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Citrus peel extract and powder attenuate hypercholesterolemia and hyperglycemia using rodent experimental modeling

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ABSTRACT

Objective: To investigate hypocholesterolemic and hypoglycemic potential of citrus peel extract and powder using rodent experimental modeling.

Methods: Considering the fact, rat feeding trial was carried out for a period of 56 d to access the prophylaxis of citrus peel flavonoids by employing normal (study I), hyperglycemic (study II) and hypercholesterolemic (study III) rats. Each study was further divided into three groups to ensure the provision of selected diets, *i.e.*, control, functional and nutraceutical diets. Each study was further divided into three groups to ensure the provision of selected diets, *i.e.*, control, functional and nutraceutical diets.

Results: Declining trend for total cholesterol was observed in all studies with maximum reduction (8.55%) in rat group fed on nutraceutical diet in study III. Likewise, levels of low density lipoproteins and triglycerides reduced 11.39% and 7.89% respectively in hypercholesterolemic rats. Moreover, nutraceutical diet alleviated the sera glucose level by 8.96% in study II.

Conclusions: Conclusively, inclusion of citrus peel bioflavonoids in dietary therapies is a promising strategy to modulate lipidemic and glycemic attributes without imparting any deleterious effect on hematological parameters.

1. Introduction

Globally, emerging trends are shifting consumer's cognizance towards the peculiar role of food in diseases management such as cardiovascular complications, cancer, arthritis and diabetes. This veracity has made gap between food and drugs very narrow in curtailing the life associated ailments [1]. In this reverence, dietary tools place emphasis on the dynamic facets of phytonutrients as they put beneficial effects on human health [2]. Taking in account the current scenario, novel health strategies assenting to the supplementation of phytochemicals to curb the onset of chronic disorders are dominating. Epidemiological studies have proved a healthy connection between functional ingredients of food and well-being of vulnerable group of the people [3]. This has drawn the concept of functional and nutraceuticals components in food, which wield beneficial effects beyond basic nutrition [4]. Amongst these ingredients of food, plant based functional components are extensively being employed for ameliorating non-communicable diseases owing to their ease to access, safety, acceptability and low cost [5,6].

Citrus peel, a byproduct of food processing industry, has a wide array of nutraceutical moieties that play significant roles against various physiological threats. It was noticed that citrus peel (orange) is a rich source of phenolic compounds that include phenolic acids and flavonoids constituting 147.6 mg/g of dry orange peel [7]. There are more than 4 000 structural variants of flavonoids that have been identified and characterized for their prophylactic potential. Among them, citrus peel derived

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flavonoids and their metabolites are of prime importance in providing therapeutic effects against various health related disorders [8]. There are two important glycosylated flavanones in citrus peel, namely, narirutin and hesperidin. However, hesperidin is more imperative for its therapeutic effects [9]. Considering numerous varieties of citrus family, *Citrus sinensis* is the major source of hesperidin ranging from 6.98 to 10.80 mg/g dried matter [10]. Studies have revealed its pharmacological and biological viewpoints such as hypolipidemic, hypoglycemic and anti-inflammatory perspectives. Due to its ease in accessibility of fruits by-products, citrus peel is one of the cheap sources of polyphenols for value added and designer food products [11].

In developing countries, hyperlipidemia resulting from fluctuations in lipid homeostasis, is the leading cause of cardiovascular diseases or atherosclerosis. Many factors are involved in the maintenance of blood cholesterol level that effect both intracellular and extracellular cholesterol metabolism [12]. Two important enzymes, 3-hydroxy-3-methyl-glutaryl-CoA 3 reductase as well as acyl CoA: cholesterol *O*-acyltransferase, present in the body that regulate cholesterol synthesis and modulate triglyceride level [13]. In this context, citrus peel flavonoids have ability to influence vascular endothelial cells in experimental models of hypercholesterolemia. In most animal species including human, inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase are effective in lowering plasma cholesterol level thus normalize both intracellular and extracellular cholesterol metabolism. Furthermore, acyl CoA: cholesterol O-acyltransferase is involved in catalysis of cholesterol esterification, hepatic secretion of very low-density lipoproteins, cholesterol absorption and its accumulation in vascular wall. Similarly, acyl CoA: cholesterol O-acyltransferase inhibitors are being used in cholesterol lowering drugs due to its hypocholesterolemic potential [14]. It is revealed from the previous data that hesperidin (0.08%) reduces weight of fatty tissues & liver, hepatic steatosis, retinol binding protein (involved in lipid metabolism) and total plasma cholesterol [15]. Among polymethoxylated flavones, nobiletin (0.1%) restored plasma and hepatic high density lipoproteins (HDL) cholesterol level with simultaneous decrement in hepatic triglycerides in diet induced hypercholesterolemic rats [16].

Diabetes is the most common metabolic syndrome related with expansion of coronary diseases. It is a multifunctional disorder that is characterized by hyperglycemia, abnormality in lipoproteins and increased oxidative stress due to which insulin secreting pancreatic beta cells become damaged [17]. Research has confirmed that polyphenolic and flavonoids rich diet has a potential to alleviate blood glucose level [18]. Substantial facts have divulged the role of citrus peel as an anti-diabetic agent by the reason of its strong antioxidant potential. The citrus peel allied bioflavonoids: hesperidin and nobiletin, attenuate hyperglycemic state by alleviating activities of phosphoenol pyruvate, glucose-6-phosphatase and α amylase whilst ameliorating glucokinase action and insulin secretion in blood [19]. Interestingly, citrus peels are considered as agro-waste material and are thought to impart negative impact by aggravating the legal boundaries and hygienic status of metropolitans. Nonetheless, their exploitation in dietary regimen will not only offer as source of cost effective and innovative generation therapeutics but also enhance nutritional value of conventional edibles [20].

2. Material and methods

2.1. Procurement of raw material and powder preparation

The research project was conducted in Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad (UAF), Pakistan. Citrus (Orange) was procured from local market, Faisalabad, considering the quality attributes like uniformity in color, size, shape and abrasion free trailed by washing. For the efficacy study, diagnostic kits were purchased from Sigma–Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Citrus peels were separated from fruits and sun dried followed by grinding to a fine powder using grinder. Resultant peel powder was stored for the extraction of biomolecules and bioevaluation trial.

2.2. Preparation of citrus peel extract

Citrus peel extract was prepared using water and methanol (50% v/v) for time interval of 45 min at 50 °C using the guidelines of Sultan *et al.* [21], with some modifications trailed by filtration and rotatory evaporation.

2.3. Efficacy study

A rodent trial was conducted to explore the therapeutic potential of the representative citrus peel based functional and nutraceutical diets against lifestyle related metabolic syndrome *i.e.* hypercholesterolemia and hyperglycemia. For the intent, 60 male Sprague Dawley rats were acquired from National Institute of Health, Islamabad, Pakistan and adjusted in the Animal Room of National Institute of Food Science and Technology, UAF. The animals were adapted by feeding the control diet for course of 7 d and by maintaining temperature (23 ± 2) °C and relative humidity 55% ± 5% throughout the trial. At the beginning, some of them were slaughtered to obtain baseline values. In rat modeling, three independent studies were executed separately; study I, study II and study III. Rats were fed with control diet, diet containing citrus peel powder and diet containing citrus peel extracts in control, functional diet and nutraceutical diet groups respectively in all three studies. Study I was consisted of rats fed on normal diet, whereas in study II & III, high glucose & cholesterol rich diets were employed, respectively. In each study, three groups of rats were formed depending on variations in their diets; control, functional and nutraceutical diets were used to assess its effect on different biological attributes like total cholesterol, HDL, low density lipoproteins (LDL), triglycerides, glucose and insulin level.

2.3.1. Ethical approval

Ethics approval was given by the head of the National Institute of Food Science and Technology, UAF, Pakistan, by reviewing the suggestions of Animal Experimentation Ethics Committee, UAF. Animal experiments were conducted in accordance with the instructions for the care and use provided by the committee and instructed by the university.

2.3.2. Feed and drink intake

The net feed and water intakes were estimated on daily basis by following the protocol of Wolf and Weidbrode [22].

2.3.3. Body weight gain

The change in body weight was monitored weekly to assess the influence of functional and nutraceutical diets on experimental rats.

2.3.4. Serum lipid profile

Serum lipid profiles including cholesterol method of Kim *et al.* [23], high density lipoproteins (HDL cholesterol precipitant) procedure of Alshatwi *et al.* [24], low density lipoproteins method of Alshatwi *et al.* [24] and triglycerides (GPO–PAP) guidelines of Demonty *et al.* [25] were measured with their respective protocols.

2.3.5. Serum glucose and insulin levels

The collected serum of rats was assessed according to glucose (GOD-PAP) method of Kim *et al.* [23] and insulin measuring procedure of Ahn *et al.* [26].

2.3.6. Hematological analysis

Red blood cells as well as white blood cell indices were estimated by following the protocol of Al Haj *et al.* [27] whilst platelets count was calculated according to the method of Kamatani *et al.* [28].

2.4. Statistical analysis

Data obtained was interpreted by statistical analysis to evaluate the level of significance and comparison of means as described by Steel *et al.* [29].

3. Results

3.1. Feed and drink intakes

Efficacy trial was conducted to explore health promoting aspects of citrus peel powder and its extract on following parameters. Mean squares regarding the feed intake elucidated significant differences as function of treatment as well as study weeks. Means relating to feed intake (Figure 1) in study I (normal diet) have revealed maximum feed intake in rat groups fed on functional and nutraceutical diets with (16.76 ± 0.45) and (16.55 ± 0.34) g/rat/day respectively, as compared to control group (15.22 ± 0.38) g/rat/day. Feed intake increased momentously in the course of time from (13.69 ± 0.25) to (19.38 ± 0.92) g/rat/day during the whole study. However, in study II (high sucrose diet), overall means indicated that rat group fed on control diet has more (17.04 ± 0.67) g/rat/day feed consumption followed by functional (16.90 ± 0.41) g/rat/day and nutraceutical diets (16.74 ± 0.87) g/rat/day. Elevation in time intervals has favored feed consumption from (14.12 ± 0.16) g/rat/day at 1st week to (21.03 ± 0.73) g/rat/day at the end of study. Likewise, in study III (high cholesterol diet), control group indicated feed intake of (18.23 ± 0.87) g/rat/day higher in contrast to functional [(17.53 ± 0.74) g/rat/day] and nutraceutical group [(16.71 ± 0.48) g/rat/day]. Moreover, time duration also has a crucial role in enhancing feed intake, as at the beginning it was (15.46 ± 0.73) g/rat/day that mounted to (20.74 ± 1.16) g/rat/day at final week.

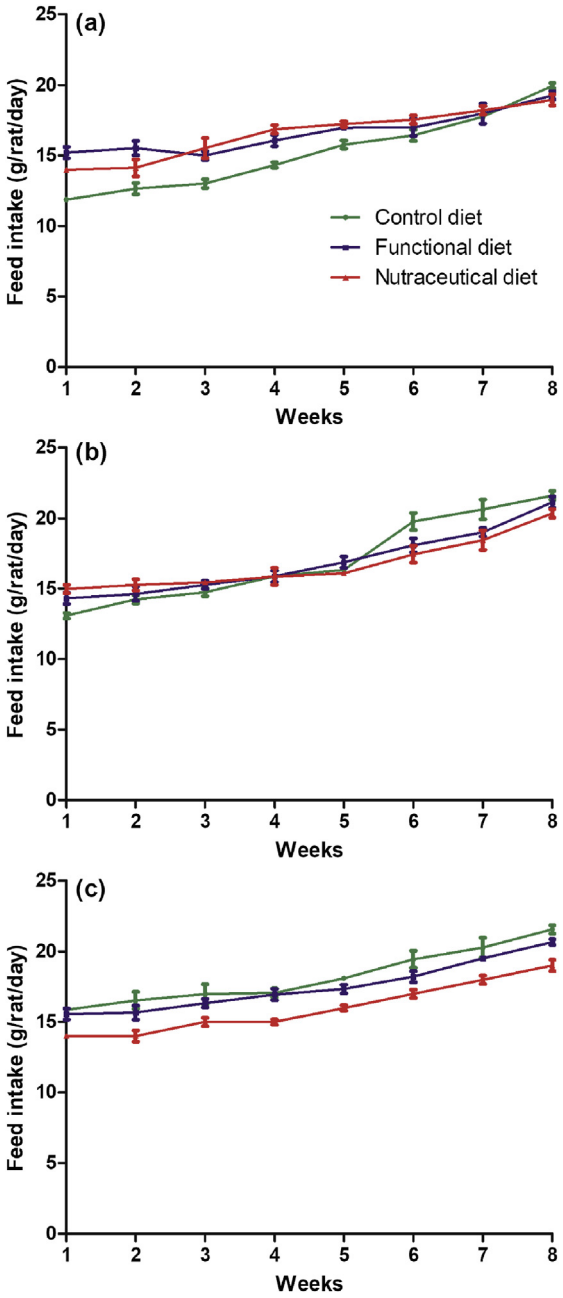

Figure 1. Feed intake in different studies (g/rat/day). (a) Study I: Normal rats; (b) Study II: Hyperglycemic rats; and (c) Study III: Hypercholesterolemic rats.

Mean squares for drink intake were affected significantly due to study intervals along with a non-significant behavior for treatments. Means relating to drink intake of rats (Figure 2) in study I, showed maximum intake in functional and nutraceutical diets based groups (23.02 ± 0.54) and (23.10 ± 0.48) mL/rat/day, correspondingly that was at par to control group (22.23 ± 0.39) mL/rat/day, nevertheless, increased from (19.43 ± 0.23) to (25.92 ± 1.02) mL/rat/day during 56 d trial. On the contrary, in study II, values observed for this trait were (23.73 ± 1.04), (23.47 ± 0.48) and (23.39 ± 0.38) mL/rat/day for control, functional and nutraceutical rat groups, respectively. Rats provided with high cholesterol diet (study III), revealed drink consumption for control, functional and nutraceutical groups as (24.33 ± 0.74), (24.23 ± 0.65) and (24.18 ± 0.43) mL/rat/day, correspondingly that enhanced from baseline value of (21.23 ± 0.86) to (27.78 ± 1.14) mL/rat/day at the end of trial (Figure 2).

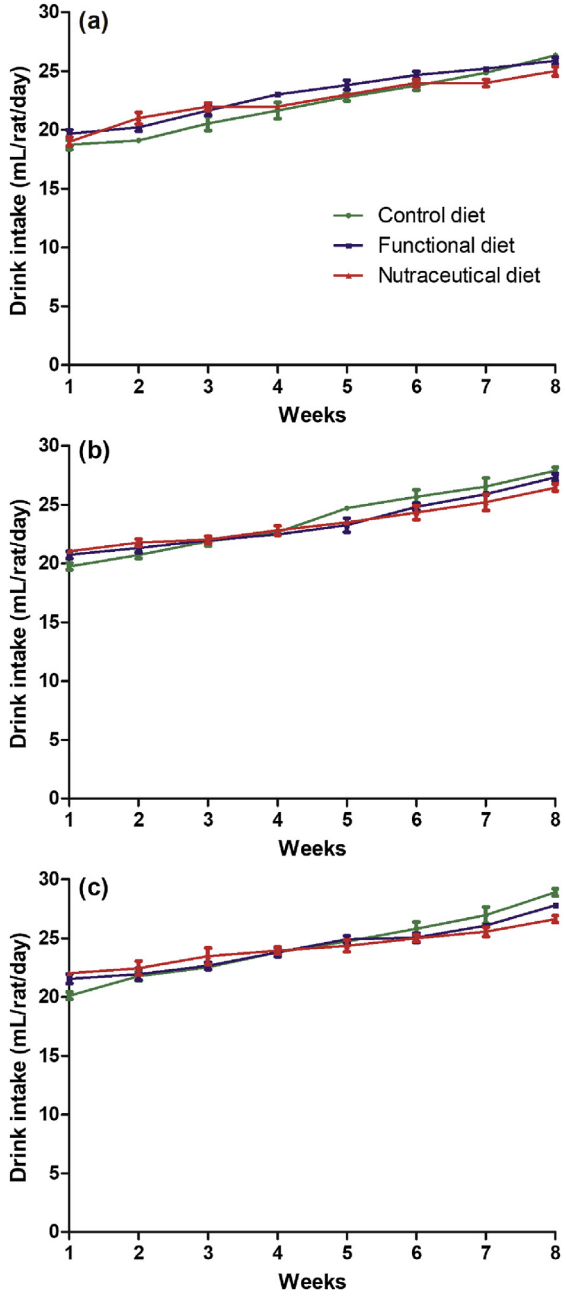

Figure 2. Drink intake in different studies (mL/rat/day). (a) Study I: Normal rats; (b) Study II: Hyperglycemic rats; and (c) Study III: Hypercholesterolemic rats.

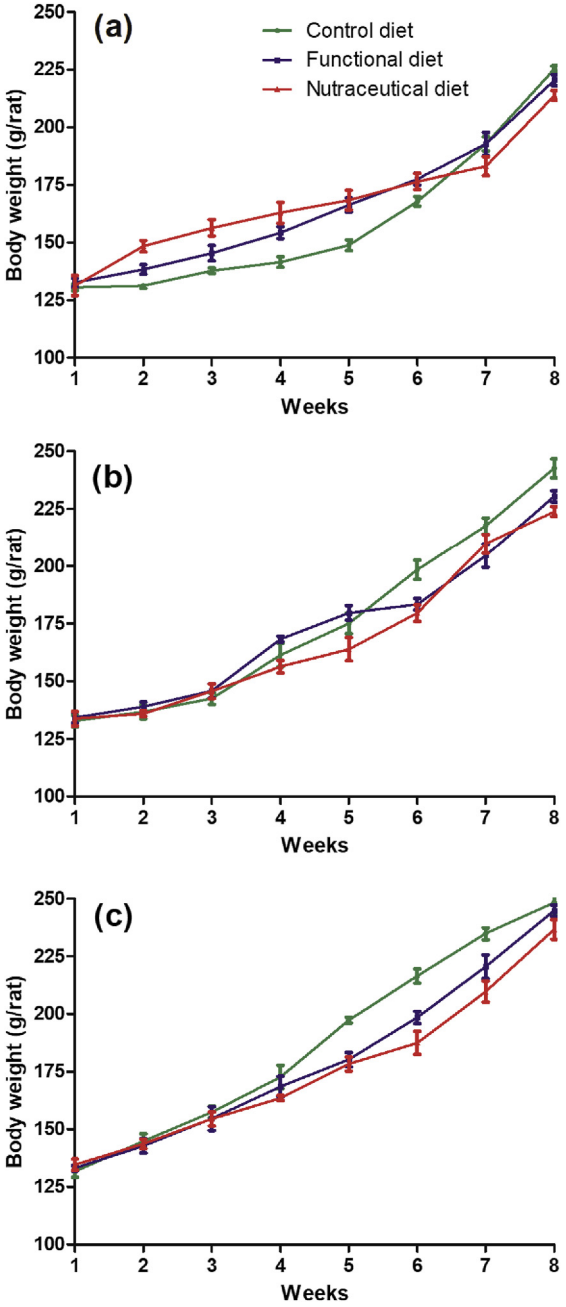

Figure 3. Body weight in different studies (g/rat). (a) Study I: Normal rats; (b) Study II: Hyperglycemic rats; and (c) Study III: Hypercholesterolemic rats.

3.2. Body weight

Mean squares for body weights of rats in different studies have illuminated significant effect of treatments and study weeks. It is evident from Figure 3 (study I) that body weight was significantly higher in functional and nutraceutical groups (165.95 ± 4.98) and (167.59 ± 5.11) g/rat, respectively in contrast to control group (159.47 ± 3.65) g/rat. Body weight in control group increased with passage of time ranging from (130.57 ± 4.76) to (225.35 ± 7.54) g/rat but in functional and nutraceutical groups increasing rate in body weight did not coincide to that of control group. In contrary to study I, the body weights of rats fed on high sucrose diet (study II) increased significantly and effect was more prominent in control group [(175.89 ± 6.32) g/rat] than in functional [(173.18 ± 7.78) g/rat] and nutraceutical [(168.60 ± 6.12) g/rat] groups. Same trend was observed in study III; rats fed on normal diet gained more body weight [(188.45 ± 7.53) g/rat] as compared to functional and nutraceutical groups [(180.40 ± 6.74) and (176.06 ± 6.24) g/rat], correspondingly. However, as function of time, increasing trend in body weight of rats fed on nutraceutical diet was lower as compared to functional and control groups from 0 to 56 d (Figure 3).

3.3. Hypercholesterolemic perspectives

3.3.1. Cholesterol

Mean squares corresponding to total cholesterol showed significant effect of diet in study II and III with non-significant impact of time intervals in all studies. While, their interaction exerted momentous effect on cholesterol level of rats in study III. In study I, maximum cholesterol was recorded in control

group [(81.39 ± 2.85) mg/dL] followed by functional [(79.29 ± 2.53) mg/dL] and nutraceutical [(79.19 ± 2.68) mg/dL] groups (Table 1). During the eight-week trial, cholesterol in control group increased from (80.45 ± 3.21) to (82.68 ± 3.35) mg/dL while decreasing trend was observed for functional from (79.45 ± 2.65) to (78.48 ± 2.29) mg/dL and nutraceutical from (80.08 ± 2.98) to (78.40 ± 3.87) mg/dL groups. Overall diets containing citrus peel powder and extract delineated percent reduction in cholesterol level by 1.15% and 2.05%, respectively. Means for cholesterol in study II (high sucrose diet) depicted the highest value for control group [(115.20 ± 4.60) mg/dL] that significantly reduced to (107.08 ± 4.28) mg/dL in functional and (106.24 ± 3.18) mg/dL in nutraceutical treated groups. Likewise, in control group cholesterol increased as function of study intervals from (108.90 ± 5.44) to (120.23 ± 6.01) mg/dL but decreased in functional and nutraceutical groups. It was calculated that cholesterol level reduced by 3.83% and 5.57% in functional and nutraceutical groups, correspondingly. Maximum reduction was found in nutraceutical group from (109.90 ± 5.49) at 0 d to (103.77 ± 3.11) mg/dL at the end of study.

However, in study III (rats fed on high cholesterol diet) showed maximum cholesterol [(138.37 ± 5.53) mg/dL] in control group but the value for this trait was reduced in functional [(124.47 ± 4.97) mg/dL] and nutraceutical groups [(122.11 ± 3.66) mg/dL]. Furthermore, rat group fed on control diet showed increment in cholesterol from (127.78 ± 3.83) to (147.65 ± 5.90) mg/dL during whole study, whilst diets containing citrus peel powder and extract decreased cholesterol level significantly from (129.18 ± 3.87) to (121.68 ± 4.86) mg/dL and (128.45 ± 5.13) to (117.46 ± 3.52) mg/dL, correspondingly. It was observed that nutraceutical diet elicited more percent decrement in cholesterol level (8.55%) in contrast to functional diet (5.80%).

3.3.2. HDL

It is obvious from mean squares that study intervals as well as interaction exhibited non-significant differences on HDL level in different studies. Mean values (Table 2) for this trait in study I (normal diet) showed that neither functional nor nutraceutical based diet altered HDL momentously and values for control, functional and nutraceutical groups were (33.23 ± 1.23), (33.86 ± 1.85) and (34.40 ± 1.61) mg/dL, respectively. However, HDL level has increased from (33.21 ± 1.32) to (33.25 ± 1.53) mg/dL, (33.78 ± 1.15) to (33.92 ± 1.49) mg/dL and (34.23 ± 1.02) to (34.55 ± 1.55) mg/dL in control, functional and nutraceutical groups, correspondingly from 0 to 56 d. Conclusively, it revealed that citrus peel extract has more tendencies to enhance HDL level by 0.95% as compared to peel powder (0.43%).

In study II (high sucrose diet), HDL values for control, functional and nutraceutical groups were (39.50 ± 1.39), (40.45 ± 2.51) and (39.25 ± 1.35) mg/dL, respectively. There was non-momentous increase in HDL level of all rat groups that was highest in nutraceutical group from (38.97 ± 1.55) at 0 d to (39.48 ± 1.44) mg/dL at termination of study. However, HDL level raised by 0.87% and 1.33% in rat groups fed on citrus peel functional and nutraceutical supplemented diets, respectively. In study III, means for control, functional as well as nutraceutical groups were (45.53 ± 1.52), (47.40 ± 2.29) and (46.86 ± 2.45) mg/dL, correspondingly. For 56 d, progressive decrease in HDL level from (45.57 ± 1.82) to (45.49 ± 1.33) mg/dL was recorded in control group, while this trait was increased from (46.34 ± 2.34) to (46.85 ± 2.54) mg/dL and (47.12 ± 2.31) to (47.98 ± 2.54) mg/dL in functional and nutraceutical groups, respectively. There was 1.11% and 1.83% rise in HDL level of hypercholesterolemic rats provided with functional and nutraceutical diets.

3.3.3. LDL

It is evident from mean squares that diet has significantly affected LDL in all studies except in normal rat group (study I). Nevertheless, LDL has changed significantly in study I and III as a function of interaction for diet and study intervals but with passage of time non-momentous trend was observed in all studies. Means for study I (Table 3) indicated LDL level as (31.83 ± 1.34) mg/dL in control group that decreased to (31.63 ± 1.63) and (31.61 ± 1.95) mg/dL in functional and nutraceutical groups, correspondingly. However, with passage of time LDL varied non-significantly from (30.31 ± 0.90) to (31.65 ± 1.58) mg/dL in control group while in functional and nutraceutical groups reduction was from (31.96 ± 1.65) to (31.29 ± 1.21) mg/dL and (32.27 ± 1.29) to (31.25 ± 1.54) mg/dL, respectively. It was concluded that LDL value decreased by 2.07% and 3.13% in rats fed on functional and nutraceutical groups, correspondingly.

LDL value for control group (study II) was (58.29 ± 1.74) mg/dL that momentously decreased in functional and

Table 1
Means for cholesterol (mg/dL) of rats in different studies.

Studies	Diet groups	Study intervals (days)			Means
		0	28	56	
Study I	Control	80.45 ± 3.21	81.05 ± 2.83	82.68 ± 3.35	81.39 ± 2.85
	Functional	79.45 ± 3.97	79.95 ± 2.36	78.48 ± 2.29	79.29 ± 2.53
	Nutraceutical	80.08 ± 2.98	79.25 ± 2.84	78.40 ± 3.87	79.19 ± 2.68
	Means	79.99 ± 2.79	80.08 ± 3.20	79.85 ± 2.56	–
Study II	Control	108.90 ± 5.44	116.47 ± 3.49	120.23 ± 6.01	115.20 ± 4.60
	Functional	109.58 ± 4.38	106.30 ± 4.25	105.38 ± 3.16	107.08 ± 4.28
	Nutraceutical	109.90 ± 5.49	105.04 ± 4.20	103.77 ± 3.11	106.24 ± 3.18
	Means	109.46 ± 4.37	109.27 ± 4.37	109.79 ± 4.31	–
Study III	Control	127.78 ± 3.83	139.69 ± 6.98	147.65 ± 5.90	138.37 ± 5.53
	Functional	129.18 ± 3.87	122.56 ± 3.67	121.68 ± 4.86	124.47 ± 4.97
	Nutraceutical	128.45 ± 5.13	120.43 ± 6.01	117.46 ± 3.52	122.11 ± 3.66
	Means	128.47 ± 3.85	127.56 ± 5.10	128.93 ± 5.15	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

Table 2
Means for HDL (mg/dL) of rats in different studies.

Studies	Diet groups	Study intervals (days)			Means
		0	28	56	
Study I	Control	33.21 ± 1.32	33.23 ± 1.41	33.25 ± 1.53	33.23 ± 1.23
	Functional	33.78 ± 1.15	33.89 ± 1.42	33.92 ± 1.49	33.86 ± 1.85
	Nutraceutical	34.23 ± 1.02	34.44 ± 1.34	34.55 ± 1.55	34.40 ± 1.61
	Means	33.74 ± 1.34	33.85 ± 1.32	33.91 ± 1.29	–
Study II	Control	39.57 ± 1.58	38.82 ± 1.43	38.52 ± 1.51	38.98 ± 1.39
	Functional	38.97 ± 2.01	39.18 ± 2.34	39.30 ± 2.39	39.15 ± 2.51
	Nutraceutical	39.97 ± 1.55	40.22 ± 1.32	40.50 ± 1.44	40.23 ± 1.35
	Means	39.50 ± 1.38	39.40 ± 1.54	39.45 ± 1.65	–
Study III	Control	45.57 ± 1.82	45.55 ± 1.65	45.49 ± 1.33	45.53 ± 1.52
	Functional	46.34 ± 2.34	46.72 ± 2.35	46.85 ± 2.54	46.63 ± 2.29
	Nutraceutical	47.12 ± 2.31	47.66 ± 2.61	47.98 ± 2.54	47.58 ± 2.45
	Means	46.34 ± 2.54	46.64 ± 2.55	46.77 ± 2.41	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

Table 3
Means for LDL (mg/dL) of rats in different studies.

Studies	Diet groups	Study intervals (days)			Means
		0	28	56	
Study I	Control	30.31 ± 0.90	30.98 ± 0.89	31.65 ± 1.58	31.83 ± 1.34
	Functional	31.96 ± 1.65	31.64 ± 1.7	31.29 ± 1.21	31.63 ± 1.63
	Nutraceutical	32.27 ± 1.29	31.32 ± 0.17	31.25 ± 1.54	31.61 ± 1.95
	Means	31.48 ± 1.34	31.31 ± 1.98	32.25 ± 1.20	–
Study II	Control	55.78 ± 1.67	57.88 ± 2.31	61.23 ± 1.83	58.29 ± 1.74
	Functional	57.63 ± 2.39	55.33 ± 1.54	54.67 ± 2.18	55.88 ± 1.32
	Nutraceutical	56.73 ± 2.26	52.63 ± 2.10	51.93 ± 2.07	53.76 ± 2.15
	Means	56.71 ± 2.45	55.28 ± 1.65	55.94 ± 1.87	–
Study III	Control	63.25 ± 1.89	68.85 ± 3.44	71.84 ± 3.59	67.98 ± 2.71
	Functional	64.86 ± 2.59	60.64 ± 2.42	59.16 ± 1.77	61.55 ± 3.07
	Nutraceutical	62.99 ± 3.14	56.94 ± 1.70	55.97 ± 2.23	58.63 ± 2.34
	Means	63.70 ± 2.54	62.14 ± 3.29	62.32 ± 3.34	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

nutraceutical groups to (55.88 ± 1.32) and (53.76 ± 2.15) mg/dL, respectively. It was interpreted that reduction in LDL was maximum in rats fed on peel extract supplemented diet (nutraceutical group) from (56.73 ± 2.26) to (51.93 ± 2.07) mg/dL. However, both functional and nutraceutical diets has significantly reduced LDL value by 5.12% and 8.45%, correspondingly. Substantial differences in LDL (study III) were measured in nutraceutical [(58.63 ± 2.34) mg/dL] and functional [(61.55 ± 3.07) mg/dL] groups as compared to control [(67.98 ± 2.71) mg/dL]. It was observed that during 56 day efficacy study, LDL increased in control from (63.25 ± 1.89) to (71.84 ± 3.59) mg/dL, nevertheless, decreasing trend was found in functional and nutraceutical groups from (64.86 ± 2.59) to (59.16 ± 1.77) mg/dL and (62.99 ± 3.14) to (55.97 ± 2.23) mg/dL, correspondingly. There was 8.78% and 11.39% reduction in LDL level of rats fed on functional and nutraceutical diets, correspondingly.

3.3.4. Triglyceride

Mean squares for the effect of diet has significantly changed triglycerides in study II and III while interaction varied this trait momentously only in study III. Mean values for triglycerides (Table 4) in study I in control, functional and nutraceutical groups were (72.11 ± 2.32), (71.73 ± 2.65) and (69.79 ± 3.65) mg/dL, correspondingly. It was observed that triglycerides in control group increased from (71.24 ± 2.10) to (72.98 ± 2.85) mg/dL but decreased in functional and nutraceutical groups from (72.67 ± 2.90) to (71.13 ± 2.34) mg/dL and (71.34 ± 2.45) to (68.82 ± 3.44) mg/dL, respectively. Overall, serum triglycerides decreased by 2.11% and 3.52% for functional and nutraceutical groups. Similarly, in study II, mean values for control, functional and nutraceutical groups differed substantially, *i.e.*, (79.80 ± 3.99), (74.06 ± 2.96) and (73.47 ± 2.78) mg/dL, correspondingly. Maximum reduction for triglycerides was noted in nutraceutical group from (76.12 ± 3.08) to (71.68 ± 2.23) mg/dL. Conclusively, it was inferred that diet containing citrus peel powder and extract has significantly reduced triglycerides by 4.16% and 5.83%, correspondingly.

However, for study III triglycerides level increased to (98.36 ± 3.93) mg/dL in control group but diet containing 10% citrus peel powder (functional) and 5% peel extract (nutraceutical) suppressed the values for this trait to (91.82 ± 3.67) and (89.37 ± 3.57) mg/dL, respectively. During course of eight-week study, nutraceutical group exhibited pronounced alleviation in triglycerides from (93.78 ± 4.68) to (86.38 ± 4.31) mg/dL. Similarly, in functional group, triglyceride decreased from (94.87 ± 2.84) to (89.71 ± 3.58) mg/dL whereas control group showed significant increase in triglyceride from baseline value to the end of study (92.14 ± 2.76) to (103.21 ± 5.16) mg/dL. However, effect of nutraceutical diet was more dominant in

Table 4
Means for triglycerides (mg/dL) of rats in different studies.

Studies	Diet	Study intervals (days)			Means
		0	28	56	
Study I	Control	71.24 ± 2.10	72.13 ± 2.43	72.98 ± 2.85	72.11 ± 2.32
	Functional	72.67 ± 2.90	71.39 ± 2.65	71.13 ± 2.34	71.73 ± 2.65
	Nutraceutical	71.34 ± 2.45	69.21 ± 3.46	68.82 ± 3.44	69.79 ± 3.65
	Means	71.75 ± 2.43	70.91 ± 2.54	70.97 ± 2.32	–
Study II	Control	76.38 ± 3.05	80.27 ± 3.21	82.75 ± 3.31	79.80 ± 3.99
	Functional	75.87 ± 3.79	73.60 ± 2.94	72.71 ± 2.18	74.06 ± 2.96
	Nutraceutical	76.12 ± 3.08	72.63 ± 2.34	71.68 ± 2.23	73.47 ± 2.78
	Means	76.12 ± 3.17	75.50 ± 3.87	75.71 ± 3.54	–
Study 111	Control	92.14 ± 2.76	99.73 ± 3.98	103.21 ± 5.16	98.36 ± 3.93
	Functional	94.87 ± 2.84	90.88 ± 3.63	89.71 ± 3.58	91.82 ± 3.67
	Nutraceutical	93.78 ± 4.68	87.95 ± 3.51	86.38 ± 4.31	89.37 ± 3.57
	Means	93.59 ± 3.74	92.85 ± 2.98	93.10 ± 3.81	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

reducing serum triglyceride level (7.89%) in contrast to functional diet (5.43%).

3.4. Hyperglycemic perspectives

3.4.1. Glucose

Statistical analysis regarding glucose delineated that diet exhibited significant differences on glucose in study II and III whereas interaction of diet and study intervals exerted momentous impact only in study II. Mean values for glucose (Table 5) in control, functional and nutraceutical groups varied as (92.45 ± 3.32), (89.92 ± 2.64) and (89.35 ± 2.76) mg/dL, respectively in study I. In control group, glucose increased from (91.87 ± 3.67) to (92.74 ± 3.98) mg/dL while in functional and nutraceutical groups decreased from (90.56 ± 3.62) to (89.22 ± 2.56) mg/dL and (91.08 ± 3.65) to (88.44 ± 2.78) mg/dL, respectively. Maximum reduction (1.69%) was observed in nutraceutical group as compared to functional group (0.98%).

In study II, maximum glucose concentration was noticed in control group [(128.44 ± 3.85) mg/dL], followed by functional [(115.08 ± 4.60) mg/dL] and nutraceutical group [(113.73 ± 5.68) mg/dL]. It was expounded that diets containing citrus peel powder and extract decreased glucose from (119.56 ± 3.58) to (111.03 ± 3.33) mg/dL and (120.12 ± 5.22) to (109.35 ± 3.28) mg/dL, correspondingly whereas increased from (118.98 ± 4.75) to (135.84 ± 5.43) mg/dL in control group. Both functional and nutraceutical diets has significantly decreased plasma glucose level by 7.13% and 8.96%, respectively. Maximum glucose value (101.78 ± 3.05) mg/dL was observed for control group in study III (high cholesterol diet) that reduced to (96.60 ± 2.98) mg/dL in functional and (94.98 ± 3.56) mg/dL in nutraceutical groups. Similarly, maximum reduction in serum glucose level was measured for nutraceutical group that changed significantly from (97.67 ± 3.90) to (93.06 ± 3.72) mg/dL as a function of study intervals. However, both functional and nutraceutical groups have percent reduction of about 2.87% and 4.71%, correspondingly.

3.4.2. Insulin

Mean squares relevant to insulin depicted significant impact of diet only in study II while this trait was no significant as function of intervals and interaction in all studies. In study I, mean values for insulin (Table 6) were (9.07 ± 0.76) (control group), (9.16 ± 0.78) (functional group) and (9.42 ± 0.47) μU/mL (nutraceutical group), respectively. Insulin level increased in functional and nutraceutical groups from (9.07 ± 0.47) to (9.25 ± 0.67) μU/mL and from (9.26 ± 0.31) to (9.55 ± 0.78) μU/mL, respectively. Both citrus peel powder and extract enhanced insulin secretion by 0.98% and 1.69% respectively as compared to control group. Likewise, in study II, means for insulin in control group was (10.20 ± 0.87) μU/mL, followed by functional and nutraceutical groups with (10.70 ± 0.65) and

Table 5
Means for glucose level (mg/dL) of rats in different studies.

Studies	Diet	Study intervals (days)			Means
		0	28	56	
Study I	Control	91.87 ± 3.67	92.75 ± 3.71	92.74 ± 3.98	92.45 ± 3.32
	Functional	90.56 ± 3.62	89.99 ± 2.69	89.22 ± 2.56	89.92 ± 2.64
	Nutraceutical	91.08 ± 3.65	88.54 ± 2.65	88.44 ± 2.78	89.35 ± 2.76
	Means	91.67 ± 3.52	90.43 ± 3.76	90.13 ± 3.98	–
Study II	Control	118.98 ± 4.75	130.52 ± 6.52	135.84 ± 5.43	128.44 ± 3.85
	Functional	119.56 ± 3.58	114.65 ± 4.58	111.03 ± 3.33	115.08 ± 4.60
	Nutraceutical	120.12 ± 5.22	111.73 ± 3.61	109.35 ± 3.28	113.73 ± 5.68
	Means	119.55 ± 3.61	118.98 ± 4.89	118.74 ± 4.76	–
Study III	Control	96.81 ± 2.90	102.68 ± 3.08	105.85 ± 3.17	101.78 ± 3.05
	Functional	98.25 ± 3.93	96.13 ± 2.78	95.43 ± 2.86	96.60 ± 2.98
	Nutraceutical	97.67 ± 3.90	94.24 ± 3.76	93.06 ± 3.72	94.98 ± 3.56
	Means	97.57 ± 3.87	97.68 ± 3.67	98.11 ± 3.87	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

Table 6
Means for insulin level (μU/mL) of rats in different studies.

Studies	Diet	Study intervals (days)			Means
		0	28	56	
Study I	Control	9.11 ± 0.47	9.07 ± 0.67	9.05 ± 0.90	9.07 ± 0.76
	Functional	9.07 ± 0.47	9.18 ± 0.45	9.25 ± 0.67	9.16 ± 0.78
	Nutraceutical	9.26 ± 0.31	9.47 ± 0.55	9.55 ± 0.78	9.42 ± 0.47
	Means	9.14 ± 0.32	9.24 ± 0.65	9.28 ± 0.98	–
Study II	Control	10.23 ± 0.51	10.19 ± 0.45	10.18 ± 0.76	10.20 ± 0.87
	Functional	10.45 ± 0.65	10.78 ± 0.55	10.89 ± 0.67	10.70 ± 0.65
	Nutraceutical	10.78 ± 0.98	11.53 ± 0.65	11.36 ± 0.61	11.22 ± 0.32
	Means	10.48 ± 0.87	10.83 ± 0.34	10.82 ± 0.76	–
Study III	Control	9.47 ± 0.49	9.45 ± 0.50	9.42 ± 0.32	9.44 ± 0.76
	Functional	9.42 ± 0.26	9.51 ± 0.64	9.54 ± 0.98	9.49 ± 0.87
	Nutraceutical	9.59 ± 0.35	9.76 ± 0.40	9.89 ± 0.67	10.08 ± 0.39
	Means	9.49 ± 0.51	9.57 ± 0.45	9.61 ± 0.62	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

(11.22 ± 0.32) μU/mL, respectively. It was observed that insulin level increased as function of time, ranging from (10.45 ± 0.65) to (10.89 ± 0.67) μU/mL in functional and (10.78 ± 0.98) to (11.36 ± 0.61) μU/mL in nutraceutical groups, but decreased in control group from (10.23 ± 0.51) to (10.18 ± 0.76) μU/mL. However, nutraceutical diet was more effective to enhance insulin secretion (5.41%) trailed by functional diet (4.23%).

Nonetheless, non-significant differences due to treatments were observed in study III comprising high cholesterol diet; nutraceutical group showed highest insulin level [(10.08 ± 0.39) μU/mL] but low in functional [(9.49 ± 0.87) μU/mL] and control groups [(9.44 ± 0.76) μU/mL], respectively. However, insulin level in functional and nutraceutical increased progressively from (9.42 ± 0.26) to (9.54 ± 0.98) and from (9.59 ± 0.35) to (9.89 ± 0.67) μU/mL, respectively. Decisively, functional and nutraceutical diet holds insulinotropic properties that improved insulin secretion by 2.07% and 3.21%, correspondingly.

3.5. Hematological study

3.5.1. Red blood cell

Statistical inferences corresponding to red blood indices have expounded non-significant effect of treatments on red blood cells in different studies while intervals led to momentous variations in study I. Keeping in view, for study I (Table 7), red blood cells counts were (8.10 ± 0.32), (8.17 ± 0.35) and (8.39 ± 0.37) × 10^6/μL in control, functional and nutraceutical groups, respectively. However, it increased significantly as a function of intervals that was highest in nutraceutical group from (7.98 ± 0.28) to (8.45 ± 0.48) × 10^6/μL. In study II, total red blood cells were (8.90 ± 0.28) × 10^6/μL in control, (8.94 ± 0.25) × 10^6/μL in functional and (9.05 ± 0.31) × 10^6/μL in nutraceutical groups. But, it improved from (8.88 ± 0.45) to (8.99 ± 0.42) × 10^6/μL and (8.97 ± 0.32) to (9.12 ± 0.44) × 10^6/μL for functional and nutraceutical groups, respectively as compared to control group. In study III, red blood cells for control, functional and nutraceutical groups were (9.41 ± 0.34), (9.66 ± 0.38) and (9.75 ± 0.45) × 10^6/μL that increased with passage of time in functional and nutraceutical groups.

3.5.2. White blood cell

Mean squares explicated that treatments as well as interaction have non-momentous changes in white blood cells of rats in different studies however intervals significantly affected white blood cells in study I and II. Mean values for white blood cells (Table 8) for control, functional and nutraceutical groups in study I were (10.17 ± 0.62), (10.18 ± 0.58) and (10.31 ± 0.72) × 10^3/μL, respectively. Functional and nutraceutical diets have significantly increased this trait with maximum variations in rat group fed on 5% citrus peel extract from (10.17 ± 0.57) to (10.40 ± 0.62) × 10^3/μL. In study II, total white blood cells were (10.73 ± 0.68) × 10^3/μL in control, (10.85 ± 0.72) × 10^3/μL in functional and (10.86 ± 0.75) × 10^3/μL in nutraceutical groups. It was observed that white blood cells increased significantly with passage of time that were highest in

Table 7
Means for red blood cells (10^6/μL) of rats in different studies.

Studies	Diet	Study intervals (days)			Means
		0	28	56	
Study I	Control	7.78 ± 0.23	8.22 ± 0.24	8.32 ± 0.33	8.10 ± 0.32
	Functional	7.85 ± 0.32	8.26 ± 0.42	8.40 ± 0.38	8.17 ± 0.35
	Nutraceutical	7.98 ± 0.28	8.32 ± 0.45	8.45 ± 0.48	8.39 ± 0.37
	Means	7.87 ± 0.25	8.26 ± 0.36	8.39 ± 0.38	–
Study II	Control	8.93 ± 0.35	8.89 ± 0.27	8.88 ± 0.34	8.90 ± 0.28
	Functional	8.88 ± 0.45	8.95 ± 0.35	8.99 ± 0.42	8.94 ± 0.25
	Nutraceutical	8.97 ± 0.32	9.08 ± 0.45	9.12 ± 0.44	9.05 ± 0.31
	Means	8.92 ± 0.37	8.97 ± 0.33	8.99 ± 0.28	–
Study III	Control	9.46 ± 0.47	9.42 ± 0.56	9.37 ± 0.37	9.41 ± 0.34
	Functional	9.57 ± 0.54	9.68 ± 0.41	9.74 ± 0.45	9.66 ± 0.38
	Nutraceutical	9.63 ± 0.23	9.79 ± 0.43	9.84 ± 0.41	9.75 ± 0.45
	Means	9.55 ± 0.48	9.63 ± 0.34	9.65 ± 0.42	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

Table 8
Means for white blood cells (10^3/μL) of rats in different studies.

Studies	Diet	Study intervals (days)			Means
		0	28	56	
Study I	Control	10.13 ± 0.53	10.17 ± 0.69	10.23 ± 0.54	10.17 ± 0.62
	Functional	10.08 ± 0.61	10.21 ± 0.59	10.26 ± 0.57	10.18 ± 0.58
	Nutraceutical	10.17 ± 0.57	10.36 ± 0.52	10.40 ± 0.62	10.31 ± 0.72
	Means	10.12 ± 0.64	10.24 ± 0.59	10.29 ± 0.65	–
Study II	Control	10.72 ± 0.87	10.73 ± 0.78	10.75 ± 0.94	10.73 ± 0.68
	Functional	10.82 ± 0.54	10.85 ± 0.82	10.90 ± 0.91	10.85 ± 0.72
	Nutraceutical	10.79 ± 0.82	10.89 ± 0.75	10.92 ± 0.85	10.86 ± 0.75
	Means	10.77 ± 0.67	10.82 ± 0.84	10.86 ± 0.87	–
Study III	Control	11.23 ± 0.91	11.26 ± 0.58	11.27 ± 0.72	11.25 ± 0.87
	Functional	11.19 ± 0.85	11.33 ± 0.65	11.49 ± 0.64	11.36 ± 0.64
	Nutraceutical	11.32 ± 0.80	11.49 ± 0.76	11.54 ± 0.59	11.39 ± 0.58
	Means	11.25 ± 0.77	11.30 ± 0.87	11.45 ± 0.97	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

Table 9
Means for platelets (10^3/μL) of rats in different studies.

Studies	Diet	Study intervals (Days)			Means
		0	28	56	
Study I	Control	1 024.0 ± 30.7	1 040.0 ± 31.2	1 047.0 ± 52.3	1 037.7 ± 31.1
	Functional	1 031.0 ± 31.2	1 044.0 ± 41.1	1 051.0 ± 31.5	1 042.4 ± 52.0
	Nutraceutical	1 034.0 ± 41.3	1 052.0 ± 52.4	1 059.0 ± 31.7	1 048.7 ± 55.8
	Means	1 029.6 ± 31.0	1 045.3 ± 41.5	1 052.3 ± 42.0	–
Study II	Control	1 063.0 ± 53.1	1 057.0 ± 43.2	1 055.0 ± 65.6	1 058.3 ± 53.9
	Functional	1 071.0 ± 32.1	1 078.0 ± 33.2	1 082.0 ± 48.7	1 077.1 ± 57.4
	Nutraceutical	1 067.0 ± 53.3	1 083.0 ± 54.7	1 086.0 ± 66.1	1 078.7 ± 54.4
	Means	1 067.0 ± 42.6	1 072.7 ± 32.6	1 074.3 ± 43.9	–
Study III	Control	1 049.0 ± 31.4	1 043.0 ± 53.3	1 041.0 ± 32.1	1 044.3 ± 63.7
	Functional	1 052.0 ± 31.5	1 063.0 ± 52.9	1 067.0 ± 63.8	1 060.9 ± 31.7
	Nutraceutical	1 061.0 ± 42.4	1 079.0 ± 41.9	1 084.0 ± 31.8	1 074.8 ± 42.2
	Means	1 054.0 ± 52.7	1 061.6 ± 42.3	1 064.4 ± 31.9	–

Study I: Normal rats; Study II: Hyperglycemic rats; Study III: Hypercholesterolemic rats.

nutraceutical group from (10.79 ± 0.82) to (10.92 ± 0.85) × 10^3/μL. In study III, mean values for white blood cells were (11.25 ± 0.87) (control group), (11.36 ± 0.64) (functional group) and (11.39 ± 0.58) × 10^3/μL (nutraceutical group).

3.5.3. Platelet count

It is deduced from mean squares that effect of treatments as well as intervals was non-significant on platelet count in different studies. In study I, mean values (Table 9) for control, functional and nutraceutical groups changed from (1 037.7) ± 31.1), (1 042.4 ± 52.0) to (1 048.7 ± 55.8) × 10^3/μL, respectively but increased non-significantly in all groups. However, in study II, higher platelet counts [(1 077.18 ± 57.40) and (1 078.73 ± 54.40) × 10^3/μL] were noted in functional and nutraceutical groups, correspondingly as compared to (1 058.33 ± 53.90) × 10^3/μL in control group. Likewise, in study III, platelet count in control group was (1 044.33 ± 63.70) × 10^3/μL, but improved to (1 060.96 ± 31.70) × 10^3/μL in functional and (1 074.81 ± 42.20) × 10^3/μL in nutraceutical groups.

4. Discussion

The concept of health and nutrition paradigm has significantly modified consumer preferences in the selection of food for the last few decades. Nowadays, phytochemical rich food is considered as a vehicle to maintain good health besides supplying nutrients for proper body functioning. Therefore, core attention was paid to illuminate the health promoting role of citrus peel; an industrial by-product of fruit processing unit. Considering the fact, present project was designed to investigate the prophylactic worth of citrus peel against various life style related disorders like hyperglycemia and hypercholesterolemia. In this context, efficacy trial was conducted by designing three different studies named as study I, II & III. The study I consisted of normal rats; rats in study II were fed on high sucrose diet to induce hyperglycemia and rats in study III were provided with high cholesterol diet to induce hypercholesterolemic conditions. Previously, the research investigation of Menichini *et al.* [30], has already confirmed that citrus peel can be effectively employed to alleviate metabolic syndrome like coronary heart diseases by decreasing free fatty acids, hepatic and plasma triglycerides and increasing fecal excretion of triglycerides. Oral administration of citrus peel extract (600 mg/kg) can reduce the plasma triglycerides in hypercholestrolemic subjects as compared to control group. It was accredited to the ability of citrus flavonoids to prevent oleic acid conjugation in triglycerides thus overall decrease plasma cholesterol level.

Current explorations were in line with the outcomes of Abdelbaky *et al.* [31], who analyzed effect of orange, grapefruit & lemon peel powder and their extracts on biochemical

parameters of hypercholesterolemic rats and reported non-significant differences in feed intake of rat groups fed separately on peel powder of various citrus fruits (orange, grapefruits and lemon) and their respective extracts. Accordingly, significant reduction was noticed in body weight gain ratio of rats fed on citrus peel supplemented diet as compared to control group. The reason behind weight reduction of treated rats is the gain in liver weight of control hyperlipidemic group while the weight of heart, kidney, lungs and spleen did not change considerably in all groups. This may be attributed to liver high cholesterol synthesis rate in control group.

Moreover, Park *et al.* [32], explored citrus peel as regulator of lipoprotein metabolism in rats against diet induced fatty liver. They provided experimental animal with escalating doses of citrus peel extracts; 278, 2.87 and 576 mL/rat/day for 6 weeks. Results depicted significant alleviation in total cholesterol of rats fed on high doses of peel extract as compared to group relying on low doses. Further, Mollace *et al.* [33] also recorded significant improvement in HDL while total cholesterol, LDL and plasma triglycerides decreased in hypercholesterolemic rats fed on citrus flavonoid rich extract (10 mg/kg and 20 mg/kg) for the course of one month. Citrus peel bioactive moieties trigger the activation of receptor cells that incorporate excess LDL and triglycerides into liver and adipose tissue rather than circulating in vascular system to develop hard plaque. Previously, Abdelbaky *et al.* [31], concluded that orange peel extract was most potent in reducing plasma LDL (12.47 mg/dL) while grapefruit peel powder was least efficient (49.65 mg/dL). It may be attributed to hesperidin contents of orange peel that are more as compared to other citrus varieties. This hypothesis was affirmed by Wang *et al.* [15], who summarized that hesperidin has potential to prevent fatty degeneration of liver thus control hepatic lipid metabolism.

Regarding the hyperglycemic perspectives of citrus peel, Fernandes *et al.* [34] observed the effect of citrus peel flavonoids to minimize adverse changes in streptozotocin induced diabetic rat. Daily intake of peel flavonoids tends to stimulate the action of pancreatic beta cells to normalize insulin level. In another experiment, Kabra *et al.* [35] studied effect of citrus flavonoid (200 mg/kg and 400 mg/kg body weight) on diabetic induced complications in rats. They assessed that oral administration of peel extract significantly decline ($P < 0.01$) blood glucose level in diabetic rat after 28 and 56 d (297 mg/dL and 133.4 mg/dL, respectively) in contrast to diabetic control (429.2 mg/dL and 485 mg/dL, correspondingly). Oral administration of citrus bioflavonoids reactivates insulin secreting cells and regularizes the disparities due to glycated Hb. Furthermore, Menichini *et al.* [30], evaluated nutraceutical effect of citrus peel extract (300 mg/kg and 600 mg/kg body weight of rat) on glucose homeostasis as well as hematological parameters. They concluded that peel extract was more effective to normalize glucose and serum lipid profile while non-significant effect was observed for all hematological attributes.

Conclusively, citrus peel extract and powder exhibited a decline in total cholesterol, triglycerides, LDL and glucose levels by the virtue of nutraceutical and functional diets. Regarding the levels of insulin and HDL, it improved by dietary inclusions. Nevertheless, citrus peel nutraceutical enriched diet regularizes glycemic and lipidemic parameters more effectively as compared to citrus peel powder. In a nutshell, proper utilization of agro-waste material like citrus peel in diet based therapies is a economical, accessible and cheap source to address life style associated diseases.

Conflict of interest statement

The authors of this publication declared that there is no conflict of interest.

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