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Protective Effect of Lycopene Rich Tomato Puree on the Development of Atherosclerosis/Heart Diseases in Animal Models

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Lycopene as an aliphatic carotenoid promulgated from tomato has gained the attention of new generation food and pharma scientists on the ground of its nutraceutical efficacy. It is a preeminent antioxidant known for its singlet oxygen quenching ability that reduces oxidative stress responsible for non-communicable diseases. In present investigation the health claim exploration ability of lycopene rich tomato puree as a dietary supplement was assessed by animal studies to project its hypolipidemic efficacy effective in reducing risk of heart diseases.
Aim: The present investigation aims to determine efficacy of tomato puree lycopene in monitoring atherogenesis mechanism through synergic mode of action of notified specialty features (total cholesterol, serum triglycerides, LDL cholesterol and HDL cholesterol) in Wistar rats.
Study Design: Wistar rats (Positive control, high fat diet fed, HFD-Stain fed and HFD-tomato puree fed) were examined for general examinations (behavioural changes and body weight), Lipid profile (total cholesterol, triglyceride, LDL and HDL cholesterol) on 0, 14 and 28th day of experiment to assess effect of tomato puree administration on wellbeing of rats against healthy, hyperlipidemic and standard drug status.

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Place and Duration of Study: MIT School of Food Technology in collaboration with Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani (MAFSU, Nagpur), Maharashtra. The overall study duration was for 3 months.

Methodology: The experimental rats (Wistar) employed in present study were acquired from authorized breeder after the approval of the experimental protocol by Institutional Animal Ethical Committee (IAEC approval resolution no.: IAEC 58/19, Date 20/08/2019). Acclimatization of the animals was undertaken for 20 days during which they were provided with natural diet, pure and safe drinking water. CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines were followed while handling the experimental animals throughout the complete study duration. The experimental animals (32 Wistar rats) were segregated into four different groups (Positive control, high fat diet fed, HFD-Stain fed and HFD-tomato puree fed), containing 8 rats (4 male and 4 female). Animals from four distinguished groups were monitored regularly for general observations (body weights, behavioural changes and mortality changes) throughout the experimental period. The lipid profile (total cholesterol, triglyceride, HDL, LDL) of blood serum samples was recorded using auto analyser on 0, 14th and 28th day of experiment.

Results: The data analysis, interpretation and reviewed literature infusion collectively underlined the efficacy of lycopene (tomato puree) in monitoring atherogenesis mechanism through synergic mode of action of notified specialty features (total cholesterol, serum triglycerides, LDL cholesterol and HDL cholesterol) and projected an effective gateway to support disease infused health claim. The cumulative decrease in total cholesterol (159.99 to 126.40mg/dl), serum triglycerides (114.81 to 107.34mg/dl), LDL cholesterol (40.19 to 32.90mg/dl) and increase in HDL cholesterol (19.99 to 23.87mg/dl) of treated rats represents hypolipidemic efficacy of tomato puree lycopene.

Conclusion: The tomato puree mass being a viable lycopene source with innate antiatherogenic efficacy has rationally updated the review base critical appraisal leading to development of prototype of CVD to launch health claim. The human clinical study as a confirmation tool, may be the best option for next generation research.

Keywords: Lycopene; tomato puree; atherosclerosis; rats; total cholesterol; triglycerides; LDL cholesterol; HDL cholesterol.

1. INTRODUCTION

Pharmaceutical value driven fruit and vegetable carotenoids recognized as bioactive compounds, have underlined their beneficial strata coiling around health wellbeing through natural functional efficacy. A lycopene as an aliphatic carotenoid promulgated from tomato [1] has gained the attention of new generation food and pharma scientists on the ground of natural and molecular reorientation driven pharmaceutical value. Lycopene, a preeminent antioxidant known for its singlet oxygen quenching ability is characterized as an unsaturated molecule providing suitable option for molecular reorientation to raise its innate potential [2,3]. Lycopene as a dietary supplement, in purified food grade or fresh matrix resource form, is being rightly utilized to compensate with vertical nutrition transition and hence provided an innovative option to lead towards personalized nutrition. Lycopene driven tomato recognition based on specialty of red tint has underlined the landmark as a rich commercial source [4,5] for production of food grade ingredient under innovation and creativity. Voluminous review base epidemiological studies projected the hypothesis summarizing as the direct relationship between high consumption of tomatoes and potentially reduced risk of non-communicable diseases [6,7].

The nutraceutical efficacy (CVD risk reduction) of lycopene through dietary mechanism is commercially explored by array of researchers [6,8-10]. In-vivo lower defensive lycopene concentration enhances the threat of vascular illness. The dietary enrichment of lycopene by tomato base products cuts the risk of coronary heart diseases [6] through platelets monitoring mechanism. The platelets monitoring process eventually regulates clotting to streamline normal blood circulatory system to reduce the risk of heart attacks and strokes [8]. Dietary lycopene helps in preventing oxidized LDL that plays a significant role in pathogenesis of atherosclerosis responsible for heart attack and ischemic strokes. The serum cholesterol level responsible for coronary heart diseases is also explored by secondary group of scientists [11,12]. Lycopene decreases cholesterol amalgamation, lipid peroxidation and LDL oxidation with linear

enhancement of HDL. Dietary supplementation of tomato products facilitates the specialty action that leads to decrease in plaque build-up in artery walls and reduce the oxidative stress [13].

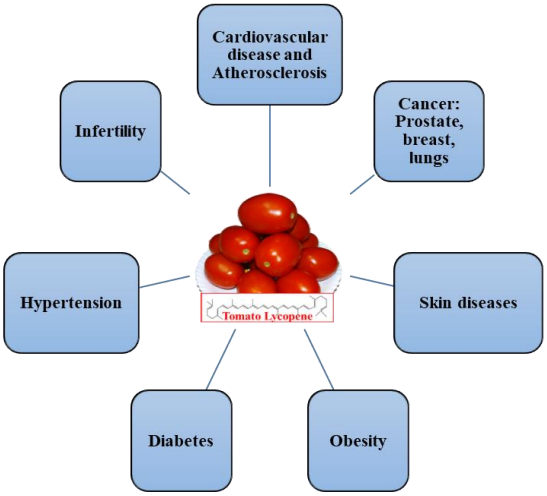

Fig. 1. Lycopene health benefits scenario [11,13]

In present experiment, study was planned to estimate the efficacy of lycopene rich tomato puree in the hyperlipidemia as it is referred to possess hypolipidemic effect and cardio tonic properties. This study has been carried out on the wistar rats which were fed on specially formulated high fat diet containing 1 percent cholesterol with the intention of inducing hyperlipidemia a basic sign of atherosclerosis and then the curable effects of tomato puree lycopene were evaluated by examining the biochemical parameters (lipid profile) and histopathological studies in animals against the high fat diet negative group and standard drug (Atorvastatin) group.

2. METHODOLOGY

2.1 Tomato Puree Preparation

Fully matured and deep red tomatoes were macerated in screw type juice extractor after preliminary processing and cutting and then passed through screening process using 100 mesh sieve (0.15mm). The enforced maceration driven fine and homogenised tomato puree mass was thermally processed at 90°C temperature for 60min time span (Mane and Pawar, 2018).

2.2 Health Claim Determination

Nutraceutical driven functionality of lycopene rich tomato puree as a core objective of the said research project was undertaken for investigation in collaboration with Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani (MAFSU, Nagpur), Maharashtra.

2.3 Experimental Animals

The experimental rats (Wistar) employed in present study were carried from authorized breeder after the approval of the experimental protocol by Institutional Animal Ethical Committee (IAEC approval resolution no.: IAEC 58/19, Date 20/08/2019). Acclimatization of the animals was undertaken for 20 days during which they were provided with natural diet, pure and safe drinking water, ad-libitum along with electrolyte powder for the rehydration purpose. CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines were followed while handling the experimental animals in the complete study duration.

2.4 Animal Grouping

The experimental animals (32 Wistar rats) were segregated into four different groups, containing 8 rats, (4 male and 4 female) as detailed below,

1. Group A (Positive control): The said group was maintained on conventional laboratory animal diet, as per NRC (1994).
2. Group B (Negative control): The said group was maintained on specially formulated high fat diet containing 1 percent cholesterol. These rats were monitored with no further experimental treatment.
3. Group C (Standard treatment- statin): The said group was maintained on high fat diet containing 1 percent cholesterol. Moreover, all the rats in this group were treated with dose of standard hypolipidemic compound statin (Atorvastatin) every day at the dose rate of 0.5 mg/kg orally from 29th day of experiment.
4. Group D (Lycopene-tomato puree base treatment): The said group was maintained on high fat diet containing 1 percent cholesterol and eventually supplemented orally with 0.16ml of lycopene rich tomato puree (lycopene content-5.51mg/100g) per rat per day from 29th day of experiment.

The pure and safe drinking water (*ad-libitum*) water supply was ensured to the rats from all the groups throughout the experiment.

Table 1. Diet intervention and treatment composition of experimental rats

Treatment	Code	Dependent variables
Positive control (normal laboratory diet)	Group A	Clinical study attributes: General examinations (Behavioural changes and body weight), Lipid profile (total cholesterol, triglyceride, LDL and HDL cholesterol)
Negative control (high fat diet-HFD)	Group B	
Standard drug (HFD- statin fed)	Group C	
Lycopene (HFD- tomato puree base treatment)	Group D	

2.5 General Examinations

Animals from four distinguished groups were observed regularly for general observations (body weights, behavioural changes and mortality changes) throughout the experimental period.

2.6 Biochemical Parameters- Lipid Profile

The blood collection of anesthetized rats was carried out from the inner canthus of eye in all animals. On scheduled 0, 14th and 28th day of experiment, lipid profile (total cholesterol, triglyceride, HDL, LDL) of blood serum samples was recorded using auto analyser on 0, 14th and 28th day of experiment.

2.7 Relative Organ Weight

The relative weight of animal organs (heart and spleen) were measured at the end of experiment to record the effect of lycopene rich tomato puree.

2.8 Histopathology

Gross and histopathological studies of the heart from sacrificed animals were undertaken on completion of the experiment.

3. RESULTS AND DISCUSSION

3.1 Behavioural and Body Weight Changes

The study experimental tenure (28 days) recorded normal behaviour of rats in all the groups. All the rats were observed to be alert, normal and healthy. The data on overall body weight of treated and control groups rats presented in Fig. 2 highlighted relatively more increase in body weights of rats in high fat diet fed groups (II, III and IV) on 14th and 28th day against 0 day. The consistent observations as increase in body weight recorded stand in conformity with the results conveyed by Bugajski et al. [14]. The rise in body weight represents hyperlipidaemia and obesity caused due to high fat diet.

3.2 Blood Biochemical Assessment- Lipid Profile

3.2.1 Total cholesterol

The diet intervention and total cholesterol (mg/dl) of Wistar rat projecting data presented in Fig. 3, directly help showcasing the lycopene efficacy determination of diet monitored rats (normal laboratory diet, high fat induced diet and statin fed diet). The significant increase (68.61 to 181.87mg/dl) in cholesterol of group II (negative control) rats recorded on 14th day with follow up of abrupt increase (241.94mg/dl) on 28th day may be the consequence of heavy fat diet. Significantly higher cholesterol in rats (groups II, III and IV) than group I recorded on 14th and 28th day, may be associated with high fat consumption. However, relative decrease in cholesterol of rats (groups III and IV) on 28th day (89.16 and 126.40mg/dl) as compared to 14th day (155.42 and 159.99mg/dl) appeared to be associated with hypolipidemic effect of statin and lycopene. The blood cholesterol reduction in rats of group IV on 28th day (126.40mg/dl) against group II (241.94mg/dl) is an indication of hypolipidemic action of tomato puree lycopene. The reduction of intracellular cholesterol by lycopene and tomato derivatives has been associated with a decrease in cholesterol synthesis through an inhibition of HMG-CoA reductase activity and expression, a modulation of LDL receptor and ACAT activity [15]. The significant reduction in cholesterol level of high fat diet induced rats on tomato/lycopene supplementation is also reported by Nouri and Rezapour [16] and Kilany et al. [17].

3.2.2 Serum triglycerides

The increased serum triglyceride level of rats (group II, III and IV) as presented in Fig. 4 is an indication of high fat diet monitoring effect. The

marginal elevation in serum triglycerides of rats (group I) recorded throughout the experimentation tenure may be associated with normal diet devoid of high fat. The significant elevation in serum triglycerides of rats from groups II (140.62mg/dl), III (100.37mg/dl) and IV (114.81mg/dl) on 14th day and successive reduction on 28th day in groups III (96.93mg/dl) and IV (107.34mg/dl) against 0 day may be associated with high fat of rats (group II) and relative effect of defined treatments. The significantly lowered serum triglyceride level of rats exhibited by groups III (100.37 and 96.93 mg/dl) and IV (114.81 and 107.34 mg/dl) on 14th and 28th day of experiment against group II (140.62 and 133.95mg/dl) found admissible to determine hypolipidemic effect of statin (standard drug) and lycopene (tomato puree base). Nouri and Rezapour [16] and Kilany et al. [17], both the groups of scientists also reported similar increasing trend in serum triglycerides of rats on high fat diet induction followed by decrease in triglycerides with tomato/lycopene supplementation.

3.2.3 Low Density Lipoproteins (LDL cholesterol)

The reduced LDL cholesterol of rats from groups III (36.51 and 39.86mg/dl) and IV (40.19 and 32.90mg/dl) recorded on 14th and 28th day against relative 0-day values (Fig. 5) underlined the LDL reducing effect of statin and lycopene. Moreover, the reduced LDL cholesterol of group IV rats on 14th and 28th (40.19 and 32.90mg/dl) day against group I (46.05 and 35.63mg/dl) also highlighted the LDL cholesterol reducing effect of lycopene. The potential antiatherogenic role of lycopene has been ascribed mainly to its antioxidant capacity, which is related to the prevention of LDL oxidation [15]. Oxidation of the circulating low-density lipoprotein (LDL(ox)) is thought to play a key role in the pathogenesis of atherosclerosis and CHD. Macrophages inside the arterial wall take up the LDL(ox) and initiate the process of plaque formation.Dietary antioxidants such as lycopene inhibits the LDL oxidation and prevents the formation of LDL(ox) as well as their uptake by microphages. Lycopene was inhibiting the activity of an essential enzyme involved in cholesterol synthesis suggesting a hypolipidemic effect [18]. The parallel LDL cholesterol reducing trend in tomato/lycopene supplemented rats is also reported by Nouri and Rezapour [16] and Kilany et al. [17]. The lower LDL cholesterol level can be linked to reduced risk of heart diseases [19].

3.2.4 High Density Lipoproteins (HDL cholesterol)

The beneficial entity, HDL cholesterol (mg/dl) of rats to combat high CVD risk (Ali et al., 2012) and its relationship with lycopene presented in Fig. 6, has underlined the significant increase in HDL cholesterol of rats from groups III (16.23 and 24.63mg/dl) and IV (19.99 and 23.87mg/dl) on 14th and 28th day against 0 day projected the HDL cholesterol stimulatory effect of statin and lycopene. The increased HDL cholesterol of rats from group IV (19.99 and 23.87mg/dl) against groups I (15.91 and 15.94mg/dl) and II (15.91 and 19.90mg/dl) on 14th and 28th day of experiment also indicated the positive effect of tomato puree lycopene treatment. These observations are in good agreement with the results reported by earlier researchers [16,17,20].

Increased oxidative stress is a prime reason in cardiovascular disease [21]. The production of excess reactive oxygen species (ROS) contributes to the reduction of nitric oxide bioavailability and vasoconstriction, initiating arterial hypertension. More importantly, ROS initiates the formation of atherosclerotic plaques [22]. Lycopene has several cardiovascular health benefits such as antioxidative, anti-inflammatory, anti-atherogenic, cardioprotective, antiplatelet effect, improving endothelial function (nitric oxide bioavailability and blood flow), metabolic profile (by impairing cholesterol synthesis) and blood pressure control [23]. Lycopene is an effective singlet oxygen quencher; it scavenges peroxynitrite resulting in oxidised lycopene products. By reducing oxidative stress and reactive oxygen species, lycopene increases the bioavailability of nitric oxide (NO), improves endothelium-dependent vasodilation and reduces protein, lipids, DNA, and mitochondrial damage [23-26].

3.3 Post Sacrificed Relative Organ Weight of Heart

The weight of heart of respective group of rats (group I to IV) was observed as 0.36, 0.33, 0.39 and 0.44g, respectively (Fig. 7). Moreover, weight of spleen of rats from groups I to IV was 0.25, 0.22, 0.16 and 0.16g, respectively. No significant alterations in relative organ weight were observed in control and treatment groups.

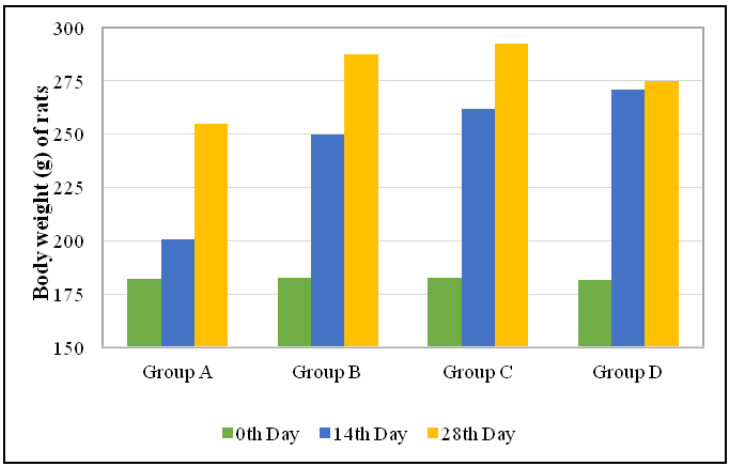

Fig. 2. Body weight (g) changes in rats

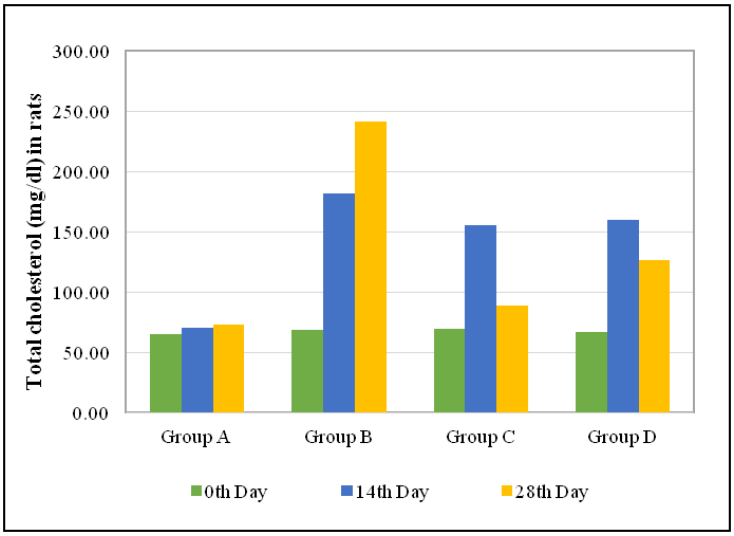

Fig. 3. Total cholesterol (mg/dl) changes in rats

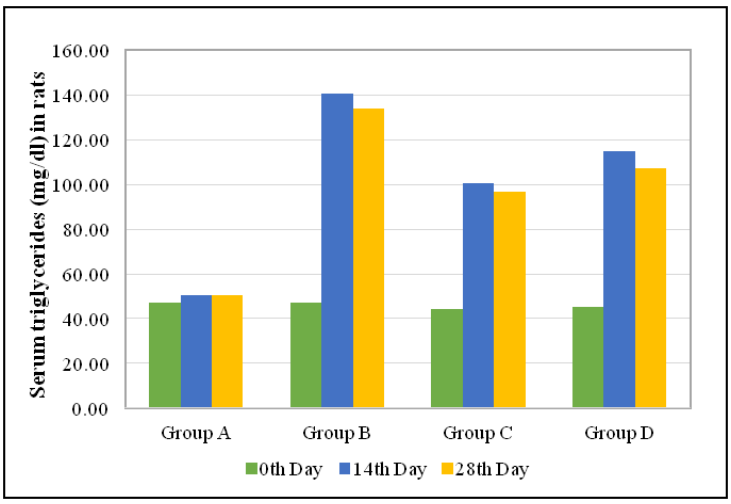

Fig. 4. Serum LDL cholesterol (mg/dl) changes in rats

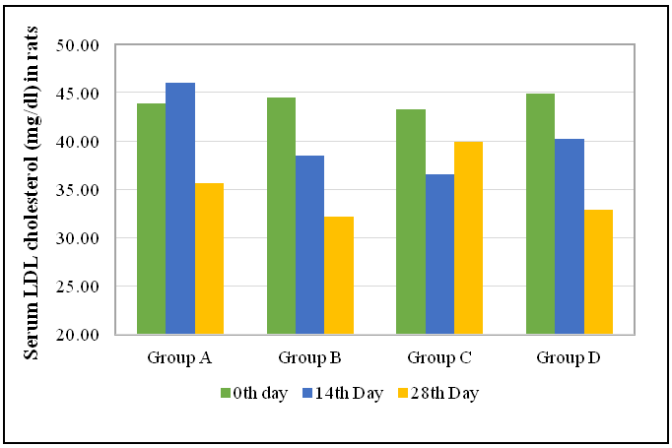

Fig. 5. Serum LDL cholesterol (mg/dl) changes in rats

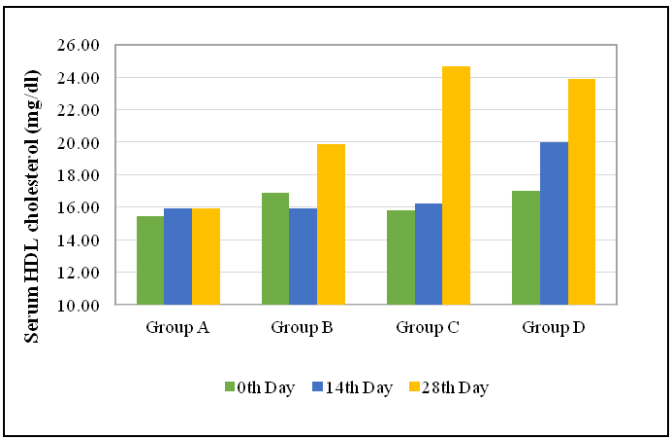

Fig. 6. Serum HDL cholesterol (mg/dl) changes in rats

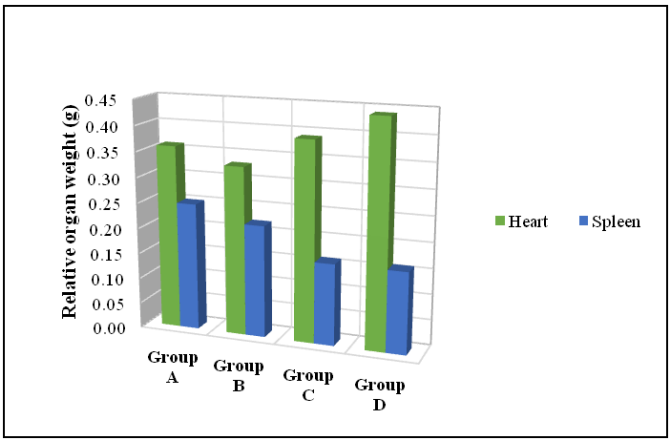

Fig. 7. Relative organ weight (g) of heart and spleen in experimental rats

3.4 Histopathological Examination of Heart and Spleen

The sections of rat heart (group I) remained far away from specific changes as compared to group II. The heart sections of group II rats observed to be with minimal to mild congestion and mononuclear cell infiltration. The sections of heart from group III and IV, did not show any changes in histomorphology architecture.

The sections of spleen of rats from all the groups (I, II, III and IV) did not record any changes in the histomorphology of the sections.

4. CONCLUSION

The animal study experimental data, confined to physiology (body weight), lipid profile (total cholesterol, serum triglycerides, LDL cholesterol and HDL cholesterol) specialty features in collaboration with existing regulatory mechanism and histopathological observations of notified organs is complementary to link with protective effect of tomato puree lycopene against physiological disorders and diseases of live being. In-vivo lipid transformation of lycopene (tomato puree) to monitor atherogenesis mechanism through synergic mode of action of notified specialty features (total cholesterol, serum triglycerides, LDL-C, HDL-C) projected an effective gateway to support disease infused health claim assessment. The cumulative decrease in total cholesterol of treated rats (159.99 to 126.40mg/dl), serum triglycerides (114.81 to 107.34mg/dl), LDL cholesterol (40.19 to 32.90mg/dl) and increase in HDL cholesterol (19.99 to 23.87mg/dl) represents hypolipidemic efficacy of tomato puree lycopene. The tomato puree mass being a viable lycopene source with innate antiatherogenic efficacy has rationally updated the review base critical appraisal leading to development of prototype of CVD to launch health claim. The human clinical study as a confirmation tool, may be the best option for next generation research.

CONSENT

It is not applicable.

ETHICAL APPROVAL

“All authors hereby declare that "The experimental rats (Wistar) employed in present study were carried from authorized breeder after the approval of the experimental protocol by Institutional Animal Ethical Committee (IAEC approval resolution no.: IAEC 58/19, Date 20/08/2019). CPCSEA (Committee for the purpose of control and supervision of experiments on animals) guidelines were followed while handling the experimental animals in the complete study duration.”

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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