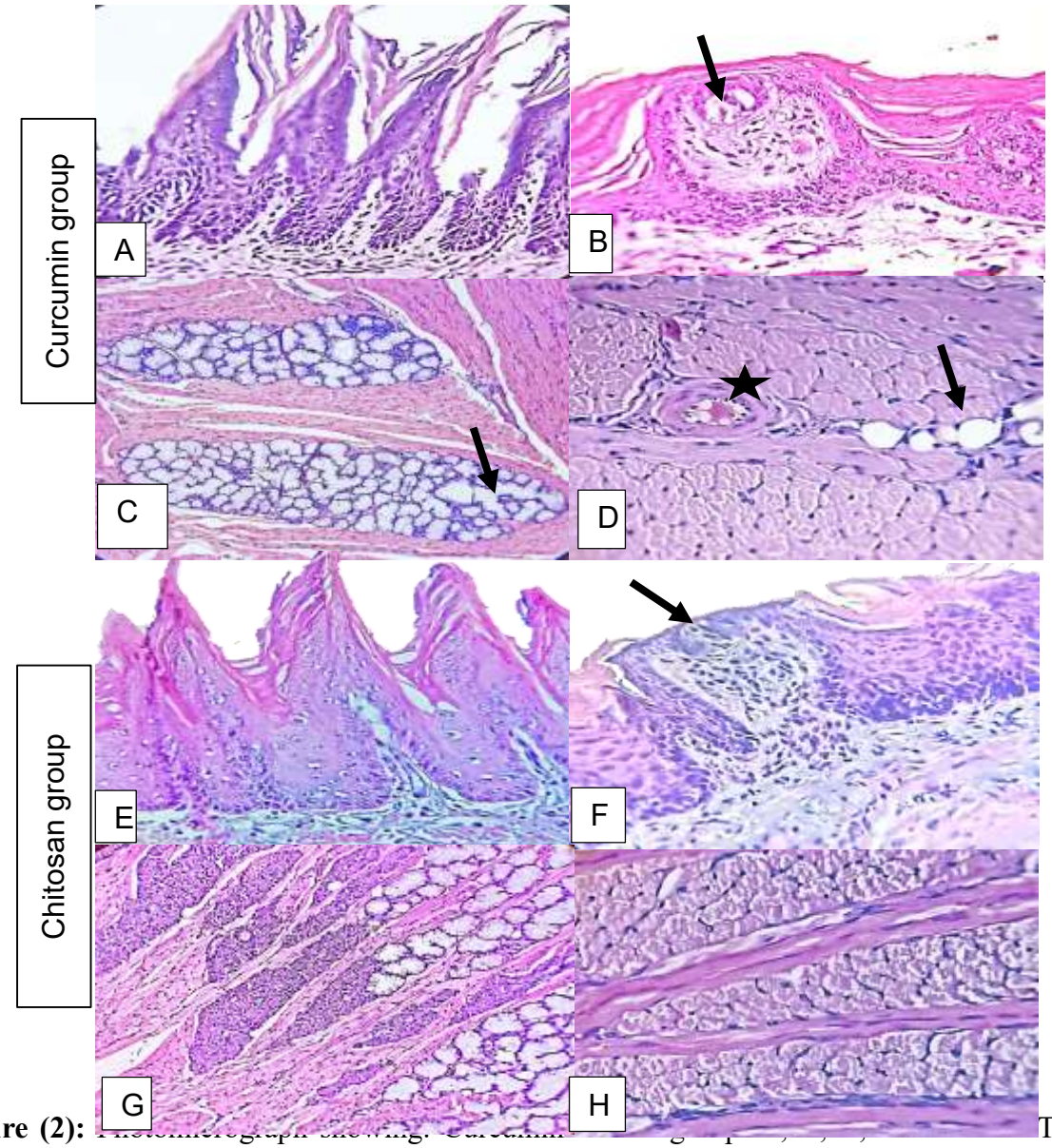

Figure (2): Photomicrograph showing: Curcumin group: A, B, C, D. Chitosan Treated group: E, F, G, H. **A:** Filiform papilla with decrease in epithelial thickness. **B:** a Fungiform papilla

with vaculed taste bud (Arrow). **C:** Mucous acini with cystic transformation (Arrow). **D:** Almost organized muscle bundles with widened blood vessel engorged with blood (Star) and connective tissue in between the muscle bundle with some vacuoles (Arrow**)**. **E**: Filiform papillae with nearly normal keratinization and underlying lamina propria. **F:** Almost normal fungiform papillae with well-organized taste bud (arrow) **G:** Von Ebner and Weber salivary of the tongue of the high fat diet rats treated with chitosan showing almost normal histological architecture of epithelial lining and lumen of ducts. **H:** well organized muscle bundles **(H&E. C, G: x160. A, B, D, E, F, H: x400.).**

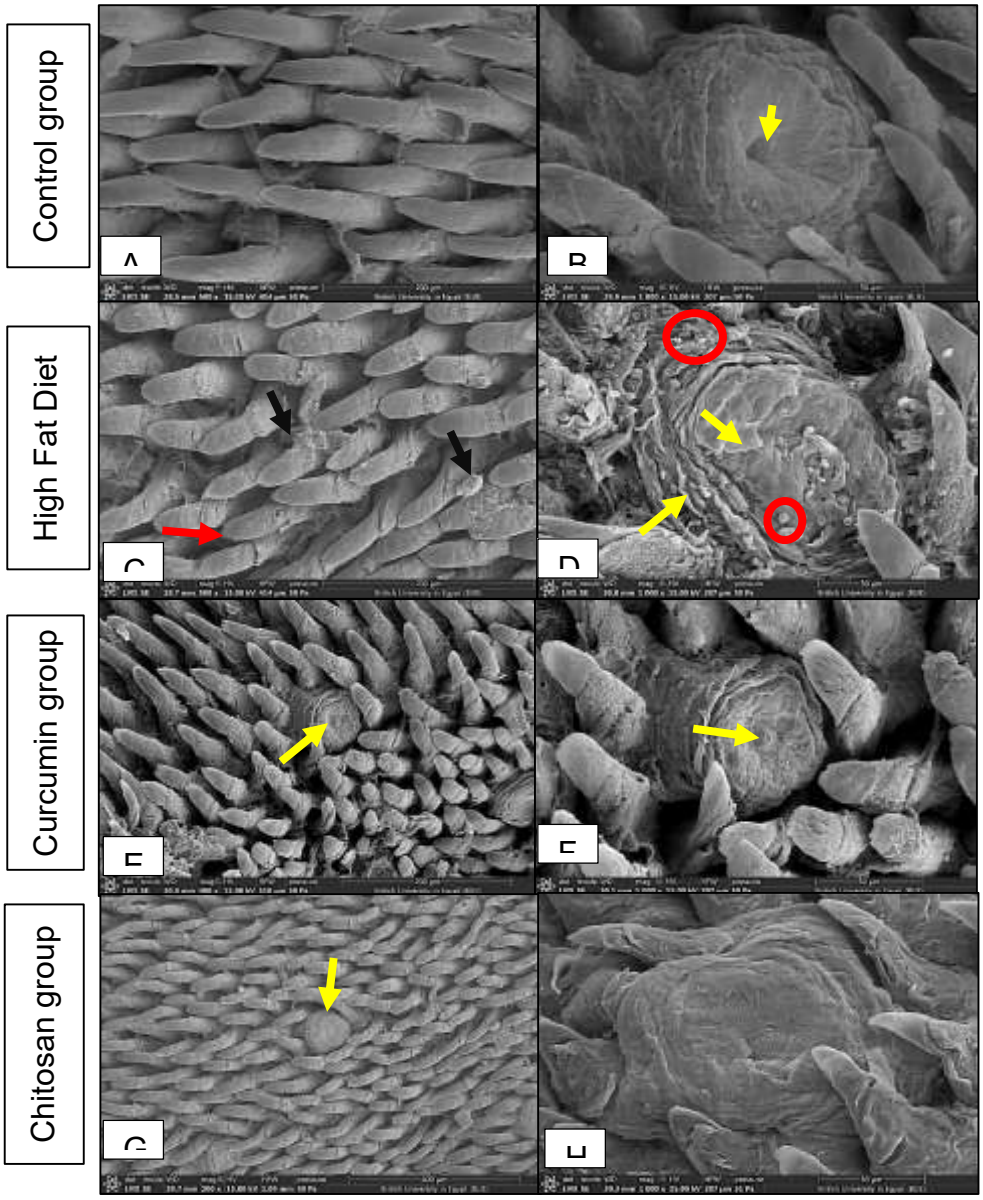

Figure (3): Photomicrograph showing: Control group: **A, B.** High fat diet group: **C, D.** Curcumin treated group **E, F**. Chitosan Treated group: **G, H. A:** filiform papillae elongated, conical in shape and regularly arranged in one direction. **B:** Fungiform papillae with mushroom shaped outline and regular surface with apparent taste pore on its upper surface (arrow) and surrounded by regular

filiform papillae **C:** filiform papillae with irregular orientations, keratin aggregations (black arrows) and conical tips distortion (red arrow). **D:** fungiform papillae with wrinkled irregular dorsal surface (arrows) and fungal infection (Circle**)**. **E**: Disorganized filiform papillae with rough keratin base. Fungiform papillae noticed between filiform papillae (arrow). **F:** Fungiform papillae in between filiform papillae with characteristic taste pore on its upper surface (arrow). **G:** Regularly oriented filiform papillae and fungiform papillae found in between (arrow). **H:** Fungiform papillae with apparently regular surface **(SEM. A, B, D, F, H: x1000. C: x500. E: x 400. G: x 200).**

III- Statistical results

Biochemical Analysis

Comparison of blood cholesterol levels among the studied groups

At day 0, there was no statistically significant difference among the groups studied in terms of cholesterol level. At day 90, a statistically significant difference was detected among groups ($P<0.001$), as cholesterol level was significantly increased in all experimental groups compared to the control group. On day 120, there was a statistically significant difference among groups ($P<0.001$) as cholesterol level was significantly lower in chitosan treated group than curcumin treated group, significantly lower in both groups compared to high fat diet group, and in comparison, to control group, it was significantly higher in high fat diet and curcumin groups but insignificantly different in chitosan treated group Table (1), (Fig. 4 A).

Comparison of blood LDL levels among the studied groups

At day 0, there was no statistically significant difference among the studied groups in terms of LDL level. At day 90, a statistically significant difference was detected among groups ($P<0.001$), as LDL level was significantly increased in treatment groups compared to the control group, but the curcumin group was the higher. At day 120, there was a statistically significant difference among groups ($P<0.001$) as LDL level was significantly lower in chitosan group than curcumin group. Also, it was significantly higher in chitosan group and curcumin group than in control group Table (1), (Fig. 4 B).

Comparison of blood HDL levels among the studied groups

At day 0, there was no statistically significant difference among the groups studied in terms of HDL level. At day 90, a statistically significant difference was detected among groups ($P<0.001$), as HDL level was significantly increased in treatment groups in compared to the control group and high fat diet group, but chitosan group was the higher. At day 120, there was a statistically significant difference among groups ($P<0.001$) as HDL level was significantly lower in control group and high fat diet group in compared with other groups. Also, chitosan group showed significant increase in HDL level in compared with curcumin group Table (1), (Fig. 4 C).

Table (1): Comparison of blood cholesterol levels, blood LDL levels and blood HDL levels among the studied groups

	Blood Cholesterol Level	**Control (n=10)**	**High Fat Diet (n=10)**	**Curcumin (n=10)**	**Chitosan (n=10)**	**P value**
Day 0	**Mean ± SD**	65.3 ± 9.8	63.4 ± 9.86	61.6 ± 10.84	65.6 ± 12.53	0.828
	Range	55 - 89	55 - 88	50 - 88	54 - 89	
Day 90	**Mean ± SD**	69.3 ± 10.07 [a]	183.4 ± 11.22 [b]	181.5 ± 9.57 [b]	179.3 ± 9.9 [b]	**<0.001***
	Range	55 - 87	169 - 198	169 - 195	169 - 195	
Day 120	**Mean ± SD**	64.2 ± 5.73 [a]	235 ± 32.91 [b]	114.2 ± 11.48 [c]	67.6 ± 9.18 [a]	**<0.001***
	Range	55 - 75	195 - 270	100 - 130	55 - 80	
	Blood LDL Level	Control **(n=10)**	High Fat Diet **(n=10)**	Curcumin **(n=10)**	Chitosan **(n=10)**	**P value**
Day 0	**Mean ± SD**	65.3 ± 9.8	63.4 ± 9.86	61.6 ± 10.84	65.6 ± 12.53	0.828
	Range	55 - 89	55 - 88	50 - 88	54 - 89	
Day 90	**Mean ± SD**	69.3 ± 10.07 [a]	183.4 ± 11.22 [b]	181.5 ± 9.57 [b]	179.3 ± 9.9 [b]	**<0.001***
	Range	55 - 87	169 - 198	169 - 195	169 - 195	
Day 120	**Mean ± SD**	64.2 ± 5.73 [a]	235 ± 32.91 [b]	114.2 ± 11.48 [c]	67.6 ± 9.18 [a]	**<0.001***
	Range	55 - 75	195 - 270	100 - 130	55 - 80	
	Blood HDL Level	Control **(n=10)**	High Fat Diet **(n=10)**	Curcumin **(n=10)**	Chitosan **(n=10)**	**P value**
Day 0	**Mean ± SD**	55 ± 3.2	57.40±4.43a	54.6 ±3.2	54.8 ±3.5	0.888
	Range	50 - 60	52 - 65	50 - 60	50 – 60	
Day 90	**Mean ± SD**	55 ± 3.2	66.40 ±5.103 a	118.7 ± 10.8[b]	94.8 ± 8.22[b]	**<0.001***
	Range	50 - 60	60 - 78	106 - 140	80-105	
Day 120	**Mean ± SD**	56.7 ± 2.4	140.80±7.036 b	114.0 ± 6.0[c]	87.2 ± 8.22[a]	**<0.001***
	Range	53 - 60	130 – 154	107 - 125	80 – 95	

*: Statistically significant as P value<0.05, Different lower-case letters indicate significant difference

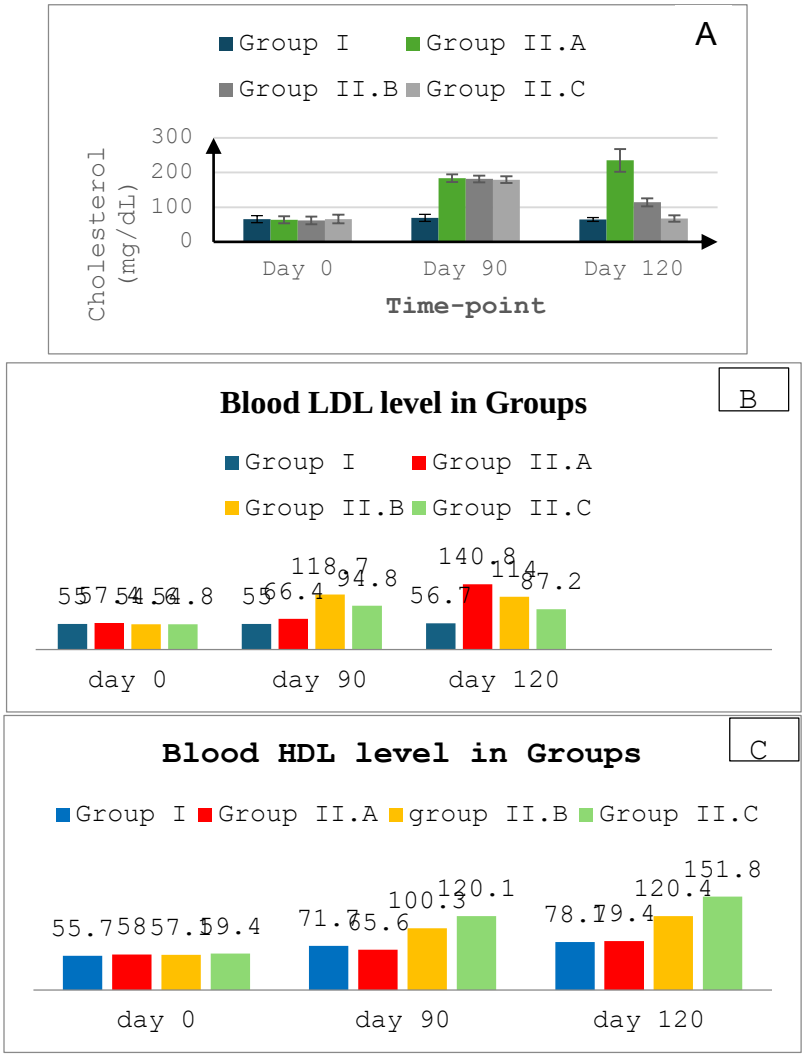

Figure (4): A histogram showing: Comparison between groups in A: Cholesterol blood level, B: blood LDL level, C: blood HDL level.

Discussion

High fat diet (HFD) is a type of diet that is characterized by higher intake of fat, particularly saturated fat. taking too much saturated fat can increase the levels of bad cholesterol in the body, which result in an increased risk of developing heart disease and other health problems.[1]

Using naturally derived additives to maintain resolving promotion has been increasing rapidly in various destructive effects on different tissues.[18]

Dietary supplements have been established as an alternative method for the prevention and/or treatment of dyslipidaemia and related cardiovascular events, with lesser side effects and toxicity.[12]

Curcumin, a natural dietary supplement, was supplied to albino rats' diets due to its cost-effectiveness, convenience. Curcumin affects cholesterol absorption, accumulation, and transport, and can decrease cholesterol precipitation in the aorta, absorption in the small intestine, and atherosclerosis in mice fed a high-fat diet. It may in a direct way controls blood lipids through anti-inflammatory and antioxidant pathways.[17]

In the present study we used chitosan as it related to dietary fibers which can aid in manipulate hypolipidemia according to the previous research which assumed that chitosan improves liver lipid metabolism and save the liver from oxidized trauma, potentially resolving hyperlipidaemia. chitosan has better water-solubility, making them tolerated to digest and absorb by the gastrointestinal tract.[14]

The study used albino rats as a model for tongue stirring by high fat diet research, as rats represent an advance in terms of cost and ease of performance (housed, bred and handled), also, they have long life span.[19]

So, the present study was designed to compare the effect of Curcumin and Chitosan (natural products) on the dorsal surface of tongue mucosa of high fat diet albino rats,

In the present study, using light microscope supported with SEM revealed obvious degenerative histological changes of the dorsal tongue mucosa after high fat diet addition to albino rats for 4 months. Epithelium of the dorsal surface showed vacuolization, atrophy, fungal infection and hyperkeratinisation.

The current results were in parallel with facts introduced by several research that observed the negative impact of high fat diet on the oral cavity and concluded that hypercholesterolemia was associated with increased hazards of periodontal diseases and dental caries and accompanied with impaired immune function which as a result lead to increased susceptibility to infection.[20]

Scanning electron microscopic examination of the dorsal surface of the tongue mucosa of high fat diet group showed fungal infection. This finding agreed with previous research which concluded that Hypercholesterolemia could affect the immune responses through disturbance in cytokine production and immune cell function.[21]

The destructive effects of high fat diet on the dorsal surface of the tongue could be explained according to Huang et al., 2015 [22] who illustrated that oxidative stress is the main principle factor in obesity-related diseases. This obesity-related oxidative stress can result in sever cellular damage and dysfunction.

Our histological findings that supported by SEM results revealed the filiform papillae with flattening in their structure, hyperkeratosis with cracks, distortion and discontinuity.

These results agreed with a study was conducted by Yasuda et al., 2011[23] investigated the effect of obesity on the mucosal epithelium of the palatal gingiva of the maxillary first molar in a rat model. The research showed a significant difference in the mucosal epithelium between high weight rats and normal rats. The high weight rats showed thicker mucosal epithelium, with more

cells in keratinized granular and prickle layers produce epithelial cells more prone to detachment. The height of epithelial papillae was also reduced in the high weight group. Basal cells lacked their normal architecture, causing basement membrane disruption and inflammatory cell infiltration.
In the current research high fat diet group showed irregular architecture of the fungiform papillae on the tongue dorsal surface.
These results come in agreement with Proserpio et al., 2016[24] who declared that obese individuals showing a significantly lower in in number of fungiform papillae and changing in its structure on their tongues and had fewer taste sensitivity for all tastes (sweet, salty, sour and bitter). This decreased fat sensitivity might be a principal factor in the pathogenesis of obesity since this could lead to the consumption of excess dietary energy and weight gain.
Hyperkeratosis in current study was noticed as there was an increase in keratin layer which may be a response to inflammation caused by high fat consumption. This increase in keratinization is a barrier against fat intake, and this is consistent with previous studies that found high-fat diets can affect the epithelial keratin layer of the skin, leading to elevate trans epidermal water loss and elevated susceptibility to external irritants.[25]
Dilatation of blood vessels and its engorgement with blood cells were observed in HFD group in lamina propria of fungiform papillae which may be due to the inflammatory consequence of the high fat diet cause propagation of blood flow to conduct inflammatory cells at the site of the inflammation. This explanation come in agreement with Pisiriciler, 2008[26] who stated that in rats fed with a diet rich with fats showed cellular infiltration and dilated blood vessels congested with red blood cells. these findings might be a part of inflammatory affection to bring more blood to the area of degeneration.
HFD group experienced significant degenerative changes in the lingual glands. These results come in consistent with Pişiriciler, 2008[26] that found high fat diet cause colossal changes in salivary gland and the accumulation of lipids in parenchymal cells of the salivary gland causes multiple inflammatory modifications which may restrict the diffusion of nutrients and oxygen and thus adversely affect the capability and survival of the parenchymal cells. Also, intracellular lipids cause numerous degenerative changes within the secretory cells greatly of fatty nature (fatty degeneration) which explained the present findings.
Degeneration of muscles cells also was recorded. These results were in one line with previous findings showed that High cholesterol levels can assist in atherosclerosis, which involves the buildup of plaque in the arteries. This can limit the blood flow to various parts of the body, including muscles which explain the destructive muscular changes in high fat diet group.[27]
Biochemical analysis of high fat diet group showed significant increase in cholesterol and LDL level and significant decrease of HDL when compared with other groups and this was in parallel way with previous finding reported by Munshi et al., 2014 who used albino rats fed on different amounts and types of fats and found increase in plasma lipid profile.[28]
In addition, Daskala and Tesseromatis, 2011[29] also found that the hyperlipidaemic diet led to significant increase of total cholesterol level, LDL with insignificant increase in rats' weight and a significant decrease HDL which proof our statistical results.

These destructive changes to the surface of the tongue, which appeared clearly in the present study, were attempted to be treated using natural materials to compare the improving effect of both. Rats fed with a high-fat diet were treated with curcumin at a daily dose (1.5 g curcumin/kg) powder for a month revealed some improving effect.

Histological examination supported by ultrastructural evaluation showed some improvement and regeneration in the epithelium of filiform and fungiform papillae although there were some vacuoles found between the muscle fibers of the tongue and infiltration of the inflammatory cells. These improvement results were coincident with previous research which revealed that adding treatment with daily diet can improve the tongue manifestations.[30]

Feeding rats with high fat diet which cause hypercholesterolemia and its treatment with curcumin showed that curcumin might lower the absorption of cholesterol and increase the activity of cholesterol-7α-hydroxylase which has an important role in cholesterol catabolism.[31]

This hypocholesterolaemic effect of curcumin may be due to its stimulatory effect on hepatic cholesterol-7α-hydroxylase enzyme, an enzyme that control cholesterol catabolism. its modulation (decreasing) of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase enzyme activity decreases the serum and liver cholesterol, triglycerides and free fatty acid levels in rats fed on high fat diet for 45 days.[32]

Curcumin also showed a proficiency in lingual gland with multiple cytoplasmic vacuoles which were in parallel with previous study showed that curcumin-enriched diet exhibited a notable hypolipidemic effect, as evidenced by its regulating effects on the serum lipid profile of rats. Curcumin lowered serum cholesterol by about 21% which decrease fats accumulations in tissues.[17]

The decrease in the keratin layer over the filiform papillae is a result of curcumin's anti-inflammatory effect, which prevents the body from originating more keratin in response to inflammation. This explanation is supported by curcumin's anti-inflammatory and cardioprotective properties, which are linked to the lowering of total plasma lipids and peroxidized lipids.[33]

Histological findings in curcumin treated group showed dilated blood vessel engorged with blood these results were explained as dilation was consistent with the response to curcumin observed for coronary arteries in previous research applied on patient treated with curcumin while adding with high fat diet.

Biochemical results showed significant decrease in blood cholesterol and LDL level and significant increase in HDL in curcumin treated group these results agreed with previous study proved that curcumin in rats fed high cholesterol diet resulted in decrease of lipid profile.[34]

Previous research also showed that cholesterol and LDL level was significantly decreased in curcumin treated group. This was explained as curcumin regulate hyperlipidaemia through lowering blood cholesterol and triglyceride levels and increase the levels of lipid removing cholesterol.[35]

In chitosan treated group rats fed on high fat diet for three months and treated by 500 mg/ Kg. BW Chitosan at the beginning of the fourth month. Histological results supported with ultrastructural evaluation showed well enhancement to the dorsal mucosa of the tongue, the

filiform and fungiform papillae showed almost normal histological appearance unless for some vacuolization.

These results were gone parallel with investigations about the possible hypolipidemic and hypocholesterolaemic cause of chitosan which cleared that chitosan had efficacious ability to increase lipid excrement into the faeces when individuals were eaten high fat diet.[36]

Lingual glands showed almost normal histological picture and muscles of the tongue were more or less organized and partially regenerated with inflammatory cells infiltrations and some vacuoles between muscle Fibers. these results agreed with previous explanation to the chitosan improvement effect as chitosan's mechanisms in preventing lipid precipitation involve accelerating lipid breakdown and forming emulsified micelles with cholesterol and lipids, interrupt their absorption and promoting excretion.[37]

Enhancement effect of chitosan on lingual glands in this group explained by Chiu et al., 2020[38] that had suggested that a soluble form of chitosan would be capable of interfering with intraluminal lipid absorption through the interaction with micelle formation or emulsification of lipids.

In chitosan treated group histological findings showed that blood vessels were almost normal, and these results agreed with previous research conducted that Chitosan has been shown to play a significant role in promoting blood vessel construction, also known as angiogenesis, which is crucial for transporting oxygen and nutrients to injured areas.[39] .

Chitosan stimulates the production of growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which are important for promoting the growth of new blood vessels.[39]

Biochemical results showed that there is a significant decrease in blood cholesterol and LDL level comparing with high fat diet group and insignificant difference between this group and control group and these results agreed with previous studies exclaimed that chitosan administration to HFD-fed rats significantly alter the significant weight gain observed compared to untreated HFD-fed rats. Aligning with previous studies indicating that chitosan is necessary to regulate body weight and appetite in HFD-induced obesity which was clearly noticed in significant decrease in serum lipid profile.[40]

Accordingly, the present study revealed that curcumin and chitosan could introduce alternative treatment for decreasing hypercholesterolemia complications. however, Chitosan representing more promising effect than Curcumin.

Conclusion

The research concluded that a high-fat diet has significant cytotoxic effects on the dorsal surface of the tongue. Both curcumin and chitosan may may hypercholesterolemia-induced tongue cytotoxicity, where chitosan showing a better preventive effect than curcumin.

Ethics approval and consent to participate:

The research has been approved from the research ethics committee (REC), Faculty of Dentistry, Suez Canal University (Ethics code number: 420/202).

Competing interests:

The author declares that there is no competing interests.

Data availability:

All datasets of the current study are available from the corresponding author on reasonable request.

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References

1. Han Y, Wu H, Sun S, Zhao R, Deng Y, Zeng S, et al. Effect of high fat diet on disease development of polycystic ovary syndrome and lifestyle intervention strategies. Nutrients. 2023;15(9):2230.
2. Lu M, Sun J, Zhao Y, Zhang H, Li X, Zhou J, et al. Prevention of High-Fat Diet-Induced Hypercholesterolemia by Lactobacillus reuteri Fn041 Through Promoting Cholesterol and Bile Salt Excretion and Intestinal Mucosal Barrier Functions. Frontiers in Nutrition. 2022;9.
3. Feingold KR, Grunfeld C. Introduction to lipids and lipoproteins [Internet]. MDText.com, Inc. eBooks. 2015.
4. Merćep I, Vujević A, Strikić D, Radman I, Pećin I, Reiner Ž. Present and Future of Dyslipidaemia Treatment—A Review. Journal of Clinical Medicine. 2023;12(18):5839.
5. Desai N, Rana D, Salave S, Gupta R, Patel P, Karunakaran B, et al. Chitosan: a potential biopolymer in drug delivery and biomedical applications. Pharmaceutics. 2023;15(4):1313.
6. Ahn CB, Je JY, Kim YS, Park SJ, Kim BI. Induction of Nrf2-mediated phase II detoxifying/antioxidant enzymes in vitro by chitosan-caffeic acid against hydrogen peroxide-induced hepatotoxicity through JNK/ERK pathway. Molecular and Cellular Biochemistry. 2016;424(1–2):79–86.
7. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The essential medicinal chemistry of curcumin. Journal of Medicinal Chemistry. 2017;60(5):1620–37.
8. Zahedi H, Hosseinzadeh-Attar MJ, Shadnoush M, Sahebkar A, Barkhidarian B, Sadeghi O, et al. Effects of curcuminoids on inflammatory and oxidative stress biomarkers and clinical outcomes in critically ill patients: A randomized double-blind placebo-controlled trial. Phytotherapy Research. 2021;35(8):4605–15.
9. Panahi Y, Ahmadi Y, Teymouri M, Johnston TP, Sahebkar A. Curcumin as a potential candidate for treating hyperlipidemia: A review of cellular and metabolic mechanisms. Journal of Cellular Physiology. 2016;233(1):141–52.
10. Amalraj A, Pius A, Gopi S, Gopi S. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives – A review. Journal of Traditional and Complementary Medicine. 2016;7(2):205–33.
11. Chen X, Yu G, Cheng G, Hao T. Research topics, author profiles, and collaboration networks in the top-ranked journal on educational technology over the past 40 years: a bibliometric analysis. Journal of Computers in Education. 2019;6(4):563–85.
12. González-Peña D, Angulo J, Vallejo S, Colina-Coca C, De Ancos B, Sánchez-Ferrer CF, et al. High-cholesterol diet enriched with onion affects endothelium-dependent relaxation and NADPH oxidase activity in mesenteric microvessels from Wistar rats. Nutrition & Metabolism. 2014;11(1).
13. Tanrıkulu-Küçük S, Başaran-Küçükgergin C, Seyithanoğlu M, Doğru-Abbasoğlu S, Koçak H, Beyhan-Özdaş Ş, et al. Effect of dietary curcumin and capsaicin on testicular and hepatic

oxidant–antioxidant status in rats fed a high-fat diet. Applied Physiology Nutrition and Metabolism. 2019;44(7):774–82.
14. Pan H, Yang Q, Huang G, Ding C, Cao P, Huang L, et al. Hypolipidemic effects of chitosan and its derivatives in hyperlipidemic rats induced by a high-fat diet. Food & Nutrition Research. 2016;60(1):31137.
15. Majeed ML, Ghafil FA, Fatima G, Hadi NR, Mahdi HF. Anti-Atherosclerotic and Anti-Inflammatory effects of curcumin on hypercholesterolemic male rabbits. Indian Journal of Clinical Biochemistry. 2019;36(1):74–80.
16. Washington University School of Medicine, Neuromuscular Lab. HEMATOXYLIN & EOSIN (H & E) STAIN PROTOCOL. 1997.
17. Fenwick M, Hurst P. Immunohistochemical localization of active caspase-3 in the mouse ovary: growth and atresia of small follicles. Reproduction. 2002;124(5):659-65.
18. Abdel-Raouf S, Rateb D, Hussein S. The Possible Healing Effect of Aloe Vera versus Silver Nanoparticles on Acid Induced Lip Ulcer in Albino Rats (Histological and Immuno-histochemical Study). Ain Shams Dental Journal. 2021;23(3):42-54.
19. Ashraf H, Butt MS, Iahtisham-Ul-Haq N, Nadeem M, Aadil RM, Rusu AV, et al. Microencapsulated curcumin from Curcuma longa modulates diet-induced hypercholesterolemia in Sprague Dawley rats. Frontiers in Nutrition. 2022;9.
20. Even PC, Virtue S, Morton NM, Fromentin G, Semple RK. Editorial: Are rodent models fit for the investigation of human obesity and related diseases? Frontiers in Nutrition. 2017;4.
21. Tibúrcio-Machado CS, Michelon C, Zanatta FB, Gomes MS, Marin JA, Bier CA. The global prevalence of apical periodontitis: a systematic review and meta-analysis. International Endodontic Journal. 2020;54(5):712–35.
22. Bernardi S, Marcuzzi A, Piscianz E, Tommasini A, Fabris B. The Complex Interplay between Lipids, Immune System and Interleukins in Cardio-Metabolic Diseases. International Journal of Molecular Sciences. 2018;19(12):4058.
23. Huang L, Chen J, Cao P, Pan H, Ding C, Xiao T, et al. Anti-Obese effect of glucosamine and chitosan oligosaccharide in High-Fat Diet-Induced obese rats. Marine Drugs. 2015;13(5):2732–56.
24. Schuurs A. Pathology of the hard dental tissues. John Wiley & Sons; 2012.
25. Proserpio C, Laureati M, Bertoli S, Battezzati A, Pagliarini E. Determinants of obesity in Italian adults: the role of taste sensitivity, food liking, and food neophobia. Chemical Senses. 2015; bjv072.
26. Kondo T, Kishi M, Fushimi T, Kaga T. Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in the liver to suppress body fat accumulation. Journal of Agricultural and Food Chemistry. 2009;57(13):5982–6.
27. Sakr M. Histology of the Salivary Glands. Surgery of the Salivary Glands. 2024;5-11.
28. Malek mohammad K, Bezsonov EE, Rafieian-Kopaei M. Role of lipid accumulation and inflammation in atherosclerosis: Focus on molecular and cellular mechanisms. Frontiers in Cardiovascular Medicine. 2021;8.

29. Munshi R, Joshi S, Rane B. Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. Indian Journal of Pharmacology. 2014;46(3):270.
30. Eid MM, Hussein RR, Shaker OG, Ibrahim SS. Effectiveness of Zinc Supplementation in management of patients with symptomatic Benign Migratory Glossitis: A randomized control clinical and biochemical Study. Ain Shams Dental Journal. 2024;35(3):244-55.
31. Daskala ID, Tesseromatis CC. Morphological changes of parotid gland in experimental hyperlipidemia. International Journal of Dentistry. 2011; 2011:1–5.
32. Feng D, Ohlsson L, Duan RD. Curcumin inhibits cholesterol uptake in Caco-2 cells by down-regulation of NPC1L1 expression. Lipids in Health and Disease. 2010;9(1).
33. Babu PS, Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (Curcuma longa) in streptozotocin induced diabetic rats. Molecular and Cellular Biochemistry. 1997;166(1/2):169–75.
34. Shin S, Ha T, McGregor RA, Choi M. Long-term curcumin administration protects against atherosclerosis via hepatic regulation of lipoprotein cholesterol metabolism. Molecular Nutrition & Food Research. 2011;55(12):1829–40.
35. Samora JB, Goodwill AG, Frisbee JC, Boegehold MA. Growth-Dependent changes in the contribution of carbon monoxide to arteriolar function. Journal of Vascular Research. 2009;47(1):23–34.
36. Jang EM, Choi MS, Jung UJ, Kim MJ, Kim HJ, Jeon SM, et al. Beneficial effects of curcumin on hyperlipidemia and insulin resistance in high-fat–fed hamsters. Metabolism. 2008;57(11):1576–83.
37. Chinchu JU, Mohan MC, Kumar BP. Anti-obesity and lipid lowering effects of Varanadi kashayam (decoction) on high fat diet induced obese rats. Obesity Medicine. 2019; 17:100170.
38. Ugbaja RN, Akinloye DI, James AS, Ugwor EI, Kareem SE, David G, et al. Crab derived dietary chitosan mollifies hyperlipidemia-induced oxidative stress and histopathological derangements in male albino rats. Obesity Medicine. 2020; 20:100300.
39. Chiu CY, Yen TE, Liu SH, Chiang MT. Comparative Effects and mechanisms of chitosan and its derivatives on hypercholesterolemia in High-Fat Diet-Fed rats. International Journal of Molecular Sciences. 2019;21(1):92.
40. Kumar R, Arya JK, Rizvi SI. Chitosan reduces inflammation and protects against oxidative stress in a hyperlipidemic rat model: relevance to nonalcoholic fatty liver disease. Molecular Biology Reports. 2022;49(10):9465–72.