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Changes in Glucose levels, Liver enzymes, and Lipid Profile in Hypercholesterolemic rats after using *Capsicum annum* L. with fast food

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Chili peppers are commonly used around the world to make a desirable taste for food, particularly fast food like spicy chicken. Studies indicate that red chili pepper makes some alterations in liver enzymes and lipid profiles. The goal of this study was to see how hypercholesterolemic rats changed their lipid profiles (Cholesterol, Triglycerides, HDL, VLDL, LDL) as well as some liver enzymes (ALP, GPT, and GOT) were evaluated in hypercholesterolemic rats. Thirty-five male rats were separated into seven groups, each with the same number of rats. First group was treated as a control negative group with no changes, while the other six groups were fed an extra 2% cholesterol for two weeks to induce hypercholesterolemia. Then there are two hypercholesterolemic groups were fed different doses of red chili pepper (RCP) extract (10, 20 and 40 mg/kg body weight (B.Wt.)), two were fed 30% (fried chicken and chicken burgers), and one remained on standard diet only (control positive). The impact of RCP on liver function and alterations in lipid profiles was investigated. The oral dose of 2% cholesterol for two weeks caused hypercholesterolemia in rats, according to our findings. The findings suggested that taking enough RCP orally as part of a standard/healthy diet or as a fast food component improves lipid profiles and liver function test values.

Keywords: *Capsicum*, spicy fast food, glucose, lipid profiles, liver function, chicken.

INTRODUCTION

Rapid urbanization, busy lifestyles, simplicity of manufacture and good taste lead to an increase in the trend of fast food consumption significantly globally (Cizza and Rother et al. 2012). Fried fast food contains a high level of refined sugar, polyunsaturated fats, salts, and numerous food additives but lacking in proteins, vitamins, and fibers. At the same time, fried fast food although it is high content of sugars and fats very popular. Unhealthy levels of lipids can cause block in blood vessels and increase the risk of heart disease and stroke. These lipids are also responsible for unhealthy weight gain (Pelletier and Laska, 2012;

Majabadi et al. 2016).

Recently, consumption of fried fast food with spices has increased dramatically among the Saudi Arabia population including young and teenagers who have gotten used to adding extra chilies to many meals (Al Moraie, 2014; Adam et al. 2014; Al-Otaibi and Basuny, 2015). Although eating a spicy meal is not always harmful to one's health, it might cause problems and trigger symptoms in those who suffer from chronic illnesses. According to certain research, chilies and spices provide health benefits such as reduced cancer incidence, hunger, obesity and cardiovascular disease (CVD) (Nilius and

Appendino, 2013; Sharma et al. 2013; Lv, et al. 2015). Spices also have antibacterial properties and impact gut microbes, which have lately been linked to diabetes, heart and liver disease, and cancer in humans (Qin et al. 2012; Tang et al. 2013; Qin et al. 2014).

The major component of red chili pepper, *Capsicum annuum* L., has been proven to have favorable effects by lowering cholesterol, triglyceride, and low-density lipoprotein (LDL) levels, resulting in less liver damage (Razavi et al. 2006; Kim et al. 2017). Scientists employed *Capsicum annuum* L. to kill nerve cells in the pancreas of mice with type 1 diabetes, allowing the insulin-producing cells to resume production (Razavi et al. 2006). Human studies found that when chili was added to the diet, the LDL cholesterol resisted oxidation for a longer amount of time, increasing the risk of cardiovascular disease (Ahuja and Ball, 2006).

When the meal contains red chili pepper, the amount of insulin required to lower blood sugar is reduced (Binshtok et al. 2007). Liver function tests are used for helping diagnose and monitor liver disease or damage. These tests can measure the levels of certain proteins and enzymes. Abnormal results of liver function tests do not always indicate liver disease. Nevertheless, they can give enough information to assess fully how the liver is functioning (Blann, 2014). In addition, it is important to keep blood sugar levels in the target range as much as possible to help prevent many serious problems, such as heart diseases, kidney diseases, liver diseases, and vision loss as well as can help improve your energy and mood.

MATERIALS AND METHODS

Preparation of *Capsicum annuum* L. extract:

The clean *Capsicum annuum* L. was ground using a porcelain grinder to pass through sieve-mesh pores of 1mm diameter. The extract was prepared by mixing 1gm powdered leaves with 100 ml distilled water. The mixture was boiled for 10 minutes and left to cool for 15 minutes. Then the aqueous extract was filtered using filter paper to remove the particulate matter (0.2mm). Finally, the filtrate was dried (Lyophilized) and then reconstituted in 1.5 ml of distilled water (100 mg/kg B.Wt.) (MacDonald's, 2019; KFC, 2019).

Study design:

All of the trials were carried out in the animal house of faculty of medicine, Umm Al-Qura University. According to the National Research

Council (1995), thirty-five male albino Sprague Dawley rats (185 ± 5 g each) were fed a standard diet that included casein (12.5%), corn oil (10%), choline chloride (0.2%), vitamin mixture (1%), cellulose (5%), salt mixture (4%), sucrose (22%), and corn starch (up to 100%) (Shaikh Omar et al. 2013; Kleinert et al. 2018). For acclimation, all animals were fed a basal diet for one week. The animals were then separated into two groups. The first group (n= 5 rats) was fed simply the basal diet as a control (-), whereas the second group (n= 30 rats) was fed an additional 2% cholesterol for 2 weeks to induce hypercholesterolemia. Hypercholesterolemic rats were disparted into six groups (n= 5 rats for each group) as the following (Kim et al. 2018; Bukhari et al. 2020): Group 1: Remained on standard diet only; positive group (+). Group 2: Fed standard diet of 10 mg/kg B.Wt. RCP extract (dose of RCP 10). Group 3: Fed standard diet of 20 mg/kg B.Wt. of RCP extract (dose of RCP 20). Group 4: Fed standard diet of 40 mg/kg B.Wt. of RCP extract (dose of RCP 40). Group 5: Fed standard diet containing 30% spicy fried chicken (dose of chicken burgers). Group 6: Fed standard diet containing 30% spicy burger chicken (dose of chicken burgers).

When experimental period was finished, all rats were fasted for 24 hours and only allowed to drink water. Blood samples have collected from all rats in a chamber containing diethyl ether. Two blood samples were collected from each animal into a plane and heparin-containing tubes. Serum samples were collected after centrifuged at 3000 rpm for 10 min. Then, all rats were sacrificed, and the liver was washed with saline and dissected to examine under a microscope to ensure inducing hypercholesterolemia.

Biochemical analysis:

Triglycerides and cholesterol were measured with enzymatic kits Allain, 1974; Young and Pestaner, 1974). High-density lipoprotein (HDL), High-density lipoprotein-cholesterol (HDLC), very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) concentrations were calculated from the Reitman and Frankel equations were determined according to previous methods (Friedewald, 1972; Gordon and Amer, 1977).

In addition, various enzymatic colorimetric methods were used to determine blood glucose (Trinder, 1969; Kim et al. 2020), glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT) activity (Reitman and Frankel, 1957), Alkaline phosphatase (ALP)

activity (Haussament, 1977), and total protein (Henry et al. 1991).

Histopathological study:

Specimens from the liver were collected from rats of all the six groups were mention above. Then specimens were fixed in 10% neutral buffered formalin (pH=7.0), were dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness sections were prepared and stained with hematoxylin and finally, eosin was added for examining the parts of the live (Figures 1 and 2).

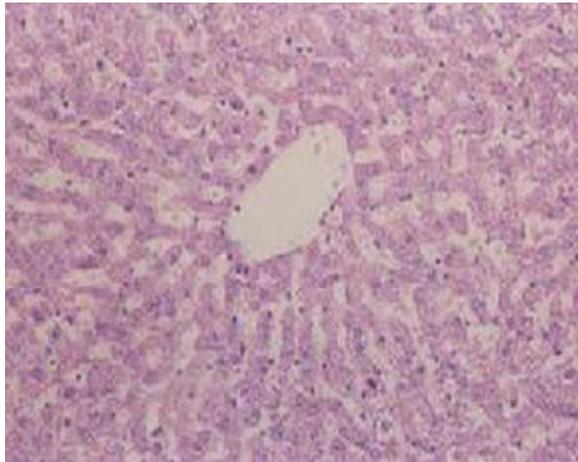


Figure 1: Rat liver tissue from the control (-) group showing the normal histological structure of hepatic lobule (H and E x 400).

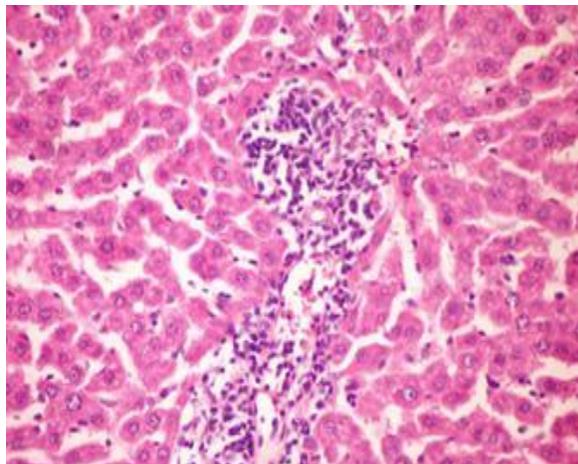


Figure 2: Rat liver tissue from the control (+) group showing infiltration of leucocytes (H and E x 400).

Statistical analysis:

The data was tabulated and analyzed using SPSS version 25 software, and the mean and standard deviation were calculated. To compare differences between hypercholesterolemic groups and the control (+), a Chi-square test and a Student's *t*-test were utilized. The results were considered significant at $P < 0.05$.

RESULTS

To determine the effect of RCP on lipid profiles (cholesterol, triglycerides, HDL, VLDL, and LDL) and liver function tests (glucose, total protein, ALP, GPT, and GOT), hypercholesterolemic groups (doses of 10 RCP, 20 RCP, 40 RCP, fried chicken and chicken burgers) were compared to control positive groups as shown in Table 1 and Table 2.

As we can see in Table 1 that rats fed with 10, 20, and 40 mg/kg B.Wt. RCP showed significant changes in both cholesterol and LDL levels ($P < 0.05$ or 0.01) construct to values of control but no significantly change in rats fed with 30% fried chicken or chicken burgers were observed. HDL levels have not significantly change only for the 10 mg/kg B.Wt. RCP group. While, it was showed significantly change ($P < 0.05$ or 0.01) in rats fed with 20, and 40 mg/kg B.Wt. RCP and 30% fried chicken or chicken burgers. On the other hand, no significant change in both triglyceride and VLDL levels were found for any of examined groups.

In experiments with results shown in Table 2 the same results showed for glucose, ALP and GPT levels no significant change in rats fed with 10 and 20 mg/kg B.Wt. RCP and 30% fried chicken or chicken burgers groups, while 40 mg/kg B.Wt. RCP showed a significant change ($P < 0.05$) in the all these three tests. For GOT test a significant change ($P < 0.05$) was observed for 20 and 40 mg/kg B.Wt. RCP, whereas not for 10 mg/kg B.Wt. RCP or 30% fried chicken or chicken burgers groups. However, no significant change in total protein level was observed for any of tested groups.

Table 1: Effect of spicy fast food on lipid profiles test.

Groups	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
control -ve	146.7 ± 11.6**	189.7 ± 5.7***	80.1 ± 1.79**	29.3 ± 2.32	80.2 ± 4.22***
control +ve	225.9 ± 7.31	255.1 ± 10.1	52.6 ± 4.43	45.2 ± 3.46	157.4 ± 9.71
Dose of RCP 10	195.3 ± 11.68	202.2 ± 10.9*	64.6 ± 7.99	39.1 ± 6.34	98.4 ± 7.59**
Dose of RCP 20	186.5 ± 6.79	201.0 ± 8.1**	71.0 ± 3.45*	37.3 ± 1.36	92.6 ± 7.74**
dose of RCP 40	195.2 ± 4.34	186.4 ± 4.3**	75.1 ± 2.13**	39.0 ± 2.87	72.2 ± 3.38**
dose of fried chicken	194.0 ± 4.36	209.3 ± 9.4	76.0 ± 3.55*	38.8 ± 2.87	94.5 ± 7.58
dose of chicken burgers	206.1 ± 5.56	228.4 ± 5.7	78.2 ± 5.69*	41.2 ± 5.11	109.0 ± 7.17
ANOVA					
F value	1.576	6.112	4.381	1.577	9.121
P value	0.191	0.001	0.003	0.191	0.001

Values are means ± standard errors, F and P value *Differences are significant at 5% (p<0.05). **Differences are significant at 1% (p<0.01). ***Differences are significant at 0.1% (p<0.001); ANOVA, analysis of variance between control positive was compared statistically to all other hypercholesterolemic groups; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein.

Table 2: Effect of spicy fast food on liver function test.

Groups	Glucose (mg/dL)	Total protein (g/dL)	ALP (U/L)	GPT (U/L)	GOT (U/L)
control -ve	87.7 ± 4.95**	6.80 ± 0.412	222.5 ± 7.42**	30.4 ± 2.9**	31.5 ± 5.2*
control +ve	135.0 ± 18.29	8.36 ± 1.172	585.4 ± 45.16	48.7 ± 0.768	44.4 ± 0.864
Dose of RCP 10	107.9 ± 12.14	8.07 ± 0.889	424.5 ± 82.75	41.8 ± 5.322	32.4 ± 4.513
Dose of RCP 20	105.0 ± 17.24	9.19 ± 0.636	424.2 ± 54.41	34.8 ± 5.06	30.5 ± 5.41*
dose of RCP 40	92.1 ± 16.59*	8.7 ± 2.147	373.4 ± 31.51*	29.5 ± 2.78**	25.7 ± 2.5**
dose of fried chicken	129.3 ± 20.27	9.55 ± 0.963	512.7 ± 68.68	49.6 ± 0.678	45.1 ± 1.018
dose of chicken burgers	123.0 ± 30.42	8.46 ± 1.778	391.0 ± 95.74	48.4 ± 0.927	44.1 ± 1.181
ANOVA					
F value	0.958	0.472	3.401	7.677	5.280
P value	0.472	0.825	0.013	0.001	0.001

Values are means ± standard errors, F and P value *Differences are significant at 5% (p<0.05). **Differences are significant at 1% (p<0.01). ***Differences are significant at 0.1% (p<0.001); ANOVA, analysis of variance between control positive was compared statistically to all other hypercholesterolemic groups; ALP, alkaline phosphatase; GPT, glutamate pyruvate transaminase; GOT, glutamic-oxaloacetic transaminase.

DISCUSSION

After examining the effect of the red chili pepper using the processes mentioned in the Materials and Methods Section, the findings of this research study must now be discussed. Because the results differed from what was expected, this has taken on added significance. The discussion will also lead to a conclusion and research recommendations.

As we know that spicy food has been consumed by many cultures like Chinese and Indian food as traditional dishes. Consumption of spicy foods in Saudi Arabia has increased over the years, particularly in fried fast food. The present study evaluated the effect of RCP extract on a standard diet or combined with fried fast food

(fried chicken and chicken burgers) on hypercholesterolemic rat models. Studies around the globe have shown conflicting effects of RCP on health (Dkhal et al. 2010), particularly with respect to liver damage (McCarty et al. 2015). Nonetheless, there is much evidence supporting their beneficial effect on the function and structure of various tissues and organs in humans and animals, as in the current study. In fact, the main active constituent in red chili pepper, *Capsicum annum* L., has been shown to affect lipid profiles and reduce liver damage (Li et al. 2013; Zhang et al. 2013; Sanati et al. 2018; Al-Jumayi et al. 2020).

The high fat content of fried fast food can increase insulin secretion, increasing fat stored in the liver. Regarding lipid profiles, all groups in our study shows some changes relative to the control

positive though not always significantly. However, triglycerides and VLDL levels did not differ much in any group compared to the control positive, in contrast to the other studies (Zhang et al., 2013; Al-Jumayi et al. 2020). Here, the addition of RCP to the standard diet caused a significant decrease in cholesterol and LDL but an increase in HDL. Furthermore, a significant increase in HDL was observed with the consumption of fried chicken and chicken burgers compared to control positive. Similar to what previous studies have identified (Sanati et al. 2018; Kim et al. 2020). Of note, this study used much higher concentrations (10, 20 and 40 mg/kg B.Wt.) of RCP than Al-Jumayi et al. (2020) 5 and 10 mg/kg B.Wt.. This difference between studies demonstrates that at higher doses, the beneficial effects of RCP are even better, as long as the animal can tolerate it throughout the experimental period. Tolerance of RCP ingestion is important as many animals and humans are not used to consuming moderate to higher doses of spice in foods (Lejeune et al. 2003).

The use of pure Capsicum would equate to a high dose compared to the consumption of natural and complete RCP (Lejeune et al. 2003). Likewise, fried fast food as if fried chicken and chicken burgers have many more components that could reduce the availability and activity of the Capsicum in RCP even more so than components found in a healthy diet. This might explain the low or absence of effect of certain spicy foods on lipid profiles or liver functions. The anti-atherogenic effect of RCP consumption found in the current study is encouraging as Cholesterol, LDL and Triglycerides ratios were lower than the control positive, with only an increase in HDL ratio. The greatest effect occurred with 40 mg/kg B.Wt. RCP. Moreover, these significant changes occurred with the consumption of spicy fried chicken and chicken burgers. These positive results are similar to those found in some previous studies (Otunola et al. 2012; Al-Jumayi et al. 2020).

Overall, the results are not conclusive, but the absorption of cholesterol could also be reduced in the intestine, with more cholesterol combining with bile salts and being excreted (Negulesco et al. 1987; Mueller et al. 2011; Li et al. 2013). In terms of liver function, the present study measured glucose, ALP, GPT, GOT, and total protein levels. Apart from total protein, all measures in all groups were significantly different from that of the control positive. However, 40 mg/kg B.Wt. RCP lowered glucose, ALP, GPT, and GOT levels the most

(Sanati et al. 2018; Kleinert et al. 2018).

CONCLUSION

This study highlights the functional of physiological properties of red chili pepper that consumption of enough RCP with a standard diet or as an ingredient of fried fast food has a positive effect on lipid profiles, reducing cholesterol, triglycerides, and LDL and increasing HDL, as well as liver function, decreasing glucose, ALP and GPT. The combination of RCP and a regular diet, on the other hand, produced the most favorable results. As a result, it is suggested that additional RCP be ingested with meals, or that more trials be conducted in order to acquire the optimal and safe RCP dose.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

OAS is the owner of the idea, designed and performed performed experiment and animal treatments and wrote the results section. He also, responsible for publication process. OSB wrote the introduction and list of references and performed statistical data analysis. YSA wrote discussion and conclusion sections. Finally, all authors reviewed and approved the final version of manuscript.

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