

# Sodium alginate-based nanoformulation: optimistic approach for enhancing the anti-obesogenic effect of rutin

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**Received:** 27 August 2024

**Revised:** 2 October 2024

**Accepted:** 3 October 2024

**Published:** 17 December 2024

**Egyptian Pharmaceutical Journal** 2024, 0:0–0

## Background

Obesity is a global epidemic that affects all age groups and social classes. Prevention and treatment strategies against obesity have gained little long-term success. Obesity has been shown to be increased rapidly in the last few decades. It is anticipated that by 2030, up to half of the human population will be classified as obese or overweight.

## Objective

This study aimed to develop a polymeric nanoformulation for the oral delivery of rutin in an obese rat model and to explore the anti-obesogenic impact of this nanosized rutin versus free rutin to provide insightful information for understanding the mechanism of action.

## Materials and methods

Sodium alginate-based nanorutin was prepared and characterized by transmission electron microscope and Zetasizer. The obtained rutin nanoformulation and its free form were applied for obesity management in rats fed a high-fat diet.

## Results and conclusion

The data demonstrated that rutin nanoparticles are made up of single spherical units with a diameter that ranged between 96.1 and 157 nm and exhibited negative zeta potential at  $-16.6$  mV. Treatment of obese rats with chitocal, free rutin, or rutin nanoformulation provoked significant reduction in the body weight, thoracic circumference, BMI, and Lee index. Also, they elicited a significant drop in serum cholesterol, triglycerides, low-density lipoprotein, glucose, insulin, insulin resistance, toll-like receptor 4, nuclear factor kappa B, and leptin levels associated with a significant rise in serum adiponectin and spexin levels. Furthermore, treatment of obese rats with chitocal or rutin nanoformulation elicited significant reduction in body length and abdominal circumference along with significant enhancement in serum high-density lipoprotein level. In conclusion, this approach provides a sparkling proof for the anti-obesogenic potential of rutin nanoformulation through its hypolipidemic, hypoglycemic, and anti-inflammatory effects as well as its inhibitory action on hyperleptinemia and adipogenesis. The superior antiobesity impact of the formulated nanorutin than its free form may be attributed to the enhancement of the solubility and bioavailability by nanoencapsulation.

## Keywords:

chitocal, nanoformulation, obesity, rats, rutin, sodium alginate

## Abbreviations:

AC, abdominal circumference, ADP, adiponectin, HDL, high density lipoprotein, LDL, low density lipoprotein, NF  $\kappa$ B, nuclear factor kappa B, SPX, spexin, TC, thoracic circumference, TG, triglycerides, TLR4, toll like receptor 4

Egypt Pharmaceut J 0:0–0

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1687-4315

## Introduction

Obesity is a common public health issue as the rates of obesity have been shown to be increased rapidly in the last few decades [1]. It is anticipated that by 2030, up to half of the human population will be classified as obese or overweight [2]. Obesity is often accompanied by an enhanced risk for different metabolic, cardiovascular, skeletal comorbidities, and cancer [3], besides a crucial influence on psychosocial health [4]. Moreover, obesity is associated with increased mortality as the latest data of the Global Burden of Disease study in 2017 showed

that a BMI more than or equal to  $25 \text{ kg/m}^2$  is linked to 2.4 and 2.3 million deaths in females and males, respectively [5]. Obesity is a multifactorial disease, with various factors contributing to the increased weight, involving dietary mode, physical activity, sleep modalities, medications, besides genetic, epigenetic, and

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environmental factors. As societies consume processed foods high in saturated fats, sugars, and low in essential nutrients, it results in cardiovascular diseases, obesity, and metabolic disorders [6].

The presence of low-grade chronic inflammation, both systemically and in adipose tissue, is a major factor in the development of chronic diseases linked to obesity. The mechanisms behind obesity-related inflammation differ from those in traditional infection-induced inflammation and involve various signaling pathways. Dysfunctional adipocytes produce inflammatory cytokines and chemokines, which attract immune cells to adipose tissue, thereby intensifying the inflammatory response [7].

Oxidative stress plays a fundamental role in obesity. The mechanisms of ROS generation, including superoxide production from oxidative phosphorylation, NADPH oxidases, protein kinase C activation, glyceraldehyde auto-oxidation, and the polyol as well as hexosamine pathways, contribute to cellular and systemic oxidative stress associated with obesity [8].

Treatment guidelines for obesity emphasize that the suitable strategy for weight management must be multidisciplinary, comprising lifestyle alterations, behavioral medication, pharmacotherapy, and/or bariatric surgery. Besides that, the high cost of obesity medication needs to be assessed from an economic perspective. The reduced availability of antiobesity medications and increased withdrawals may be attributed to the rising prices over the past two decades [9,10]. Antiobesity drugs are recommended for patients with a BMI more than or equal to 30 kg/m<sup>2</sup> or if more than or equal to 27 kg/m<sup>2</sup> in the existence of one or more comorbidities. Nevertheless, the history of antiobesity drugs was characterized by the impairment of numerous ones after their extensive use in the market, consequential to critical side events, particularly cardiovascular effects, suicidality, risk for abuse, and dependence [11] and recently cancer [12]. Thus, the European Medicines Agency (EMA) and the Food Drug Administration (FDA) upgraded the guidelines of antiobesity drugs approval, indicating the significance of cardiovascular and central nervous system safety [13]. While essentially dubbed as an adjustable as well as forbidding health issue, inhibition and management approaches against obesity have gained little long-term success [14].

Flavonoids in plant foods have generated attention owing to increasing proof of their favorable impact

on human health. Dietary plants are the primary sources of flavones [15], and the antiobesity capability of flavonoids like rutin and quercetin is actually applicable and their modulatory influence is recorded on diminishing food intake, decreasing the absorption of nutrients, ameliorating adipogenesis and the life cycle of adipocytes, promotion of thermogenesis and energy exhaustion, and regulating intestinal microbiota and exhibiting antimicrobial activity [16]. Rutin is an important flavonoid merging one molecule of quercetin and rutinose each. It is well recognized to have numerous profitable health effects, involving anti-allergic, antiviral, anticancer, anti-inflammatory, and antioxidant activity [17]. The antiobesity effect of rutin stems from its ability to increase the mitochondrial number and the consumption of oxygen during brown adipogenesis *in vitro* [18]. It has been found that rutin activated brown adipose tissue (BAT) and white fat browning [17]. The functional BAT plays fundamental roles in the metabolism of systemic energy. So, the advancement of energy expenditure by increasing the mass and activity of BAT represents a promising approach in obesity treatment [19] as the elevation of the function of mitochondria and energy expenditure may synergistically participate to proper metabolic profiles versus obesity and metabolic disorders [17].

Despite numerous activity of rutin as an antiobesity agent, it is known as a fat-soluble molecule that is dissolved in organic solvents such as pyridine, methanol, and ethanol [20]. Likewise, it possesses imperfect durability as well as bioavailability and such rutin physicochemical characteristics are particularly attributed to its scarce water dissolution [21]. To solve poor solubility of drugs, a new approach like the loading of the drug into polymeric nanosystems or hybrid polymeric systems has been emerged [22]. Also, nanotechnology-based drug nanoformulation provides the usefulness of the augmented bioavailability of active ingredients [23]. Thus, the rutin nanoformularization for the different therapeutic purposes indicated the elevated aqueous solubility and enhanced efficiency in comparison with traditional delivery of rutin [24].

This research work was constructed to develop sodium alginate-based nanorutin formulation for enhancing its therapeutic impact. This study was focused on exploring the anti-obesogenic potential of this nanosized rutin compared with free rutin in an obese rat model and tracking the associated mode of action.

## Materials and methods

### Preparation and characterization of a polymeric nanoformulated rutin

#### Chemicals

Rutin, sodium alginate, and Tween 80 (T80) were provided by Sigma-Aldrich Co. (St Louis, Missouri, USA). Miglyol 812 N, a medium chain triacylglyceride mixture, was purchased from Sassol Germany GmbH (Brunsbüttel, Germany). All other chemicals used were of analytical classification and also acquired by Sigma-Aldrich Co.

#### Nanoformulation

In the current investigation, the nanoformulated rutin was prepared using a modified procedure based on Terjung *et al.* [25]. This involved homogenization with a homogenizer PRO400 PC (SN: 04-01198, USA) in a matrix containing sodium alginate and Tween 80 (T20). Specifically, 1 g of rutin was put in 100 ml solution comprising an oil phase mass fraction of 10 wt% and an aqueous phase mass fraction of 90 wt %, with the water phase containing 1 wt% Tween 80 and sodium alginate (3 g). The emulsion was formed by blending the solution in a high-pressure homogenizer for 30 min at 18 000 rpm, with the process conducted in an ice bath to keep the temperature at 35°C. Then, the rutin nanoformulation was stored at 4°C until used.

#### Transmission electron microscopy

A drop of the nanoformulated rutin was placed into a carbon-coated copper grid and allowed to adhere to the carbon substrate for 10 min. Excess fluid was eliminated using filter paper. Subsequently, a drop of phosphotungstic acid solution (2%) was applied, and the excess liquid was again separated using the tip of the filter paper. Samples were observed by operating at 160 kV [26] under transmission electron microscopy (Jeol JEM-2100, Tokyo, Japan).

#### Zeta potential determination

Zeta potential was analyzed by the light scattering technique [27] using a Zetasizer (Nano ZS, Malvern Instrument, Worcestershire, UK). For zeta potential measurements, the sample was diluted with bidistilled water (1 : 100 v/v) [28]. Following dilution, the sample was transferred to a 1 ml quartz cuvette and measured at room temperature. Three cycles were taken for the sample.

#### Biological experimentation

##### Animal caring and housing conditions

The rats were housed in facilities that were authorized and complied with established regulations on ordering, equipment, and maintenance of the experimental

biological clinics. Fifty adult female Wistar strain albino rats, weighing 130±10 g at 90-day old, were enclosed in this work. These rats were provided by the Animal Care Unit of the National Research Centre, Giza, Egypt. They were maintained in polypropylene cages under standard environmental conditions, including a temperature of 24±1°C, a cycle of 12-h light/12-h dark, and humidity of 60±5%, and provided with *ad libitum* approach to tap water as well as standard rodent diet. The rats were permitted to acclimatize to these circumstances for 2 weeks before the experiment initiation.

#### Ethical statement

The research protocol adhered to the standards and universal ethical concepts of animal experiments. This research was performed following the principles and guidelines established by the Ethics Committee for Medical Research of the National Research Centre, Egypt (approval no: 18/175).

#### Research protocol

Following the adaptation period, 10 rats were fed a standard rodent chow with carbohydrate (40%), fat (3.8%), protein (26.5%), and crude fiber (4.5%) in 100 g of chow, as described by Buettner *et al.* [29] for 18 weeks and assigned as the control group. The remaining 40 rats were fed with a high-fat diet (HFD) comprising carbohydrate (45.7%), fat (38.8%), and protein (15.5%) by calories for 12 weeks, according to Soliman *et al.* [30]. The diet was prepared as recorded by Levin and Dunn-Meynell [31] with slight modification as follows: 72% powdered rat feed, 18% milk powder, 5% corn oil, and 5% ghee. All ingredients of the HFD were thoroughly mixed and baked in an oven at 65°C overnight.

The obese rats were classified to four groups (10 rats/group). Group 1: untreated obese group; group 2: obese rats treated orally with 50 mg/kg body weight of chitocal (Chitocal capsules, which contain 500 mg chitosan, 100 mg ascorbic acid, and 50 mg *Gymnema sylvestre* leaves powder extract, dBK Pharma S.A.E.) [32], administered daily for 6 weeks; group 3: obese rats treated orally with of 20 mg/kg body weight free rutin suspended in 1 ml distilled water/rat [33], administered daily for 6 weeks; group 4: obese rats treated orally with 20 mg/kg body weight rutin nanoformulation dissolved in 1 ml distilled water/rat, administered daily for 6 weeks.

#### Anthropometric parameters

At the end of the experimental period (18 weeks), the abdominal circumference (AC), measured immediately

anterior to the forefoot and the thoracic circumference (TC), measured immediately behind the foreleg, along with the body length (nose–anus length or nose-to-anus), were estimated in rats under anesthesia. Body weight and body length measurements were recorded for the calculation of BMI [34]:

$$\text{BMI (g/cm}^2\text{)} = (\text{body weight (g)/length}^2 \text{ (cm}^2\text{)})$$

Also, Lee index was estimated according to the following equation:

Lee index = cube root of body weight (g)/nose-to-anus length (cm).

Both BMI and Lee index values are indicators for the existence of obesity.

#### Blood sampling

Following completion of the animal treatment regimen, the rats were fasted overnight (12–14 h), and blood specimens were withdrawn from the tail vein into clean, dry centrifuge tubes. These tubes were then let to coagulate at room temperature for 45 min to get sera. Serum specimens were collected by centrifugation at 4° for 15 min at 1800g using a cooling centrifuge. Collected serum was then cryopreserved at –20°C till further biochemical analyses. Subsequently, after blood collection, rats were killed by cervical dislocation under full anesthesia induced by intraperitoneal injection with 90 mg/kg of ketamine and 5 mg/kg xylazine [35].

#### Biochemical analyses

Total cholesterol (Chol), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glucose concentration were determined in serum by colorimetric methods with kits procured by Reactivos GPL (Barcelona, Spain). The methods used were based on Meattini *et al.* [36] for Chol; Bucolo and David [37] for TG; Naito [38] for HDL and LDL as well as Young and McEneny [39] for glucose.

Quantitative estimation of serum insulin concentration was carried out by an ELIZA kit obtained from Immunoscopoc Co. CA (the Netherlands), following the method outlined by the National Committee for Clinical Laboratory Standards procedures for the collection of diagnostic blood specimens by venipuncture [40]. The homeostasis model assessment of basal insulin resistance was calculated to estimate the index from the product of the fasting concentration of serum glucose (mmol/l) and serum insulin (mU/ml) divided by 22.5, as described by

Duncan *et al.* [41]. The quantitative evaluation of serum toll-like receptor 4 (TLR4) level was performed according to manufacturer's manual using an ELIZA kit purchased from Sunlong Biotech. Co. Ltd (Hangzhou, China). Serum nuclear factor kappa B (NF-κB) levels were estimated using an ELIZA kit purchased from SinoGeneClon Biotech. Co. Ltd (Hangzhou, China) according to manufacturer's protocol. Furthermore, quantitative assessment of serum leptin, adiponectin (ADP), and spexin (SPX) levels were carried out using ELIZA kits provided by SunRed Biotechnology Co. Ltd (Shanghai, China), following the operating instructions.

#### Statistical procedure

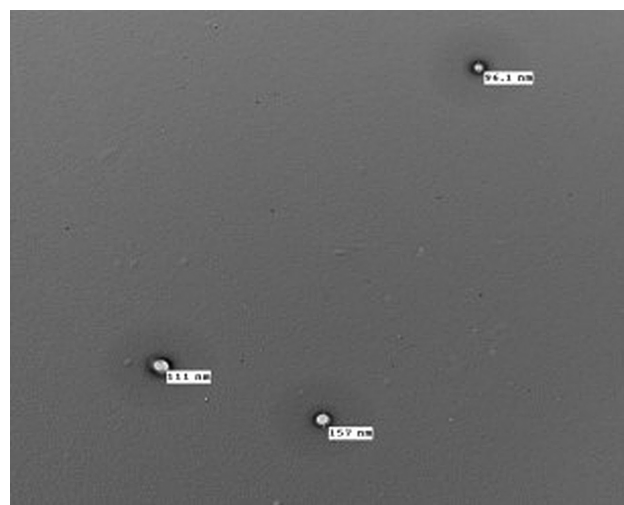
The obtained data were presented as arithmetic means with their standard errors (mean±SE). Statistical analysis was performed using one-way analysis of variance with the Statistical Package for the Social Sciences program, version 20 (Chicago, USA). Post-hoc analysis was conducted using the least significant difference method to compare significance between groups [42]. A significance level of *P* value less than 0.05 was estimated statistically significant.

## Results and discussion

#### Transmission electron microscopy observations

The shape of the formulated rutin nanoparticles is illustrated in Fig. 1. The observed structures consist of single spherical units with diameters ranging between 96.1 and 157 nm, indicating a nanoscale size. The external surface exhibited a smooth and regular appearance, suggesting that sodium alginate gel polymer creates a continuous film around rutin.

Figure 1



Transmission electron microscopy (TEM) morphology and size of the formulated rutin nanoparticles incorporated in sodium alginate gel.

### Zeta potential findings

The rutin nanoformulation zeta potential is recorded in Fig. 2, which showed a negative zeta potential at  $-16.6$  mV.

Rutin, a bioflavonoid commonly found in citrus fruits and leafy vegetables, possesses a vast range of biological practices. Some studies have highlighted its pharmacological advantages for the treatment of different chronic diseases such as cancer, diabetes, hypertension, and hyperlipidemia [43]. The common obstacle accompanied with rutin is its poor water solubility [44], which is responsible for its poor bioavailability. Improving its bioavailability will introduce this optimistic botanical product to the primacy of medication for the treatment of different chronic diseases. For poorly water-soluble agents, particle size often performs an essential impact in accomplishing sufficient oral bioavailability. Reduction of particles of poorly water-soluble drugs to the nanosize range is a beneficial strategy to enhance saturation solubility, dissolution rate, and consequently to improve oral bioavailability. During the last decades, nanoparticle-based formulations have protruded as a solution for improving the bioavailability of inherent therapeutic candidates [45].

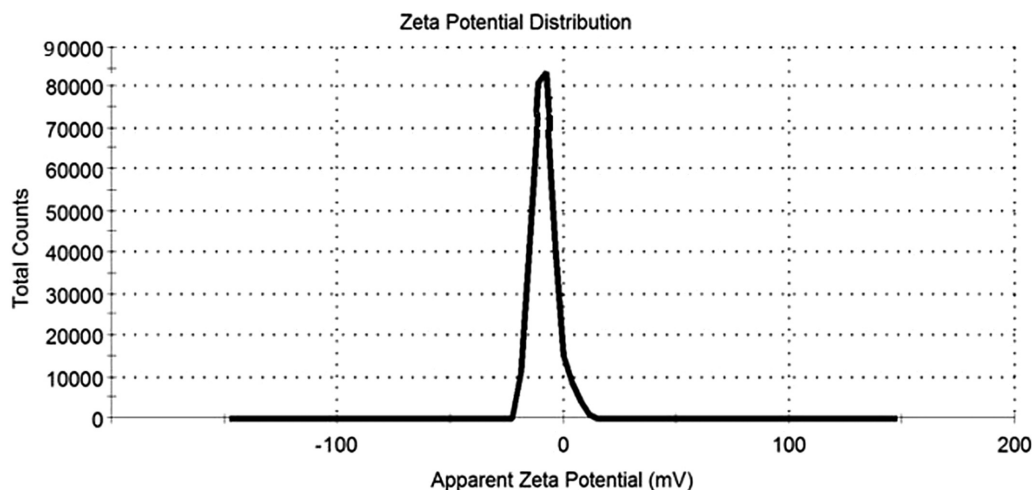
Sodium alginate, a naturally occurring polyanionic polysaccharide derived from marine algae, is soluble in an aqueous medium. It consists of two  $\beta$ -D mannuronic acid (M), hexuronic acid residues, and  $\alpha$ -L-guluronic acid (G), linked together by 1,4-glycosidic bonds. Sodium alginate possesses degradation properties *in vivo*. Its common properties, including controlled minimum toxicity, biocompatibility, gelation, and relatively low cost,

reserve it for use in drug delivery systems and various biological applications [46]. In the current investigation, rutin was incorporated in sodium alginate gel to form rutin nanoformulation and the obtained data clarified that the developed rutin nanoparticles were made up of single spherical units with a diameter that ranged between 96.1 and 157 nm and exhibited negative zeta potential at  $-16.6$  mV. These results fit with those of Gagliardi *et al.* [47], who recorded that poly(lactide-coglycolide) nanoparticles containing rutin are characterized by a smooth spherical shape and negative zeta potential. Furthermore, Ali *et al.* [45] registered that the designed chitosan–alginate nanoparticles of rutin exhibited an average particle size of  $141.29 \pm 5.11$  nm. Electron microscopy images showed all nanoparticles with a spherical shape and appearance as a nonaggregated smooth.

### Effectiveness of chitocal, free rutin, and nanorutin administration on anthropometric parameters of obese rats

Table 1 presents the influence of treatment with chitocal, free rutin, and rutin nanoformulation on anthropometric measurements of obese rats. Prolonged feeding of rats with HFD motivated significant ( $P < 0.05$ ) rise in body weight, body length, TC, AC, BMI, and Lee index compared with the control group. However, treatment of obese rats with the proposed treatments provoked significant ( $P < 0.05$ ) drop in body weight, TC, BMI, and Lee index compared with the untreated obese group. Furthermore, treatment of obese rats with chitocal or rutin nanoformulation elicited a significant ( $P < 0.05$ ) decline in body length and AC when compared with those left without treatment.

Figure 2



Zeta potential of rutin nanoformulation.

**Table 1 Effectiveness of chitocal, free rutin, and nanorutin administration on anthropometric parameters of obese rats**

| Parameters groups | Body weight (g)        | Body length (cm)      | TC (cm)               | AC (cm)               | BMI (g/cm <sup>2</sup> ) | Lee Index                |
|-------------------|------------------------|-----------------------|-----------------------|-----------------------|--------------------------|--------------------------|
| Control group     | 120.1±3.1              | 20.1±0.3              | 11.1±0.2              | 12.4±0.2              | 0.29±0.01                | 0.246±0.004              |
| Obese group       | 283.8±3.3 <sup>a</sup> | 21.1±0.1 <sup>a</sup> | 13.2±0.4 <sup>a</sup> | 14.9±0.4 <sup>a</sup> | 0.72±0.02 <sup>a</sup>   | 0.341±0.01 <sup>a</sup>  |
| Ob+chitocal       | 175.5±3.4 <sup>b</sup> | 20.2±0.2 <sup>b</sup> | 11.3±0.2 <sup>b</sup> | 13.6±0.4 <sup>b</sup> | 0.43±0.01 <sup>b</sup>   | 0.275±0.004 <sup>b</sup> |
| Ob+free rutin     | 181.5±4.1 <sup>b</sup> | 20.5±0.2              | 11.7±0.4 <sup>b</sup> | 14.2±0.4              | 0.45±0.01 <sup>b</sup>   | 0.290±0.004 <sup>b</sup> |
| Ob+nanorutin      | 180.5±1.9 <sup>b</sup> | 20.3±0.3 <sup>b</sup> | 11.4±0.2 <sup>b</sup> | 13.9±0.3 <sup>b</sup> | 0.44±0.01 <sup>b</sup>   | 0.287±0.004 <sup>b</sup> |

Data were expressed as mean±SE of 10 rats/group. AC, abdominal circumference; TC, thoracic circumference. <sup>a</sup>Significant change at *P* value less than 0.05 as compared to the control group. <sup>b</sup>Significant change at *P* value less than 0.05 as compared to the obese group.

However, treatment of obese rats with free rutin or rutin nanoformulation caused insignificant ( $P>0.05$ ) increase in the measured anthropometric parameters in comparison with obese rats treated with chitocal. Also, obese rats treated with rutin nanoformulation showed an insignificant ( $P>0.05$ ) decrease in these parameters relative to those treated with free rutin.

The data of the current study documented successful induction of obesity in experimental rats after being fed on HFD for 12 weeks, which is indicated by the increase in body weight, body length, TC, AC, BMI, and Lee index. These findings are in harmony with those of Pérez-Corredor *et al.* [48] and could be explained by the high deposition of fat due to the HFD regime [49].

Chitocal is a mixture of chitosan, ascorbic acid, and *G. sylvestre*. It consists of high-density chitosan, which is a natural polysaccharide containing copolymers of glucosamine and N-acetyl glucosamine, obtained through fractional deacetylation of chitin, from crustacean shells [50]. The present data revealed that treatment of obese rats with chitocal reduces body weight, length, TC, AC, BMI, and Lee index. These findings are in line with other reports published by Ahmed *et al.* [51]. Also, Bukhari *et al.* [52] found that rats treated with chitocal display a highly significant reduction in body weight gain versus the control positive counterparts. Furthermore, Safavi *et al.* [53] recorded that using antiobesity medication, such as chitocal, resulted in significantly higher mean percentage decrease in baseline weight than nonusers.

Reduction in measured parameters of anthropometry is demonstrated among the treated experimental groups with free rutin or rutin nanoformulation contrary to the untreated obese group indicating the antiobesity impact of the proposed medications. This could be referred to their higher content of the antiobesity major metabolites. Anti-inflammatory, antioxidant, antiplatelet, and antihypertensive characteristics of rutin have been established *in vitro* and *in vivo* [54,55]. By mitigating inflammation, lipogenesis,

oxidative stress, and regulating glucose as well as lipid metabolism in both liver and adipose tissue [56–59] rutin could refine obesity and its attributes. It has been reported that feeding of rats with rutin shows a significant decline in body weight without altering food intake [58,60]. Moreover, rutin consumption reduced adipose size, adipogenic gene expression of sterol regulatory element-binding protein 1c (SREBP-1c), peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), AP2 adaptor complex, and AMP-activated protein kinase activity of epididymal adipose tissue, evidencing the rutin antiobesity characteristics [61]. Moreover, it has been reported that rutin administration reduces HFD-induced weight gain and adipose tissue mass, associated with enhanced mitochondrial DNA and genes expression contributed in muscular mitochondrial biogenesis and function. These data suggest that the antiobesity properties of rutin may be linked to its ability to activate muscle mitochondria [62].

#### **Effectiveness of chitocal, free rutin, and nanorutin administration on serum lipid profile of obese rats**

The data illustrated in Table 2 shows the impact of treatment with chitocal, free rutin, and rutin nanoformulation on serum lipid profile of obese rats. Prolonged feeding of rats with HFD triggered significant ( $P<0.05$ ) elevation in serum Chol, TG, and LDL levels, accompanied with significant ( $P<0.05$ ) depletion in serum HDL level compared with control rats. However, treatment of obese rats with chitocal, free rutin, or rutin nanoformulation caused a significant ( $P<0.05$ ) drop in serum Chol, TG, and LDL levels when compared with untreated obese rats. Moreover, treatment of obese rats with chitocal or rutin nanoformulation emerged significant ( $P<0.05$ ) enhancement in serum HDL level versus those left without treatment. However, in comparison with obese rats treated with chitocal, the rats treated with free rutin or rutin nanoformulation showed an insignificant ( $P>0.05$ ) increase in serum Chol, TG, and LDL levels along with an insignificant ( $P>0.05$ ) decrease in serum HDL level. Furthermore,

**Table 2 Effectiveness of chitocal, free rutin, and nanorutin administration on serum lipid profile of obese rats**

| Parameters groups | Chol (mg/dl)           | TG (mg/dl)             | HDL (mg/dl)           | LDL (mg/dl)           |
|-------------------|------------------------|------------------------|-----------------------|-----------------------|
| Control group     | 72.8±4.5               | 75.4±2.3               | 38.6±1.3              | 26.3±2.4              |
| Obese group       | 135.9±3.3 <sup>a</sup> | 122.4±4.5 <sup>a</sup> | 27.7±0.6 <sup>a</sup> | 83.7±2.6 <sup>a</sup> |
| Ob + chitocal     | 79.8±3.1 <sup>b</sup>  | 86.2±4.3 <sup>b</sup>  | 35.5±1.4 <sup>b</sup> | 27.9±2.2 <sup>b</sup> |
| Ob + free rutin   | 93.9±6.7 <sup>b</sup>  | 92.9±2.9 <sup>b</sup>  | 31.8±2.3              | 29.2±2.2 <sup>b</sup> |
| Ob+ nanorutin     | 88.2±6.6 <sup>b</sup>  | 89.7±4.5 <sup>b</sup>  | 32.4±1.6 <sup>b</sup> | 28.1±1.6 <sup>b</sup> |

Data were expressed as mean±SE of 10 rats/group. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

<sup>a</sup>Significant change at *P* value less than 0.05 as compared to the control group. <sup>b</sup>Significant change at *P* value less than 0.05 as compared to the obese group.

the group of obese rats treated with rutin nanoformulation triggered insignificant ( $P>0.05$ ) decline in serum Chol, TG, and LDL levels and an insignificant ( $P>0.05$ ) rise in serum HDL level in comparison with that treated with free rutin.

Table 3 demonstrates the influence of treatment with chitocal, free rutin, and rutin nanoformulation on serum glucose, insulin, and insulin resistance values of obese rats. Supplementation with HFD brought about a significant ( $P<0.05$ ) rise in serum glucose, insulin levels, and insulin resistance values relative to control rats. However, treatment of obese rats with the offered treatments created a significant ( $P<0.05$ ) decline in serum glucose, insulin, and insulin resistance values compared with untreated obese rats. Also, treatment of obese rats with free rutin or rutin nanoformulation elicited an insignificant ( $P>0.05$ ) increase in serum glucose, insulin, and insulin resistance values when compared with obese rats treated with chitocal. However, treatment of obese rats with rutin nanoformulation caused an insignificant ( $P>0.05$ ) drop in serum glucose, insulin, and insulin resistance values in comparison with those treated with free rutin.

In this work, rats that administered an HFD manifested high serum Chol, TG, and LDL levels parallel low serum HDL level. Also, extended administration of HFD to rats triggered augmentation of serum glucose and insulin levels as

well as insulin resistance value. These findings are consistent with Sowmya and Ananthi [63], Godea Lupei *et al.* [64], and Faraji *et al.* [65]. The deleterious effects of HFD on the lipid serum profile could be attributed to the induced impairment in lipid metabolism that is indicated by an increment of cholesterol, TGs, LDL, and very low density lipoprotein (VLDL) levels [63]. Rezvani-Kamran *et al.* [66] registered that the levels of Chol and LDL are increased following the suppression of receptor-dependent LDL transport in the liver. Furthermore, Videla *et al.* [67] cited that lipid accumulation initially produces insulin resistance and visceral obesity, which trigger the synthesis of fatty acids from glucose and suppress their  $\beta$ -oxidation resulting in overflowing of fatty acids leading to an increase in TG synthesis. Also, the dysregulation of adipokines by obesity and inflammation caused systemic insulin resistance [68].

Supplementation of HFD-fed rats with chitocal generated a reduction of serum Chol, TG, and LDL levels paralleled by an elevation of serum HDL level. Moreover, treatment of HFD-fed rats with chitocal motivated a decline in serum glucose and insulin levels as well as insulin resistance value. These findings are greatly supported by those of Bukhari *et al.* [52]. It has been reported that chitocal triggered hypolipidemic effect by the suppression of fatty acid absorption in rats [69]. Furthermore, the strong positive charge carried by the chitocal molecule (amino groups)

**Table 3 Effectiveness of chitocal, free rutin, and nanorutin administration on serum glucose, insulin, and insulin resistance values of obese rats**

| Parameters groups | Glucose (mg/dl)        | INS (mIU/ml)          | Insulin resistance value |
|-------------------|------------------------|-----------------------|--------------------------|
| Control group     | 64.9±2.1               | 11.2±0.1              | 1.8±0.1                  |
| Obese group       | 166.3±3.8 <sup>a</sup> | 25.6±1.4 <sup>a</sup> | 10.1±0.2 <sup>a</sup>    |
| Ob+chitocal       | 66.8±4.0 <sup>b</sup>  | 14.4±1.0 <sup>b</sup> | 2.3±0.2 <sup>b</sup>     |
| Ob+free rutin     | 75.2±3.8 <sup>b</sup>  | 15.4±0.6 <sup>b</sup> | 2.8±0.2 <sup>b</sup>     |
| Ob+nanorutin      | 69.4±2.9 <sup>b</sup>  | 14.7±0.9 <sup>b</sup> | 2.6±0.2 <sup>b</sup>     |

Data were represented as mean±SE of 10 rats/group. <sup>a</sup>Significant change at *P* value less than 0.05 as compared to the control group.

<sup>b</sup>Significant change at *P* value less than 0.05 as compared to the obese group.

enables it to bind with the negatively charged compounds such as lipids [70]. The possible interpretation for enhancing lipid metabolism by chitocal is related to the ability of chitocal to promote the peripheral tissue Chol transport to the liver and thereby reduce Chol in the blood [71]. Moreover, the composition of chitocal regarding the polysaccharide contents is believed to inhibit fat and Chol absorption [72]. Meanwhile, the possible underlying mechanism for increasing serum HDL by chitocal may be owing to the increase in lecithin activity [71]. However, the mechanism behind the reduction of LDL-Chol by chitocal may be related to the oxidation of LDL [69]. However, the *G. sylvestre* (present in chitocal) has been found to decrease glucose and some nutrient absorption in the gastrointestinal tract [73]. Gymnemic acid (the active compound of *G. sylvestre*) has similar configuration of glucose molecules but with a larger molecular weight giving it a high affinity to bind with glucose receptors and competitively inhibits the binding of glucose to its receptor in the small intestine [74]. Moreover, it has been cited that chitosan (present in chitocal) elicited an improvement of insulin resistance and impaired glucose tolerance in high-fructose-diet-fed rats [75]. Muanprasat and Chatsudthipong [76] registered that chitosan treatment upregulates GLUT4 gene expression in the soleus muscle and adipose tissue in type 2 diabetes mellitus-bearing rats leading to the enhancement of muscle glucose uptake. Furthermore, Bai *et al.* [77] stated that chitosan improves the impaired glucolipid metabolism in HFD-fed mice through suppressing the upregulated pro-inflammatory cytokines.

The data from the current study showed that supplementation of HFD-fed rats with free rutin or rutin nanoformulation induces a depletion of serum Chol, TG, and LDL levels paralleled by the promotion of serum HDL level. These outcomes are consistent with those of Livingston Raja *et al.* [78], who observed that rutin shows significant lipid-lowering effectiveness on Triton WR-1339-induced hyperlipidemia in rats. The lipid-lowering effects of polyphenols are attributed to multiple mechanisms including the suppression of lipid synthesis and absorption, inhibition of hepatic production of apolipoprotein B and foam cell formation, transcription factor PPAR $\gamma$  stimulation, LDL receptor upregulation, enhancement of HDL, and stimulation of fatty acids metabolizing enzymes such as lipoprotein lipase (LPL), 3-hydroxy-3-methylglutaryl coenzyme A, cholesterol-7 $\alpha$ -hydroxylase, and hepatic lipase. It has been found that rutin increases plasma HDL Chol and decreases

LDL and VLDL Chol. This could be explained by the enhancement of plasma lecithin cholesterol acyltransferase enzyme and LPL enzyme, which is an essential enzyme for upgrading lipoprotein metabolism and accelerating the maturation of HDL elements [79,80]. The lecithin cholesterol acyltransferase enzyme mode of action is related to the conversion of free Chol to cholesteryl ester, which is further sequestered into the core of lipoprotein particle, facilitating the formation of new HDL. Subsequently, HDL can undergo transformed back into the liver as intermediate density lipoprotein and VLDL [81]. Furthermore, the decreased TG levels due to rutin or rutin nanoformulation administration could be attributed to the intensified impact of the endothelium LPL enzyme. The LPL functions by hydrolyzing TG into fatty acids and inhibiting the reformation of fatty acids into TG [82].

Prior researches indicated that rutin has a significant impact on reducing glucose in the blood [83,84]. In line with these studies, our findings showed that treatment of HFD-fed rats with free rutin or rutin nanoformulation caused a detraction of serum glucose and insulin levels and insulin resistance value. Huang *et al.* [85] registered that rutin reduces blood glucose level and generation of advanced glycation end products that aggravate insulin resistance and oxidative stress response. When insulin binds to cell membrane insulin receptor (IR), it triggers the phosphorylation of the receptor itself and the phosphorylation of the tyrosine site of IRS-2 protein. Activation of IRS-2 by phosphorylation leads to the subsequent phosphorylation of downstream signaling factors, including Akt and glycogen synthase kinase-3 beta (GSK-3 $\beta$ ), and phosphoinositide 3-kinase. GSK-3 $\beta$  phosphorylates glycogen synthase (GS) to suppress its activity in a non-phosphorylated state. However, phosphorylation of GSK-3 $\beta$  at Ser9 reduces GS phosphorylation, thereby increasing its activity and promoting glycogen synthesis, ultimately leading to depleted blood glucose levels [86,87]. Also, Liang *et al.* [88] elucidated that rutin has a favorable function in insulin signal transduction pathway, efficiently mitigating insulin resistance, encouraging uptake and utilization of glucose.

#### **Effectiveness of chitocal, free rutin, and nanorutin administration on inflammatory markers of obese rats**

The results in Table 4 describe the impact of treatment with chitocal, free rutin, and nanorutin on serum TLR4 and NF- $\kappa$ B levels of obese rats. Extended



feeding of rats with HFD elicited significant ( $P < 0.05$ ) augmentation in serum TLR4 as well as NF- $\kappa$ B levels contrary to control rats. On the opposite side, treatment of obese rats with chitocal, free rutin, and nanorutin triggered significant ( $P < 0.05$ ) depletion in serum TLR4 and NF- $\kappa$ B levels versus the untreated obese rats. Obese rats treated with free or nanorutin showed insignificant ( $P > 0.05$ ) elevation in serum TLR4 and NF- $\kappa$ B levels relative to those treated with chitocal. Moreover, obese rats treated with nanorutin exhibited an insignificant ( $P > 0.05$ ) drop in serum TLR4 and NF- $\kappa$ B levels in comparison with those treated with free rutin.

The data of the current study revealed that feeding rats with HFD for a long duration displayed an elevation in serum TLR4 and NF- $\kappa$ B levels. TLRs, a family of transmembrane proteins, played a fundamental role in the innate immune system [89]. They exhibit differential expression across various cell compartments. TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11 are predominantly expressed on the cellular membrane, whereas TLR3, TLR7, TLR8, and TLR9 are primarily found in intracellular compartments like endosomes and the endoplasmic reticulum [90]. Specifically, TLR4 is expressed in innate immune cells such as monocytes, macrophages, dendritic cells, and in other cell types including enterocytes, adipocytes, and muscle cells [91]. Notably, the direct release of free fatty acids (FFA) and adipokines from visceral adipose tissue to the liver can trigger immune responses, leading to the secretion of inflammatory compounds [92]. The possible mechanism through which FFAs, and particularly dietary saturated fatty acids, can intercede adipose tissue dysfunctions participating to the onset of inflammation induces the TLR4 [93]. Indeed, the adipocyte secretion profile alterations, induce the recruitment and activation of immune cells [94]. Particularly, in obese individuals, adipose

tissue macrophages relocate from the anti-inflammatory profile (like that observed in normal weight individuals) toward a pro-inflammatory phenotype [95], producing a change in the output/stimulation of key factors that aggravate local and systemic inflammation [96], such as TNF $\alpha$ , IL-6, IL-1 $\beta$ , TLR4, and NF- $\kappa$ B, that may intensify the inflammatory status [97]. It has been supposed that FFAs can join and activate TLR4; thus, the increased plasma level of FFAs demonstrated in obesity could stimulate TLR4. A recent study of McKernan *et al.* [98] denoted that stimulation of TLR4 can also induce inflammatory responses by impairing adipogenesis, which subsequently leads to dysfunctions in adipocytes and resident immune cells.

Obesity-associated inflammation arises from changes in the communication between adipocytes and macrophages as a result of increased infiltration of macrophages into adipose tissue, activation of pro-inflammatory pathways, alterations in adipokine secretion, and upregulation of expression and release of various inflammatory cytokines [7]. The TLR4 pathway upregulates the pro-inflammatory cytokine expression, like TNF- $\alpha$ , IL-1, and IL-6, by stimulating the transcription factors NF- $\kappa$ B and activator protein-1 [99]. Therefore, the expression of pro-inflammatory cytokines is regulated by the stimulation of the transcription factor NF- $\kappa$ B. In its inactive form, NF- $\kappa$ B binds the NF- $\kappa$ B light polypeptide gene enhancer in B-cells inhibitor alpha (I $\kappa$ B $\alpha$ ) in the cytoplasm. The inhibitor of  $\kappa$ B kinase (IKK) complex, composed of subunits IKK $\alpha$  and IKK $\beta$ , regulates the activity of I $\kappa$ B $\alpha$  [100]. FFAs can stimulate the IKK kinase complex, leading to the proteasomal degradation of I $\kappa$ B $\alpha$  and subsequent translocation of NF- $\kappa$ B into the nucleus, where it induces the expression of various inflammatory mediator genes. Obese individuals often exhibit elevated activation of the NF- $\kappa$ B pathway, likely contributing to the increased release of pro-inflammatory cytokines [101].

Treatment of obese rats with chitocal instigated decline in both serum TLR4 and NF- $\kappa$ B levels. Chitosan has been demonstrated to have specific immunomodulatory impact. It can polarize the balance of cytokines toward Th1 cytokines, decreases the generation of the pre-inflammatory cytokines IL-6 and TNF- $\alpha$ , downregulates the expression of CD44 and TLR4 receptor, and inhibits the proliferation of T cells [102]. The study of Xiao *et al.* [103] indicated that chitosan supplementation decreases the expression of TLR4 mRNA and Xiao *et al.* [104] extended these

**Table 4 Effectiveness of chitocal, free rutin, and nanorutin administration on serum toll-like receptor 4 and nuclear factor kappa B levels of obese rats**

| Parameters groups | TLR4 (pg/ml)                    | NF- $\kappa$ B (ng/l)         |
|-------------------|---------------------------------|-------------------------------|
| Control group     | 1631.5 $\pm$ 134.9              | 314.9 $\pm$ 10.4              |
| Obese group       | 3593.5 $\pm$ 223.8 <sup>a</sup> | 596.6 $\pm$ 15.4 <sup>a</sup> |
| Ob+chitocal       | 1857.0 $\pm$ 84.0 <sup>b</sup>  | 330.6 $\pm$ 19.9 <sup>b</sup> |
| Ob+free rutin     | 2204.8 $\pm$ 141.1 <sup>b</sup> | 364.9 $\pm$ 22.9 <sup>b</sup> |
| Ob+nanorutin      | 2133.0 $\pm$ 86.2 <sup>b</sup>  | 341.9 $\pm$ 13.9 <sup>b</sup> |

Data were expressed as mean $\pm$ SE of 10 rats/group. NF- $\kappa$ B, nuclear factor kappa B; TLR4, toll-like receptor 4. <sup>a</sup>Significant change at  $P$  value less than 0.05 as compared to the control group. <sup>b</sup>Significant change at  $P$  value less than 0.05 as compared to the obese group.

findings by demonstrating that chitosan supplementation decreases TLR4 protein expression in weaned pigs challenged with enterotoxigenic *Escherichia coli*.

Chitosan or its derivatives have been found to play an important role in mitigating oxidative stress inflammatory reaction by the suppression of NF- $\kappa$ B signaling pathways under an inflammatory stimulus [105]. The more recent study of Mohyuddin *et al.* [106] reported the inhibitory effect of chitosan on mRNA expression level of NF- $\kappa$ B in rats under heat stress. Furthermore, TLR4 stimulates NF- $\kappa$ B through mobilization of the adaptor proteins toll/interleukin 1 receptor domain-containing adaptor protein inducing IFN- $\beta$  (TRIF) and myeloid differentiation primary response gene 88 (MyD88), which results in the successive induction of NF- $\kappa$ B signaling genes, like TNF- $\alpha$ , IL-6 and IL-1 [107]. Thus, one can postulate that the inhibitory effect of chitosan on TLR4 as shown in the current approach could result in the reduction of NF- $\kappa$ B level.

Treatment of obese rats with free rutin or rutin nanoformulation lessened serum TLR4 and NF- $\kappa$ B levels. It has been cited that rutin acts as a TLR4 inhibitor in the activated hepatic stellate cells (HSCs) [108]. The anti-inflammatory mechanisms of rutin were further confirmed through its effects on TLR4, TNF receptor-associated factor 6 (TRAF6), MYD88, and NF- $\kappa$ B signaling pathways. The regulatory impact of rutin on TLR4, MyD88, and TRAF6 protein expression was similar to the trend observed in gene expression levels. Rutin has been found to exhibit anti-inflammatory influence by suppressing MyD88, TRAF6, and TLR4 genes as well as protein expression in LPS-induced RAW 264.7 cells [109].

It has been demonstrated that the suppression of TLR4 pathway activation leads to the inhibition of NF- $\kappa$ B activation [110]. Moreover, rutin can inhibit the generation of TNF- $\alpha$  and activation of NF- $\kappa$ B induced by LPS, playing a crucial role in the treatment of vascular inflammatory diseases [111]. Rutin displayed significant attenuating effect in cyclophosphamide-induced inflammation by downregulating the levels of inflammatory mediators, like IL-6 and TNF- $\alpha$ , and the expression levels of NF- $\kappa$ B, p38-mitogen-activated protein kinase (MAPK), COX-2, and iNOS [112]. Furthermore, rutin could ameliorate the inflammatory reaction by repressing the phosphorylation of I $\kappa$ B and p65 in LPS-induced RAW 264.7 cells [109].

#### Effectiveness of chitocal, free rutin, and nanorutin administration on serum leptin, adiponectin, and spexin levels of obese rats

The data presented in Table 5 elucidate the impact of treatment with chitocal, free rutin, and nanorutin on serum leptin, ADP, and SPX levels of obese rats. Feeding of rats with HFD for a long period of time promoted significant ( $P<0.05$ ) increase in serum leptin along with significant ( $P<0.05$ ) reduction in ADP and SPX serum levels compared with control rats. However, the recommended treatments brought about a significant ( $P<0.05$ ) decline in leptin serum level along with a significant ( $P<0.05$ ) rise in ADP and SPX serum levels of obese rats relative to their untreated counterparts. Meanwhile, treatment of obese rats with free or nanorutin triggered insignificant ( $P>0.05$ ) increase in serum leptin levels associated with an insignificant ( $P>0.05$ ) drop in serum ADP and SPX levels when compared with obese rats treated with chitocal. However, obese rats treated with nanorutin exhibited an insignificant ( $P>0.05$ ) decline in serum leptin level and an insignificant ( $P>0.05$ ) increase in serum ADP and SPX levels in comparison with those treated with free rutin.

Feeding rats with HFD for an extensive time elevated serum leptin levels and reduced serum ADP and SPX levels significantly. Leptin is a hormone generated by the adipose tissue, mainly by the white adipose tissue; it consists of 167 amino acids. The level of circulating leptin in the body is proportional to the amount of fat of the same body [113]. This means that leptin level is positively correlated with fat mass, being elevated in obesity [114].

ADP is synthesized as a 28–30 kD monomer, which assembles into homooligomers of varying molecular weights: low molecular weight trimeric form, medium molecular weight hexameric form, and high molecular weight oligomers [115]. Both total ADP and high molecular weight ADP oligomers are negatively

**Table 5 Effectiveness of chitocal, free rutin, and nanorutin on serum leptin, adiponectin and spexin levels of obese rats**

| Parameters groups | Leptin (pg/ml)                | ADP (mg/l)                 | SPX (pg/ml)                  |
|-------------------|-------------------------------|----------------------------|------------------------------|
| Control group     | 130.0 $\pm$ 9.8               | 7.5 $\pm$ 0.1              | 208.2 $\pm$ 2.1              |
| Obese group       | 214.7 $\pm$ 14.7 <sup>a</sup> | 3.7 $\pm$ 0.3 <sup>a</sup> | 136.7 $\pm$ 6.5 <sup>a</sup> |
| Ob+chitocal       | 158.8 $\pm$ 9.4 <sup>b</sup>  | 6.6 $\pm$ 0.3 <sup>b</sup> | 179.7 $\pm$ 3.5 <sup>b</sup> |
| Ob+free rutin     | 176.4 $\pm$ 6.9 <sup>b</sup>  | 6.1 $\pm$ 0.4 <sup>b</sup> | 167.7 $\pm$ 4.2 <sup>b</sup> |
| Ob+nanorutin      | 165.0 $\pm$ 9.8 <sup>b</sup>  | 6.3 $\pm$ 0.1 <sup>b</sup> | 176.4 $\pm$ 6.5 <sup>b</sup> |

Data were represented as mean $\pm$ SE of 10 rats/group. ADP, adiponectin; SPX, spexin. <sup>a</sup>Significant change at  $P$  value less than 0.05 as compared to the control group. <sup>b</sup>Significant change at  $P$  value less than 0.05 as compared to the obese group.

correlated with BMI, glucose, insulin, and TG levels, as well as the degree of insulin resistance and visceral fat deposition [116]. The process by which adipose tissue expands through increases in cell size (hypertrophy) and/or cell count (hyperplasia) regulates the synthesis and production of ADP. Drolet *et al.* [117] observed a negative correlation between the mean diameter of adipocytes and ADP production. It has been found that *adipoR1* and *adipoR2* expression is significantly decreased in type 2 diabetes mellitus and obesity. In type 2 diabetes mellitus and obesity, the changes in ADP expression and its *adipoRs* diminish the sensitivity of ADP resulting in insulin resistance, which in turn exacerbates hyperinsulinemia [116].

SPX, also known as neuropeptide Q, is an endogenous neuropeptide. The SPX gene and protein are excessively expressed in the CNS and peripheral tissues in humans, rodents, goldfish, etc. [118]. The prepropeptide SPX consisting of 116 amino acid residues was encoded by the *C12ORF39* gene [119]. The amino acid sequence of SPX is highly preserved across different species, suggesting that this peptide has a fundamental role through biological development and may be actively contributed in the regulation of different pathological and physiological functions [120]. In the diet-induced obesity (DIO) in rats, chronic subcutaneous injection with SPX decreased the food intake [121]. These findings denote a scientific evidence for the inhibitory influence of exogenous SPX on food intake and indicate a close correlation between SPX and food control. It is well known that the uptake and storage of long-chain fatty acid are the key players for controlling body weight [122]. Treatment with SPX induced an inhibitory influence on long-chain fatty acid uptake into adipocytes isolated from untreated DIO in mice. Chronic intraperitoneal injection of SPX decreased the respiratory exchange ratio at night and elevated locomotor activity in DIO in mice [121]. Indeed, the treatment with the synthetic SPX in DIO in mice has been found to decrease body weight probably by its inhibitory effect on fatty acid uptake into hepatocytes [123,124]. In human adipocytes and murine 3 T3-L1 cells, SPX displayed a prompting impact on lipolysis and a suppressing impact on lipogenesis [125]. A study on a healthy adult woman shows that serum SPX levels are inversely correlated with age, BMI, fasting glucose, and TG. This suggests that SPX could independently predict the risk of high BMI and elevated fasting glucose [126]. Moreover, in obese women and children serum SPX levels are considerably decreased compared with the lean ones, indicating the relation between SPX and body weight regulation [125]. Also,

SPX has a possible relation with glucose homeostasis; the reduction of fasting glucose also modifies the levels of SPX. The significant decrease in fasting glucose levels correlated with a significant elevation in circulating SPX levels [127]. Another study advocates the SPX role in fat tissue metabolism and lipid homeostasis [125]. In addition, a clinical study on obese individuals reveals that the SPX gene and protein are downregulated in omental and subcutaneous fat [121]. Moreover, in obese individuals the level of serum SPX was lesser than that of the healthy counterparts [128]. Furthermore, the serum SPX is negatively correlated with leptin in obese patients [121,128].

Treatment of obese rats with chitocal reduced serum leptin and elevated serum ADP and SPX levels significantly. The suppression of leptin is essential because leptin is turned on by epigenetic modulation when preadipocytes are activated to initiate the process of adipogenesis [129]. Chitosan supplementation has been found to decrease leptin level in obese adolescents. Apart from the anorexigenic (appetite suppressant) function of leptin, experimental findings offer a theoretical basis for the systemic impact of chitosan treatment in enhancing the expression of liver leptin receptor b (*LepRb*) and promoting signal transducer and activator of transcription 3 (STAT3) and the phosphorylation of janus kinase 2 (JAK2) [130]. Thus by activation of the JAK2-STAT3 signaling pathway chitosan could reduce leptin resistance while partially inhibiting adipogenesis.

Regarding the stimulatory action of chitocal on ADP level in obese rats in the current investigation, Fatahi *et al.* [131] supported our finding in obese adolescents. Chitosan supplementation has antiobesity and antidiabetic impacts in ob/ob mice, in addition to its role in improving ADP plasma concentrations along with its ability to increase the expression of PPAR- $\gamma$  in the adipose tissue, a key regulator of ADP production [132].

The increase in serum SPX level in obese rats administered with chitocal in the current study is linked to the negative correlation between SPX level and adiposity markers (BMI and waist and lip circumferences) as well as lipid profile markers (LDL, TG, and Chol) [133]. Moreover, a negative correlation has been found between SPX and leptin based on the serum data obtained from obese versus nonobese individuals [134]. Therefore, the inhibitory influence of chitosan on adiposity and lipid profile markers as well as leptin level in obese rats in the

present work may be the cause of elevated levels of SPX in this group.

Treatment of obese rats with free rutin or rutin nanoformulation lessened serum leptin level and enhanced serum ADP and SPX levels significantly. It has been demonstrated that rutin reduces hyperleptinemia and inhibits leptin secretion from adipose tissue as rutin improved hyperinsulinemia and dyslipidemia, and in turn regulated leptin dysfunction in fructose-fed rats [135]. Moreover, Ganjaji *et al.* [136] recorded that the treatment of 3T3-L1 adipocytes with rutin leads to reduced levels of leptin and increased levels of ADP, which might be due to increased lipolysis and fatty acid oxidation through AMP-activated protein kinase activation. In addition, rutin substantially inhibited the activity of lipase and glucosidase, enhanced glucose uptake, and decreased lipid content and adipogenesis in 3T3-L1 cells by acting on various targets [136]. Rutin could increase ADP mRNA together with promoting ADP secretion from differentiating 3T3-L1 cells. This indicates that the motivating influence of rutin on adipocyte differentiation probably takes place occurs through the upregulation of adipogenic transcription factors and downregulation of adipocyte-specific gene expression [137].

It has been demonstrated that the decreased expression of SPX gene due to elevated serum leptin in obese individuals is accompanied by impaired intake of food and energy metabolism [121]. Therefore, elevated serum leptin levels can decrease SPX expression in obese individuals. Rutin administration in fructose-fed rats could reduce hyperleptinemia and inhibit leptin secretion from the adipose tissue [135]. This finding together with our results regarding the inhibitory effect of rutin on serum leptin level in obese rats suggest that rutin can upregulate SPX gene expression by decreasing serum leptin levels and consequently enhance serum SPX level in obese rats in our study.

## Conclusion

In conclusion, the results of the present attempt contribute to developing a new strategy using natural products as a therapeutic agent combined with nanobiotechnology to control obesity. The sodium alginate-based nanoformulation of rutin exhibited prominent anti-obesogenic potential through its hypolipidemic, hypoglycemic, and anti-inflammatory actions as well as its inhibitory effect on hyperleptinemia and adipogenesis. Noteworthy, the

formulated nanorutin has promising antiobesity action than its free form, which may be due to the enhancement of the solubility and bioavailability by the development of rutin nanoplatform.

## Limitation of the study

Although the fabricated sodium alginate-based nanoformulation of rutin proved its usefulness in the management of obesity in the present investigation, it would be better to design other similar formulations with different ratios for comparison to establish the most promising one in the treatment of obesity. Moreover, the potential toxicity or side effects of the designed nanoformulation should be performed to find out the optimal therapeutic dosage and long-term safety profile. Furthermore, the duration time for the treatment of obese rats with the nanorutin in the current study could be insufficient to achieve significant effects; and so a longer treatment duration could be more effective.

## Future directions

The biomarkers assessed in the current study could be determined at the adipose and liver tissues at the protein and gene levels. Furthermore, it is important to develop functional ligand-targeted nanocarriers as antiobesity strategies that target the white adipose tissues and its vasculature for the reversal of obesity.

## Acknowledgements

Authors' contributions: S.E.K.: performed the *in vivo* antiobesity experiment on the rat model, conducted biochemical analyses, and handled statistical analysis and data interpretation. H.H.A.: proposed the idea, designed the study, and contributed to paper preparation and final revision. O.A.M.M.: performed the *in vivo* antiobesity experiment on the rat model and contributed to the assessment of biochemical results. K.F.M.: responsible for nanoformulations and preparation. H.A.A.: proposed the idea, contributed to the study design, and participated in paper preparation and revision.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

- Mendagudli VG, Sarawad SS. Obesity and its impact on health. *Int J Nurs Educ Res* 2021; 9:485–487.
- Chandrakala M. Lifestyle modification to combat adolescent obesity. *Asian J Nur Educ Res* 2011; 1:82–84.

- 3 Mr. Manjunatha DM. Obesity and growing health risks – a review. *Asian J Nurs Educ Res* 2022; 12:350–352.
- 4 Jarali A., Radhakrishnan G. Stress, obesity and selected health problems among professionals. *Asian J Nurs Educ Res* 2013; 3:154–163.
- 5 Dai H, Alsalhe TA, Chalghaf N, Riccò M, Bragazzi NL, Wu J. The global burden of disease attributable to high body mass index in 195 countries and territories, 1990-2017: an analysis of the Global Burden of Disease Study. *PLoS Med* 2020; 17:e1003198.
- 6 Nikam GV, Kengar MD, Kalyani NV, Patil AA. Review on: diet and its disease. *Asian J Res Pharma Sci* 2024; 14:146–148.
- 7 Vari R, Scazzocchio B, Silenzi A, Giovannini C, Masella R. Obesity-associated inflammation: does curcumin exert a beneficial role? *Nutrients* 2021; 13:1021.
- 8 Manna P, Jain SK. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metab Syndr Relat Disord* 2015; 13:423–444.
- 9 Prashar D, Singh P. Obesity medications: pharmaco-economical outline. *Asian J Res Pharma Sci* 2020; 10:195–198.
- 10 Garvey WT, Mechanick JL, Brett EM. American Association of Clinical Endocrinologists and American College of Endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity. *Endocr Pract* 2016; 22:1–203.
- 11 Müller TD, Blüher M, Tschöp MH, DiMarchi RD. Anti-obesity drug discovery: advances and challenges. *Nat Rev Drug Discov* 2022; 21: 201–223.
- 12 Administration FaD. Guidance for industry developing products for weight management. Draft guidance. Available at: <https://www.federalregister.gov/documents/2007/02/15/E7-2581/draft-guidance-for-industry-developing-products-for-weight-management-availability>; 2007. [Accessed May 2022].
- 13 Administration USFaD. FDA requests the withdrawal of the weight-loss drug Belviq, Belviq XR (lorcaserin) from the market. Available at: <https://www.fda.gov/drugs/fda-drug-safety-podcasts/fda-requests-withdrawal-weight-loss-drug-belviq-belviq-xr-lorcaserin-market#:~:text=On%20February%2013%2C%202020%20FDA,an%20increased%20occurrence%20of%20cancer;2022>. [Accessed May 2022].
- 14 Mahboob A, Samuel SM, Mohamed A, Wani MY, Ghorbel S, Miled N, *et al.* Role of flavonoids in controlling obesity: molecular targets and mechanisms. *Front. Nutr* 2023; 10:12.
- 15 Tian C, Liu X, Chang Y, Wang R, Lv T, Cui C. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. *S Afr J Bot* 2021; 137:257–264.
- 16 Ashok Kumar BS, Lakshman K, Jayaveera KN, Vamshi Krishna N, Manjunath M, Suresh MV, *et al.* Estimation of rutin and quercetin in terminalia chebula by HPLC. *Asian J Res Chem* 2009; 2:388–389.
- 17 Vijaya Sri K, Santhoshini G, Ravi Sankar D, Niharika K. Formulation and evaluation of rutin loaded nanosponges. *Asian J Res Pharm Sci* 2018; 8:21–24.
- 18 You Y, Yuan X, Lee HJ, Huang W, Jin W, Zhan J. Mulberry and mulberry wine extract increase the number of mitochondria during brown adipogenesis. *Food Funct* 2015; 6:401–408.
- 19 Liu X, Zheng Z, Zhu X, Meng M, Li L, Shen Y, *et al.* Brown adipose tissue transplantation improves whole-body energy metabolism. *Cell Res Vol* 2013; 23:851–854.
- 20 Narasagoudr SS, Hegde VG, Chougale RB, Masti SP, Vootla S, Malabadi RB. Physico-chemical and functional properties of rutin induced chitosan/poly (vinyl alcohol) bioactive films for food packaging applications. *Food Hydrocolloids* 2020; 109:106096.
- 21 Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: a review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends Food Sci Technol* 2017; 67:220–235.
- 22 Júlio A, Caparica R, Costa Lima SA, Fernandes AS, Rosado C, Prazeres DM, Fonte P. Ionic liquid-polymer nanoparticle hybrid systems as new tools to deliver poorly soluble drugs. *Nanomaterials* 2019; 9:1148.
- 23 Joye IJ, Davidov-Pardo G, McClements DJ. Nanotechnology for increased micronutrient bioavailability. *Trends Food Sci Technol* 2014; 40:168–182.
- 24 Negahdari R, Bohlouli S, Sharifi S, Dizaj SM, Saadat YR, Khezri K, Jafari S, Ahmadian E, *et al.* Therapeutic benefits of rutin and its nanoformulations. *Phytother Res* 2021; 35:1719–1738.
- 25 Terjung N, Löffler M, Gibis M, Hinrichs J, Weiss J. Influence of droplet size on the efficacy of oil-in-water emulsions loaded with phenolic antimicrobials. *Food Funct* 2012; 3:290–301.
- 26 Abd-Elsalam KA, Khokhlov AR. Eugenol oil nanoemulsion: antifungal activity against *Fusariumoxysporum f. sp. vasinfectum* and phytotoxicity on cottonseeds. *Appl Nanosci* 2015; 5:255–265.
- 27 Zheng-hong W, Qi-neng P, Yi W, Jia-ming L. Hypoglycemic efficacy of chitosan-coated insulin liposomes after oral administration in mice. *Acta Pharmacol Sin* 2004; 25:966–972.
- 28 DiMarzio L, Marianecchi C, Petrone M, Rinaldi F, Carafa M. Novel pH-sensitive non-ionic surfactant vesicles: comparison between Tween 21 and Tween 20. *Colloids Surf B Biointerf* 2011; 82:18–24.
- 29 Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Scholmerich J, Bollheimer LC. Defining high-fat diet rat models: metabolic and molecular effects of different fat types. *J Mol Improv Serum Lipid Profile Diab Obes Metab* 2006; 7:65–72.
- 30 Soliman MM, Attia HF, El-Shazly SA, Saleh OM. Biomedical effects of cinnamon extract on obesity and diabetes relevance in Wistar rats. *Am J Biochem Mol Biol* 2012; 2:133–145.
- 31 Levin BE, Dunn-Meynell AA. Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. *Am J Physiol* 2002; 282:46–54.
- 32 Al-Hilfy JHY, Amer N. Effect of weight reduction drug chitocal contained *Gymnema sylvestre* extract on body weight, lipid profiles, kidney function and histological structure of kidney and intestine in male albino rats. *Med J Babylon* 2012; 9:200–208.
- 33 Janbaz KH, Saeed SA, Gilani AH. Protective effect of rutin on paracetamol- and CCl4-induced hepatotoxicity in rodents. *Fitoterapia* 2002; 73:557–563.
- 34 Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F. Anthropometrical parameters and markers of obesity in rats. *Lab Anim* 2007; 41:111–9.
- 35 Othman ZA, Zakaria Z, Suleiman JB, Ghazali WSW, Mohamed M. Anti-atherogenic effects of orlistat on obesity-induced vascular oxidative stress rat model. *Antioxidants* 2021; 10:251.
- 36 Meattini F, Prencipe L, Bardelli F, Giannini G, Tarli P. The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. *Clin Chem* 1978; 24:2161–2165.
- 37 Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 1973; 19:476–82.
- 38 Naito HK., Cholesterol HD. *Clinical Chemistry*. St Louis, Toronto, Princeton: The C.V. Mosby Co 1984:1207–1213.
- 39 Young IS, McEnery J. Lipoprotein oxidation and atherosclerosis. *Biochem Soc Trans* 2001; 29:358–362.
- 40 National committee for clinical laboratory standards procedures for the collection of diagnostic blood specimens by venipuncture: Approved standards 4<sup>th</sup> ed NCC LS Document H3–A4, Wayne, PA 1998.
- 41 Duncan MH, Singh BM, Wise PH, Carter G, Alagband-Zadeh J. A simple measure of insulin resistance. *Lancet* 1995; 346:120–121.
- 42 Armitage P, Berry G. Comparison of several groups. In: statistical method in medical research. 2nd ed. Oxford: Blackwell Significant Publication; 1987:186–213.
- 43 Al-Dhabi NA, Arasu MV, Park CH, Park SU. An up-to-date review of rutin and its biological and pharmacological activities. *EXCLI J* 2015; 14: 59–63.
- 44 Lauro MR, Torre ML, Maggi L. Fast- and slow-release tablets for oral administration of flavonoids: rutin and quercetin. *Drug Dev Ind Pharm* 2002; 28:371–379.
- 45 Ali M, Hassan KAM, Ansari MI. Chitosan-alginate nanoparticles as a novel drug delivery system for rutin. *Int J Adv Biotechnol Res* 2018; 9:1895–1903.
- 46 Nalini T, KhaleelBasha S, MajeethMohamed Sadiq A, SuganthaKumari V, Kaviyarasu K. Development and characterization of alginate/chitosan nanoparticulate system for hydrophobic drug encapsulation. *J Drug Deliv Sci Technol* 2019; 52:65–72.
- 47 Gagliardi A, Paolino D, Costa N, Fresta M, Cosco D. Zein- vs PLGA-based nanoparticles containing rutin: a comparative investigation. *Mater Sci Eng C Mater Biol Appl* 2021; 118:111538.
- 48 Pérez-Corredor PA, Gutiérrez-Vargas JA, Ciro-Ramírez L, Balcazar N, Cardona-Gómez JP. High fructose diet-induced obesity worsens post-ischemic brain injury in the hippocampus of female rats. *Nutr Neurosci* 2022; 25:122–136.
- 49 Othman ZA, Wan Ghazali WS, Noordin L, Mohd. Yusof NA, Mohamed M. Phenolic compounds and the anti-atherogenic effect of bee bread in high-fat diet-induced obese rats. *Antioxidants* 2020; 9:33.

- 50 Illum L. Chitosan and its use as a pharmaceutical excipient. *Pharm Res* 1998; 15:1326–1331.
- 51 Ahmed ABA, Rao AS, Rao MV. Role of *in vivo* and *in vitro* callus of *Gymnema sylvestre* (Retz) R. Br. Ex. Roemer & Schultes in maintaining the normal levels of blood glucose and lipid profile in diabetic Wistar rats. *Biomedicine* 2008; 28:134–138.
- 52 Bukhari H, Zahran S, Bakr E, Sahibzadah F, Header E. Comparison study between drugs (orlistat and chitocal) and food supplements (green tea and apple cider vinegar) for weight loss and hepatoprotection in rats. *Egypt J Hosp Med* 2021; 83:1218–1223.
- 53 Safavi R, Lih A, Kirkpatrick S. Impact of anti-obesity medication initiation and duration on weight loss in a comprehensive weight loss programme. *J Obes Sci Pract* 2019; 5:468–478.
- 54 La Casa C, Villegas I, de la Lastra CA, Motilva V, Calero MM. Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *J Ethnopharmacol.* 2000; 71:45–53.
- 55 Sheu JR, Hsiao G, Chou PH, Shen MY, Chou DS. Mechanisms involved in the antiplatelet activity of rutin, a glycoside of the flavonol quercetin, in human platelets. *J Agric Food Chem* 2004; 52:4414–4418.
- 56 Choi I, Park Y, Choi H, Lee EH. Anti-adipogenic activity of rutin in 3T3-L1 cells and mice fed with high-fat diet. *Biofactors* 2006; 26:273–281.
- 57 Hsu CL, Yen GC. Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L1 adipocytes. *J Agric Food Chem* 2007; 55:8404–8410.
- 58 Panchal SK, Poudyal H, Arumugam TV, Brown L. Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. *J Nutr* 2011; 141:1062–1069.
- 59 Wu CH, Lin MC, Wang HC, Yang MY, Jou MJ, Wang CJ. Rutin inhibits oleic acid induced lipid accumulation via reducing lipogenesis and oxidative stress in hepatocarcinoma cells. *J Food Sci* 2011; 76:T65–T72.
- 60 Hsu CL, Wu CH, Huang SL, Yen GC. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. *J Agric Food Chem* 2009; 57:425–431.
- 61 Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev* 2000; 14:1293–1307.
- 62 Seo S, Lee MS, Chang E, Shin Y, Oh S, Kim IH, Kim Y. Rutin increases muscle mitochondrial biogenesis with AMPK activation in high-fat diet-induced obese rats. *Nutrients* 2015; 7:8152–8169.
- 63 Sowmya A, Ananthi T. Hypolipidemic activity of *Mimosa pudica* Linn on butter induced hyperlipidemia in rats. *Asian J Res Pharm Sci* 2011; 1:123–126.
- 64 Godea Lupei S, Ciubotariu D, Danciu M, LupuÈoru RV, Ghiciuc CM, Cernescu I, et al. Improvement in serum lipids and liver morphology after supplementation of the diet with fish oil is more evident under regular feeding conditions than under high-fat or mixed diets in rats. *Lipids Health Dis* 2020; 19:162.
- 65 Faraji N, Ganji A, Heshami N, Salehi I, Haddadian A, Shojaie S, Komaki A. Hypolipidemic effects of *Hypericum scabrum* extract on the serum lipid profile and obesity in high-fat diet fed rats. *Human Antibod* 2021; 29:55–61.
- 66 Rezvani-Kamran A, Salehi I, Shahidi S, Zarei M, Moradkhani S, Komaki A. Effects of the hydroalcoholic extract of *Rosa damascena* on learning and memory in male rats consuming a high-fat diet. *Pharma Biol* 2017; 55:2065–2073.
- 67 Videla LA, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quiñones L, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin Sci (Lond)* 2004; 106:261–268.
- 68 Mohd YK, Gupta P, Bihari B, Verma VK. A review on diabetes and its management. *Asian J Pharma Res* 2013; 3:27–32.
- 69 Wang LF, Luo H, Miyoshi M, Imoto T, Hiji Y, Sasaki T. Inhibitory effect of gymnemic acid on intestinal absorption of oleic acid in rats. *Can J Physiol Pharmacol* 1998; 76:1017–1023.
- 70 Gallaher C.M., Munion J., Hesslink J., Wise J, Gallaher D.D. Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. *J Nutr* 2000; 130:2753–2759.
- 71 Mitra SK, Gopurdhavan TS, Mualidhar SD, Anturikar Sujata MB. Effect of a herbomineral preparation D-400 in streptozotocin –induced diabetic rats. *J Ethnopharmacol* 1996; 54:41–46.
- 72 Muzzarelli R, Orlandini F, Pacetti D, Boselli E, Frega NG, Tosi G, Muzzarelli C. Chitosan taurocholate capacity to bind lipids and to undergo enzymatic hydrolysis: An *in vitro* model. *Carbohydr Polym* 2006; 66:363–71.
- 73 Daisy P, Eliza J, Farook KAM. Anovel dihydroxy gymnemic triacetate isolated from *Gymnema sylvestre* possessing normoglycemia and hypolipidemic activity on STZ- induced diabetic Rats. *J Ethnopharma Col* 2009; 126:334–344.
- 74 Mitra SK, Gopumadhavan S, Muralidhar TS, Anturlikar SD, Sujatha MB. Effect of D-400 herbomineral preparation on lipid profile glycated haemoglobin and glucose tolerance in streptozotocin induced diabetes in rats. *Indian J Exp Biol* 1995; 33:798–800.
- 75 Liu SH, Cai FY, Chiang MT. Long-term feeding of chitosan ameliorates glucose and lipid metabolism in a high-fructose-dietimpaired rat model of glucose tolerance. *Mar Drugs* 2015; 13:7302–7313.
- 76 Muanprasat C, Chatsudthipong V. Chitosan oligosaccharide: Biological activities and potential therapeutic applications. *Pharmacol Ther.* 2017; 170:80–97.
- 77 Bai Y, Zheng J, Yuan X, Jiao S, Feng C, Du Y, Liu H, Zheng L. Chitosan oligosaccharides improve glucolipid metabolism disorder in liver by suppression of obesity-related inflammation and restoration of peroxisome proliferator-activated receptor gamma (PPARgamma). *Mar. Drugs* 2018; 16:455.
- 78 Livingston Raja NR, Ravindran Nair A, Senthilpandian S, Ravi V. Hypolipidemic action of Rutin on Triton WR-1339 induced hyperlipidemia in rats. *J Pre Clin Clin Res* 2021; 15:51–55.
- 79 Agrawal S, Zaritsky J, Fornoni A. Dyslipidaemia in nephrotic syndrome: mechanisms and treatment. *Nat Rev Nephrol* 2018; 14:57–70.
- 80 Kuang H, Yang F, Zhang Y, Wang T, Chen G. The impact of egg nutrient composition and its consumption on cholesterol homeostasis. *Cholesterol* 2018; 2018:1–22.
- 81 Kunnen S, Van Eck M. Lecithin: cholesterol acyltransferase: old friend or foe in atherosclerosis? *J Lipid Res* 2012; 53:1783–1799.
- 82 Sheneni VD, Shaibu IE, Okpe JM. In-vivo biological effect of Carica papaya leaf extracts on P-407 induced hyperlipidemic Wistar rats. *MOJ Food Process Technol* 2018; 6:409–412.
- 83 Fernandes AA, Novelli EL, Okoshi K, Okoshi MP, Muzio BP, Guimarães JF. Influence of rutin treatment on biochemical alterations in experimental diabetes. *Biomed Pharmacother* 2010; 64:214–219.
- 84 Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin. *Biomed Pharmacother* 2017; 96:305–312.
- 85 Huang R, Shi Z, Chen L, Zhang Y, Li J, An Y. Rutin alleviates diabetic cardiomyopathy and improves cardiac function in diabetic ApoEknockout mice. *Eur J Pharmacol* 2017; 814:151–160.
- 86 Sheelagh F, Daniella Z. Targeting glycogen synthase kinase-3 in insulin signalling. *Expert Opin Ther Targets* 2006; 10:429–444.
- 87 Liu QP, Li X, Li CY, Zheng YF, Wang F, Li HY. 1-deoxyynojirimycin alleviates liver injury and improves hepatic glucose metabolism in db/db mice. *Molecules* 2016; 21:279.
- 88 Liang W, Zhang D, Kang J, Meng X, Yang J, Yang L, et al. Protective effects of rutin on liver injury in type 2 diabetic db/db mice. *Biomed Pharmacother* 2018; 107:721–728.
- 89 Carvalho FA, Aitken JD, Vijay-Kumar M, Gewirtz AT. Toll-like receptor-gut microbiota interactions: Perturb at your own risk! *Annu. Rev. Physiol.* 2012; 74:177–198.
- 90 Connolly DJ, O'Neill LA. New developments in Toll-like receptor targeted therapeutics. *Curr Opin Pharmacol* 2012; 12:510–518.
- 91 Dobrovolskaia MA, Vogel SN. Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes Infect* 2002; 4:903–914.
- 92 Stern JH, Rutkowski JM, Scherer PE. Adiponectin, leptin, and fatty acids in the maintenance of metabolic homeostasis through adipose tissue crosstalk. *Cell Metab* 2016; 23:770–784.
- 93 Rogero MM, Calder PC. Obesity, inflammation, toll-like receptor 4 and fatty acids. *Nutrients* 2018; 10:432.
- 94 Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AWJr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; 112:1796–1808.
- 95 Boulouvar S, Michelet X, Duquette D, Alvarez D, Hogan AE, Dold C, et al. Adipose type one innate lymphoid cells regulate macrophage homeostasis through targeted cytotoxicity. *Immunity* 2017; 46:273–286.
- 96 Sukanami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, et al. Role of the Toll-like receptor 4/NF-κB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol* 2007; 27:84–91.
- 97 Luna-Vital D, Luzardo-Ocampo I, Cuellar-Nunez ML, Loarca-Pina G, Gonzalezde Mejia E. Maize extract rich in ferulic acid and anthocyanins prevents high-fat-induced obesity in mice by modulating

- SIRT1, AMPK and IL-6 associated metabolic and inflammatory pathways. *J Nutr Biochem* 2020; 79:108343.
- 98 McKernan K, Varghese M, Patel R, Singer K. Role of TLR4 in the induction of inflammatory changes in adipocytes and macrophages. *Adipocyte* 2020; 9:212–222.
- 99 Wu Y, Potempa LA, El Kebir D, Filep JG. C-reactive protein and inflammation: conformational changes affect function. *Biol Chem* 2015; 396:1181–1197.
- 100 Shi H, Kokoeva MV, Inouye K, Tzamelis I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 2006; 116:3015–3025.
- 101 Shoelson SE, Lee J, Yuan M. Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. *International journal of obesity and related metabolic disorders. J Int Assoc Study Obes* 2003; 27:S49–S52.
- 102 Chen CL, Wang YM, Liu CF, Wang JY. The effect of water-soluble chitosan on macrophage activation and the attenuation of mite allergen-induced airway inflammation. *Biomaterials* 2008; 29:2173–2182.
- 103 Xiao D, Tang Z, Yin Y, Zhang B, Hu X, Feng Z, Wang J. Effects of dietary administering chitosan on growth performance, jejunal morphology, jejunal mucosal sIgA, occluding, claudin-1 and TLR4 expression in weaned piglets challenged by enterotoxigenic *Escherichia coli*. *Int Immunopharmacol* 2013; 17:670–676.
- 104 Xiao D, Wang Y, Liu G, He J, Qiu W. Effects of chitosan on intestinal inflammation in weaned pigs challenged by enterotoxigenic *Escherichia coli*. *PLoS ONE* 2014; 9:e104192.
- 105 Guan G, Azad MAK, Lin Y, Kim SW, Tian Y, Liu G, Wang H. Biological effects and applications of chitosan and chito-oligosaccharides. *Front. Physiol.* 2019; 10:516.
- 106 Mohyuddin SG, Qamar A, Hu C, Chen SW, Wen J, Liu X, et al. Effect of chitosan on blood profile, inflammatory cytokines by activating TLR4/NF-κB signaling pathway in intestine of heat stressed mice. *Sci Rep* 2021; 11:20608.
- 107 Dou W, Zhang J, Sun A, Zhang E, Ding L. Protective effect of naringenin against experimental colitis via suppression of Toll-like receptor 4/NF-kappa B signalling. *Br J Nutr* 2013; 110:599–608.
- 108 Hou LS, Cui ZY, Sun P, Piao HQ, Han X, Song J, et al. Rutin mitigates hepatic fibrogenesis and inflammation through targeting TLR4 and P2x7 receptor signaling pathway in vitro and in vivo. *J Funct Foods* 2020 2020; 64:103700.
- 109 Tian C, Liu X, Chang Y, Wang R, Yang M, Liu M. Rutin prevents inflammation induced by lipopolysaccharide in RAW 264.7 cells via conquering the TLR4-MyD88-TRAF6-NF-κB signalling pathway. *J Pharm Pharmacol* 2021; 73:110–117.
- 110 Huang S, Rutkowski JM, Snodgrass RG, Ono-Moore KD, Schneider DA, Newman JW, Adams SH, Hwang DH. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. *J Lipid Res* 2012; 53:2002–2013.
- 111 Lee W. Barrier protective effects of rutin in LPS-induced inflammation in vitro and in vivo. *Food Chem Toxicol* 2012; 50:3048–55.
- 112 Tian CL. The regulatory effect of flavonoids extracted from leaves of *Abutilon theophrasti* on gene expression in LPS-induced ALI mice via the NF-κB and MAPK signalling pathways. *Pharm Biol* 2019; 57:514–18.
- 113 Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; 392:398–401.
- 114 Ozata M, Ozdemir IC, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. *Clin Endocrinol Metab* 1999; 84:3686–3695.
- 115 Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. Plasma adiponectin complexes have distinct biochemical characteristics. *Endocrinology* 2008; 149:2270–2282.
- 116 de Rosa A, Ludovica Monaco M, Capasso M. Adiponectin oligomers as potential indicators of adipose tissue improvement in obese subjects. *Eur J Endocrinol* 2013; 169:37–43.
- 117 Drolet R, Bèlanger C, Fortier M. Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women. *Obesity* 2009; 17:424–430.
- 118 Lv SY, Zhou YC, Zhang XM, Chen WD, Wang YD. Emerging roles of NPQ/spexin in physiology and pathology. *Front Pharmacol* 2019; 10:457.
- 119 Wan B, Wang XR, Zhou YB, Zhang X, Huo K, Han ZG. C120RF39, a novel secreted protein with a typical amidation processing signal. *Biosci Rep* 2010; 30:1–10.
- 120 Wong MK, Sze KH, Chen T, Cho CK, Law HC, Chu I.K. Goldfish spexin: solution structure and novel function as a satiety factor in feeding control. *Am J Physiol Endocrinol Metab* 2013; 305:E348–E366.
- 121 Walewski JL, Ge F, Lobdell H. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. *Obesity* 2014; 22:1643–1652.
- 122 Petrescu O, Fan X, Gentileschi P, Hossain S, Bradbury M, Gagner M. Long-chain fatty acid uptake is upregulated in omental adipocytes from patients undergoing bariatric surgery for obesity. *Int J Obes (Lond)* 2005; 29:196–203.
- 123 Jasmine FG, Walewski J, Anglade D, Berk P. Regulation of hepatocellular fatty acid uptake in mouse models of fatty liver disease with and without functional leptin signaling: roles of NFκB and SREBP-1C and the effects of spexin. *Semin Liver Dis.* 2016; 36:360–372.
- 124 Lin CY. Spexin acts as novel regulator for bile acid synthesis. *Front Physiol* 2018; 9:378.
- 125 Kolodziejcki PA, Pruszyńska-Oszmialek E, Micker M, Skrzypski M, Wojciechowicz T, Szwarzkopf P. Spexin: a novel regulator of adipogenesis and fat tissue metabolism. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018; 1863:1228–1236.
- 126 Lin CY, Huang T, Zhao L, Zhong LLD, Lam WC, Fan BM. Circulating Spexin levels negatively correlate with age, BMI, fasting glucose, and triglycerides in healthy adult women. *J Endocr Soc* 2018; 2:409–419.
- 127 Sassek M, Kolodziejcki PA, Strowski MZ, Nogowski L, Nowak KW, Mackowiak P. Spexin modulates functions of rat endocrine pancreatic cells. *Pancreas* 2018; 47:904–909.
- 128 Walewski JL, Berk PD. Obesity-related genes and their proteins and uses thereof (Google Patents). 2013. doi: US20130274181 A1
- 129 Melzner I, Scott V, Dorsch K, Fischer P, Wabitsch M, Bröderlein S, Hasel C, Möller P. Leptin gene expression in human preadipocytes is switched on by maturation-induced demethylation of distinct CpGs in its proximal promoter. *J Biol Chem* 200; 277:45420–45427.
- 130 Pan H, Fu C, Huang L, Jiang Y, Deng X, Guo J. Anti-obesity effect of chitosan oligosaccharide capsules (COSC) in obese rats by ameliorating leptin resistance and adipogenesis. *Marine Drugs* 2018; 16:198.
- 131 Fatahi S, Sayyari AA, Salehi M, Safa M, Sohoul M, Shidfar F, Santos HO. The effects of chitosan supplementation on anthropometric indicators of obesity, lipid and glycemic profiles, and appetite-regulated hormones in adolescents with overweight or obesity: a randomized, double-blind clinical trial. *BMC Pediatrics* 2022; 22:527.
- 132 Kumar SG, Rahman MA, Lee SH, Hwang HS, Kim HA, Yun JW. Plasma proteome analysis for anti-obesity and anti-diabetic potentials of chitosan oligosaccharides in Ob/Ob mice. *Proteomics* 2009; 9:2149–2162.
- 133 Khadir A, Kavalakatt S, Madhu D, Devarajan S, Abubaker J, Al-Mulla F, Tiss A. Spexin as an indicator of beneficial effects of exercise in human obesity and diabetes. *Sci Rep* 2020; 10:10635.
- 134 Kumar S, Hossain MJ, Javed A, Kullo IJ, Balagopal PB. Relationship of circulating spexin with markers of cardiovascular disease: a pilot study in adolescents with obesity. *Pediatr Obes* 2018; 13:374–380.
- 135 Jian-Mei L, Chuang W, Qing-Hua HU, Linglong K. Fructose induced leptin dysfunction and improvement by quercetin and rutin in rats. *Chin J Natl Med* 2008; 6:466–473.
- 136 Ganjavi MS, Karunakaran RS, Gandham S, Meriga B. Quercetin-3-O-rutinoside from *moringa oleifera* down regulates adipogenesis and lipid accumulation and improves glucose uptake by activation of AMPK/Glut-4 in 3T3-L1 Cells. *Rev Brasil Farmacog* 2023; 33:334–343.
- 137 Naowaboot J, Chung CH, Choi R. Rutin stimulates adipocyte differentiation and adiponectin secretion in 3T3-L1 adipocytes. *J Med Assoc Thai* 2015; 98 3:S1–S6.