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INFLUENCE OF TOMATO JUICE ON BODY WEIGHT AND SOME BLOOD BIOCHEMICAL PARAMETERS OF RATS

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Abstract

Oxidative stress is thought to play an important role in the pathogenesis of numerous degenerative and chronic diseases. Some antioxidants in tomato juice were found to have a powerful antioxidant effect. Dietary supplementation of tomato juice which is enriched with antioxidants especially lycopene may be effective in reducing oxidative stress during hyperlipidemia condition. In this study 10 male albino rats from Sprague Dawlly (Rattus rattus norvegicus) were used, their ages ranged between 8-10 weeks and their weights ranged between 150-200 grams; before treatment, they are considered as control. The animals were placed in plastic cages individually, their body weights were taken before and at the end of trial; blood samples were taken from all rats. The aim of this study was to investigate the protective effect of tomato juice on some blood parameters. The results showed that there were positive and significant ($p<0.05$) effects on improving lipid values after trail, reducing the level of Total cholesterol (TC), Low-Density Lipoprotein (LDL), Triglycerides levels (TG), Phospholipids (PL), Total Lipids (TL), Atherogenic indices and blood glucose; while it increases the High-Density lipoprotein (HDL); but for blood hemoglobin there was no significant difference ($p>0.05$) between the values before and after trail.

Keywords: Tomato juice, biochemical parameters, rats

1. INTRODUCTION

Behavioral risk factors, including a plethora of dietary risks, including low intakes of fruit and vegetables, are asserted to possess the greatest potential to promote disease and impair human health, globally (Forouzanfar et al., 2015; Gorji et al., 2019; Rahmani et al., 2019). One of the most preponderant healthy diets followed, globally, is the Mediterranean diet, and one of its main constituents is tomato which is the most abundant source of lycopene within the diet (Ursini and Sevanian, 2002). Lycopene may be defined as a carotenoid that contains a long-conjugated chain of double bonds, organized linearly; moreover, the antioxidative of lycopene is attributed to its' structural organization (Cheng et al., 2017). It has been shown that, in healthy humans given tomato juice, the levels of cholesterol and low-density lipoprotein are significantly decreased (Lundman et al., 2007; Lundman et al., 1997). Increased serum LDL level and decreased HDL level, is one of the main factors involved in cardiovascular disease, especially coronary atherosclerosis, the development that causes inflammation and reduces of endothelial function and wide vascular lesions (Ross, 1993).

Epidemiologic studies suggest that tomato and tomato products such as tomato juice presumably might have lipid-lowering and antioxidant effect (Hsu et al., 2008; Hassan and Edrees, 2004). Tomato contains antioxidants such as lycopene, vitamin A, vitamin C, vitamin E, carotenoids, phytochemicals, polyphenols and flavonoids (Campbell et al., 2004) and can act in synergy in oxidative stress mechanism (Clinton. 1998). It has been suggested that there is a role of lycopene in protecting LDL or phospholipid in LDL from oxidation and in up

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regulating LDL receptor activity in macrophages (Arab and Steck.2000); and inhibiting reductase a key enzyme in the biosynthesis of cholesterol (Fuhrman et al., 1997).

Furthermore, tomatoes showed high lipid metabolism levels that have been induced by genes over expression; that have been involved in more efficient fatty acid oxidation (Mohammed et al., 2020). The regular consumption of tomato juice and tomato-based products have been correlated to reduction in risk of contracting cardiovascular diseases confers cardiovascular benefits (Cheng et al., 2017). Several studies support the notion that intake of tomato juice and tomato-based foods improves serum lipids and reduces the cardiovascular risk better than lycopene supplementation (Burton-Freeman and Sesso, 2014; Canene et al., 2005). However, the best to our knowledge, no published data has yet examined the association between fresh tomato juice consumptions and hypoglycemic due to its atherogenic index in association to lipid profile and kidney functions within any diabetic model.

This work aims to evaluate the influence of tomato juice on lipid profile, glucose, and hemoglobin and body weight of adult rats before and after using the juice.

2. MATERIAL AND METHODS

2.1 Tomato Juice Collection And Preparation

Fresh ripe tomatoes were acquired from the local marketplace of Duhok; washed 250 g with running water, then it has been cut into small pieces and finally blended for 10 minutes with 25 mL distilled water in a Braun blender (Model No 4979, Germany) at maximum speed; after that, the seeds were removed by a household filter, then parked in a special package and placed in the freezer until was analyzed. The prepared tomato juice (TJ) was used daily (1mL it /day) for feeding the experimental animals.

2.2 Chemical Analysis for Tomato Juice

Humidity: Humidity was estimated using the electric oven equipped from the German Mermmert Company at a temperature of 105 C, according to the method mentioned by (AOAC, 2000).

Total Soluble Solid (TSS): The Total Solid percentage of tomato juice was measured by Hand Refractometer, ranging from 0-50 percent.

Protein Determination: Protein was determined by the Macro-Kjeldahl method and according to what was reported by (AOAC, 2000).

Crud fat Determination: Fat was measured using the Sexhelt apparatus and according to what was mentioned by (AOAC, 2000).

Fiber Determination: Fibers were estimated also according to the method reported by (AOAC, 2000).

Ash Determination: Ashe was estimated using a (Muffle Furance) incineration furnace equipped by the American company Thermolyn according to (AOAC, 2000) at a temperature of 550 C overnight until the ash turned white.

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Total Carbohydrates: The total carbohydrate was estimated by calculating the difference between the components, i.e.:100- (fat + protein +ash + moisture).

However, Table 1, presents the chemical composition of tomato juice.

Table 1: Chemical Composition for Tomato Juice (%)

Moister	TSS	Protein	Fat	Fiber	Ash	Carbohydrates	Energy (Kcal/100 gm.)
93.90	5.2	0. 89	0.30	0.64	0.91	4.0	24.82

Some Constants and Properties of Tomato Juice (g/100g)

Total Carbohydrates: Total Carbohydrates mentioned above in chemical analysis for tomato juice.

Total Sugars: Total Sugars were estimated using Lane and Eynon method mentioned in Ranganna (1977).

Reducing Sugars: Reducing sugars were estimated using the DNS -3,5 Dinitro saliciy acid method reported in AOAC (2000).

Determination of glucose sugar According to AOAC (2000).

Determination of fructose sugar According to AOAC (2000).

Determination of non-reducing sugars: The non-reducing sugars were estimated mathematically as follows:

Total sugars –reducing sugars = non reducing sugars.

Vitamin C determination: Determination of Vitamin C by visual bleaching method using 2, 6-dichlorophenol indophenol (DCPI) according to the method mentioned in AOAC (2000).

Determination of pH: The pH was estimated by Chinese pH-meter,Modl Microcomputer pH/mv/TEMPMeter 6171,NO 6171,after adjusting the device with a buffer solution of potassium phthalate of pH 4 and 7 to lend the device at a temperature of 20 degrees Celsius and according to what was mentioned in the AOAC (2000).

Total Acidity: The percentage of total acidity was estimated by the titration method with sodium hydroxide according to Ling (1963).

Table 2: Some Constants and Properties of Tomato Juice (g/100g)

Carbohydrates	Total sugars	Reducing sugars	Glucose	Fructose	Non reducing sugars	Vitamin C mg for 100g	PH	Total acidity
4.0	4.0	3.40	1.90	1.50	0.60	28	4.40	3.80

Moreover, Table3, illustrating the information representing the nutrients obtained from an average round tomato and how these relate to daily recommended intakes (Frusciante et al., 2007; NHS, 2020)

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Table 3: The Nutrients Obtained About Tomato and How These Relate To Daily Recommended Intakes

Nutrients obtained from an average round tomato	Nutritional content (Per100g)	% of Recommended Daily Allowance (RDA)
Fiber	Fiber 1.2 g	4% RDA
Carbohydrates	3.89g	1.50% RDA
Sugar	2.6g	2.89% RDA
Protein	0.9g	1.8% RDA
Fat	0.2g	0.29%RDA
Potassium	237mg	6.77%RDA
Phosphorus	24mg	4.36%RDA
Magnesium	11mg	3.66%RDA
Sodium	5mg	0.21%RDA
Iron	0.27mg	3.10%RDA
Zinc	0.17mg	1.79%RDA
Energy	18 kal	

(Frusciante et al., 2007; NHS, 2020)

2.3 Basal Diet Preparation

To prepare the basal diet according to what was mentioned in NAS/NRC (1978) in order to meet the physiological and nutritional requirements of the rats; the basal diet contained 12% protein, 10% fat,10% sucrose, 5% cellulose,0.50 calcium carbonate, 1.2% mixture of elements and mixture of vitamins 2% and completed the volume to 100gm of corn starch. As for the sources of additives for the preparation of the basal diet, they are as follows:

1- Casein is free of vitamins and minerals prepared by BDH chemical (England) Ltd, Pool Company.

2- Cellulose produced by Merchery and Nagel Company Germany.

3- Sucrose produced by BDH chemical (England) Ltd, Pool Company.

4- Calcium carbonate (CaCO3) from BDH chemical (England) Ltd, Pool Company.

5- Corn starch of Turkish origin, supplied from local markets.

6- Corn oil of Turkish origin, supplied from local markets.

7- Vitamins and Minerals Mixtures: The mixture of vitamins and minerals from the pharmaceutical laboratory according to NAS/NRC (1978).

To prepare the basal diet, the dry ingredients mentioned in table 4 were weighed and mixed with each other using an electric mixer, taking in to account not adding all the starch with the ingredients, due to the difficulty of kneading and homogenizing the ingredients together, therefore third of the calculated amount of starch was boiled in a glass beaker with a sufficient amount of distilled water, so a gelatinous substance was formed for the purpose of homogenizing the diet ingredients together.

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Table 4: The Components of the Basal Diet Prepared For Feeding Rats (Gm /100gm)

Casein	Corn oil	Sucrose	Cellulose	Mixture of vitamins	Mixture of minerals	Calcium carbonate	Starch
	10	10	5	2	1.2	0.50	59.3

After diet ingredients in Table 4 were mixed well, then placed in a suitable plastic kneader, and the above-prepared gelatinous substance was added to it with an appropriate amount of distilled water, mixed well until homogeneous, then kneaded in to fingers with weights of approximately 5 grams, after that, it was placed in stainless steel trays and dried in the electric oven at an initial temperature of 30-40 degrees Celsius with continuous good ventilation to get rid of high humidity and to prevent surface drying .Then the temperature was raised to 50 degrees Celsius for period of 12 hours in order to avoid damage vitamins and nutrients; after cooling they were packaged in plastic bags and then frozen until used in the experiment.

Table 5: The estimated chemical composition of the basal diet intended for feeding rats (g/100g)

Moister	Protein	Fat	Fiber	Ash	Carbohydrate	Energy Kcal/100
12.44	11.30	9.83	4.87	1.98	59.58	372

2.4 Experimental Animals And Experiment Implementation

In this study 10 male albino rats, are Sprague Dawlly (Rattus rattus norvegicus) were used, their ages ranged between 8-10 weeks and their weights ranged between 150-200 grams; before applying treatment, the measures were considered as control. The animals were placed in plastic cages individually; they were equipped with basal diet and water according to the animals need, they were also subjected to appropriate laboratory conditions for rearing in terms of temperature 25 degrees Celsius and a light cycle of 12 hours and 12 hours of darkness. Food and water were submitted ad libitum. Animals were acclimated to the laboratory environment for 5-7 days before used in study. After this duration the entail body weight, and blood samples were taken, the animals were starved (overnight fasting), all animals were anesthetized by diethyl ether and then blood samples were taken from the animals according to (Timm, 1979); Samples were centrifuged for preparing blood serum.

The rats receiving basal diet were dosed with tomato juice 1ml daily for one month period.

At the end of 30 days, final body weight of the rats was taken and blood samples were taken.

Total Cholesterol [TC], High Density Lipoprotein [HDL] and Triglyceride [TG], serum glucose were measured by (Kit). Low density lipoprotein [LDL] and VLDL were calculated and as stated by (Glew and Peters,1987). Level of phospholipids was calculated using the equation developed by (Tietz, 1987), Serum total lipids were determination according to (Toro and Ackermann, 1975).Atherogenic indices TC/HDL and LDL /HDL were calculated arithmetically according to (Yousif, 2000) and VLDL +LDL /HDL according to (Tietz, 1987).

2.5 Statistical Analysis

The collected data submitted to SPSS program (SPSS, 2019) for statistical analysis, where

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descriptive statistics (Means and Mean Standard Error-MSE) and **paired t-test** were used to compare between the studied parameters before and after applying the treatment on studied animals.

3. RESULTS AND DISCUSSION

The results presented in Table 6, are illustrating some blood parameters of the studied rates, measured before and after dosing the animals with tomato juice**.** The differences between them are significant ($p<0.05$) for all studied parameters; where the applied Tomato juice treatment resulted in lower values for all studied parameters except HDL which is significantly ($p<0.05$) recorded higher value after applying the treatment (12.04 vs. 13.68) as shown in Table 6. These results indicating the benefits from tomato juice because it decreased the bad blood parameters that affect negatively on the health, this due to Lycopene possesses lipid-lowering effects that inhibit the oxidation of TC, TG, LDL.c, VLDL-c and the formation of dysfunctional HDL.c (Mozos et al., 2018). These findings also are in agreement with the findings reported by Li et al. (2014), who found that lycopene reduces cholesterol, TG and LDL.c levels and also, increases HDL.c in diabetic rats.

The results of current experiment showed that tomato juice was the superior in lowering the serum levels of TC, LDL-c and VLDL-c and TG. This data agrees with the other findings which suggest that healthy human subjects who ingested lycopene in the form of tomato juice or tomato paste for a week had a significantly lower level of LDL-c than controls (Blum et al., 2005). Interestingly, tomato juice was shown to have the lower levels of these lipids and the higher levels of HDL-c, this may be due to the lycopene compounds inhibiting the activity of an essential enzyme involved in cholesterol synthesis (macrophage 3- hydroxy-3-methyl glutaryl coenzyme A reductase) as reported by Witztum (1994). Our results are in agreement with many studies which showed that, the tomato decreased amount of Total Cholesterol, LDL-c and Triglycerides and increased HDL-c levels (Nouri and Rezapour, 2011).

Table 6: Effect of Tomato Juice on Serum Lipid Profile in Healthy Rats at the End Experimental Period

	TC mg/100ml	TG mg/100ml	HDL-C mg/100ml	LDL-C mg/100ml	VLLDL mg/100ml
Before trial	64.54 a	50.14 a	12.04 b	42.48 a	10.03 a
After trial	56.33 b	40.34 b	13.68 a	34.58 b	8.06 b
MSE	3.1	2.2	0.36	2.07	2.24
Sig.	*	*	*	*	*

Means having different letters are differed significantly.

**= Significant ($p<0.01$); *= Significant ($p<0.05$); NS= non-significant ($p>0.05$)

Table 6, showed significant decrease ($p<0.05$) in serum cholesterol level, this may relate with the value of the first atherogenic index, because it was found from the same results that there were significant differences ($p<0.05$) in atherogenic index represented by the concentration of total cholesterol to high lipoproteins density TC/HDL between the beginning and end of the

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experiment, as mentioned by the value of TC/HLD in healthy rats. While, the non-treated tomato juice group recorded (5.36mg/100ml) compared to significant decrease ($p<0.05$) in the treated ones as value of (40.12mg/100ml). Indeed, Story et al. (2010) found that consuming more lycopene lowers CVD risks, additionally to early results within in vitro investigations that indicated lycopene molecules have anti-atherogenic properties (Hung et al., 2008). It appears from the results given in Table7, that there are significant differences ($p<0.05$) in the value of the second atherogenic index VLDL+LDL/HDL, for rats dosing tomato juice ,where it was (4.36mg/100ml) before treated by tomato juice and decreased to (3.13 mg/100ml) after applying trail's treatment, due to the affect tomato juice on LDL and VLDL, Moreover, a high intake of tomato juice prevents low density lipoprotein (LDL) oxidation and thiobarbituric reactive species (TBARS) formation in healthy men (Bub et al., 2000; Gitenay et al.,2007).

The result presented in the Table7, also showed that there was significant difference ($p<0.05$) in the value of third atherogenic index represented by the concentration of low density lipoproteins to the concentration of high density lipoproteins LDL/HDL, where the value was in not-treated rats (3.53mg /100ml), while it decreased at the end of the experiment to (2.53mg/100ml), from mentioned results we found that administration of tomato juice to healthy rats led to reduce in the level of TC,LDL, TC/HDL,LDL/HDL,VLDL+LDL/HDL and in other hand increase in HDL which is believed to be protective factor for lipoproteins, these results are in agreement with Yousif (2000). Also, the current results were consistent with those of Tripathi (2003) that showed Diabetes mellitus (DM) that is linked to impaired lipid metabolism and an elevation in the atherogenic index, which are significant predictors of CVD risk; the greater of the atherogenic indices value, the greater of the CVD risk occurrence (Chigozie and Chidinma, 2013). Additionally, these findings are consistent with Choi et al. (2013), who stated that tomato consumption reduced CVD incidence that may be due to its antioxidant activities and lycopene levels.

Table 7: Effect of Tomato Juice on Atherogenic Index in Healthy Rats at the End Experimental Period

	TC/HDL mg/100ml	LDL/HDL mg/100ml	VLDL+LDL/HDL mg/100ml
Before trial	5.36 a	3. 53 a	4.36 a
After trial	4.12 b	2.53 b	3.13 b
MSE	0.1	0.08	0.09
Sig.	*	*	*

Means having different letters are differed significantly.

**= Significant ($p<0.01$); *= Significant ($p<0.05$); NS= non-significant ($p>0.05$)

Table 8, shows the effect of tomato juice consumption on the level of the phospholipids and total lipids in healthy rats, it is noted from the table that there is a significant difference ($p<0.05$) in the level of phospholipids, where as its level decreases from 125.44 to 118.13mg/100ml, between beginning and end of the experiment, the significant change found in the level of phospholipids before and after trial, and it was as parallel to the level of total cholesterol shown in Table 6, because the level of phospholipids (PL) was calculated depending on the level of total cholesterol according to the equation developed by (Tietz, 1987), which represents the

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regression line for the fat and its relationship with the level of total cholesterol (TC) **as** following model.

PL (mg/dl) = 68 + (0.89*TC).

It is also noted from the same table that there is a significant reduction ($p<0.05$) in the level of total lipids between the beginning and end of the experiment, and when comparing the result after giving rats tomato juice, it was noted that tomato juice had a significant effect, as the level of total lipids deceased from 240.12 to 214.80mg /100ml, this could be due to that lipid lowering properties of tomato juice have been associated with suppression of cholesterol synthesis by inhibiting HMG-coA –reductase and activation LDL-receptors (Palozza et al., 2010).

Table 8: The Effect of Tomato Juice on Phospholipids and Total Lipids in Healthy Rats at the End Experimental Period

	PL mg/100ml	TL mg/100ml
Before trial	125.44 a	240.12 a
After trial	118.13 b	214.80 b
MSE	3.05	6.2
Sig.	*	*

Means having different letters are differed significantly.

**= Significant ($p<0.01$); *= Significant ($p<0.05$); NS= non-significant ($p>0.05$)

The initial and final body weight of the animal (gm), the level of initial and final hemoglobin (gm/100ml) and the level of blood glucose (mg/100ml) are presented in Table 9. The results indicated that there are significant differences ($p<0.05$) for the initial body weight, where it was at the beginning 165.22 grams, but it reached 183.5 grams at the end of the experiment (after dosing it with tomato juice), perhaps the reason is due to the chemical composition of the juice used, especially for the total soluble solids (TSS) which amounted as 5.2. When comparing the final hemoglobin concentration with the initial one, it was found that there were non-significant differences ($p<0.05$) in the hemoglobin concentration between the beginning and end of the experiment, as the rate ranged between (13.5-13. 64 gm/100ml), the reason may be due to the fact that the rats used in the experiment have a normal hemoglobin level because they had good physiology activity (Shraaf, 1998).

It is noted also, from Table 9, that there are significant differences ($p<0.05$) in the level of the glucose between the beginning and end of the experiment, as the glucose level was before trail (104.77mg /100ml), and after dosing tomato juice was (90.17mg/100ml). The present result is consistent with what reported by (Ali & Agha, 2009) who found that Lycopene's hypoglycemic effect shown to be attributed to a number of processes, including increased insulin production, increased repair and proliferation of β–cells, enhanced insulin and adrenaline effects, and increased anti oxidative capabilities, thus increasing tomato juice as a Lycopene source by time or levels consumed could be attributed to decrease the blood glucose levels.

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Table 9: The Effect of Tomato Juice on Body Weight, Hemoglobin and Blood Glucose In Healthy Rats at the End Experimental Period

	Animal Weight gm	Hb gm/100ml	Glucose mg/100ml
Before trial	165.22 b	13.5	104.77 a
After trial	183.5 a	13.64	90.17 b
MSE	4.8	0.42	2.46
Sig.	*	NS	*

Means having different letters are differed significantly.

**= Significant ($p<0.01$); *= Significant ($p<0.05$); NS= non-significant ($p>0.05$)

4. CONCLUSION

It could be concluded from the current study that dosing rat's tomato juice has healthy benefits. Tomato juice decreasing bad blood biochemical parameters and increasing the good healthy parameters.

5. RECOMMENDATION

The present investigation suggests utilizing tomato juice in human food or instead of some fruit juice, due to its health benefits.

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