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Original Research Article

Olive Cake *Prevents Inflammation,* Modulates Glomerulopathy, Glomerular Filtration Rate and Improves Renal Functions in Adult Obese Rats Fed a High-Fat Diet Since Weaning

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ABSTRACT

Obesity-related glomerulopathy (ORG) is a type of kidney disease induced by obesity, which can start in obese children and continue into adulthood. This work investigated the effects of olive cake (OC) on obesity-related renal complications in obese rats fed a high-fat diet (HFD) since weaning. Post-weaned male rats were divided into a control (C) rat fed a standard diet and obese (Ob) rat fed HFD for 14 weeks. Ob rats were divided into groups treated (HFD-OC) or not (HFD) with OC at 7.5% for four weeks. In comparison to the C group, the HFD group presented significant increases in kidney index, serum creatinine, urea, proteinuria, and a decrease in glomerular filtration rate. The OC treatment improved these parameters. The HFD group exhibited elevated values of triglycerides, total cholesterol, lipid, and protein oxidation, while superoxide dismutase, glutathione peroxidase, and catalase activities were decreased in the kidneys. OC improved oxidative stress markers, the lipid profile, and increased kidney antioxidant defenses. Additionally, it lowered pro-inflammatory cytokine levels in the serum and dipeptidyl peptidase-4 (DPP4) activity. Histomorphometric analysis showed glomerulopathy, mesangial cell proliferation, tubular distortion, and the lipid accumulation in the renal of adult obese rats in the HFD group, which was attenuated by OC. A molecular docking study showed strong binding affinities of vanillic acid and ferulic acid with 38-kilodaltons of mitogen-activated protein kinase and DPP4. This study reveals that OC delays obesity and prevents ORG in adult rats by reducing oxidative stress, renal lipid accumulation, proteinuria, inflammatory response, and DPP4 activity.

Keywords: Rat, Obesity-related kidney injury, Olive cake, Redox status, Inflammation, Morphometric.

Introduction

Obesity is a major health problem worldwide that impacts people of any ages.[1] Childhood obesity is especially concerning as it increases the risk of adult obesity[2] and associated complications, including cardiometabolic disease, non-alcoholic fatty liver disease and kidney disease.[3] One form of obesity-related kidney illness that can develop in obese children and persist into adulthood is called obesity-related glomerulopathy (ORG).[4] Chronic renal failure can result from this condition, which is characterized by enlarged glomeruli, protein in the urine, and reduced glomerular filtration rate.[5]. Over the past thirty years, ORG has seen a tenfold increase in incidence,[6] and is associated with significant health issues such as chronic kidney disease, end-stage renal disease (ESRD), and higher mortality rates.[7]

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Although the exact mechanisms responsible for the development of ORG are not fully understood, it is evident that inflammation, oxidative stress, lipotoxicity, hypertension, and hyperglycemia play a significant role.[5]

In the current state of knowledge, there is no specific treatment for ORG. Instead, therapeutic approaches target underlying risk factors, like body weight and blood pressure.[7] However, these strategies are not effective in preventing the progression of kidney disease. It is therefore necessary to find effective prevention or treatment strategies to reduce the prevalence of ORG. Given that lipotoxicity, oxidative stress, and inflammation are causally linked to the development and progression of ORG, interventions targeting these parameters may prove useful in its treatment.

Recently, there has been an important interest in the utilization of fruit and vegetable by-products generated by the food industry as therapeutic agents in the prevention and treatment of cardiovascular disease. Skins, pulp residues, stone fragments, water, and residual oil are the components of olive cake (OC) that come from the olive oil extraction process. They are excellent sources of health-promoting nutrients; including minerals, vitamins, dietary fiber, and polyphenols.[8] They contain phenolic compounds such as oleuropein, hydroxytyrosol, caffeic acid, and verbascoside. It's a source of antioxidants that could prevent oxidative stress and tissue damage induced by oxidative stress. Olive pomace extracts and phenolic compounds are suggested as remedies against inflammatory diseases.[9] Our previous research showed that olive cake (OC) offers significant benefits for a variety of pathological conditions. It reduces adiposity and improves redox balance in obese rats,[10] attenuates renocardiopathy in hypertensive rats[11] and improves markers of kidney damage in diabetic rats.[12] Although the health benefits of olive oil are well known, its specific effect on obesity-related kidney disease is yet to be studied. Thus, in

this work we evaluated the effect of olive cake "*in vivo*" on glomerulomegaly, oxidative stress, renal lipotoxicity, and inflammatory markers in adult obese rats submitted to a diet rich in fat since weaning. In addition, we evaluated "*in silico*" the effect of the bioactive compounds on the modulation of the 38-kilodaltons mitogen-activated protein kinase (p38 MAPK) and dipeptidyl peptidase-4 (DPP4).

Materials and Methods

Experiments were conducted in accordance with the Council of European Communities (1987)[13] guidelines on the use of living animals in scientific investigations, and the protocol and use of rats were approved by our Institutional Committee on Animal Care and Use (Approval number: D00L01UN310120190002).

Olive cake collection
Olive cake (OC) was obtained immediately after oil extraction in an oil mill in Sig (Mascara, Algeria: 35° 23' 47.90" N, 0° 08' 24.97" E). It was then transported to the laboratory, where it underwent a drying process at 60°C. The dried OC was crushed to obtain a homogeneous powder and stored in airtight containers at room temperature until its use as an ingredient for the preparation of the experimental diets.

Qualitative analysis of phenolic compounds in OC by HPLC-DAD
The analysis of phenolic compounds was done through the use of high-performance liquid chromatography and diode array detection (HPLC-DAD) (Agilent 1100 Series, Englewood, CO, USA) employing an RP C18 column (4.6 mm x 250 mm internal diameter, 5 µm). Mobile Phase A was acidified water with 0.1% formic acid, and Mobile Phase B was acetonitrile. The Mobile Phase was programmed in a linear gradient as follows: 0 min (87% A); 0-18 min (45% A); 18-23 min (40% A); 23-25 min (87% A); 25-28 min (87% A). The injection volume was 10 µl with a flow rate of 0.7 ml/min. The wavelength detector was controlled at 320 nm, and the column temperature was maintained at 25°C.

Experimental animal model
Post-weaned male Wistar rats (n=18; 3 weeks old; weighing 45 ± 5 g) were kept under a lighting cycle of 12 h/12 h of darkness at constant room temperature (25°C±1°C) with free access to food and tap water. The rats were divided into 02 groups: a control (C) group (n=6), fed a standard diet, and a second group (n=12), fed a high-fat diet (HFD), for 14 weeks. After this period, HFD rats whose body weights were 20% ≥ of the mean value of the control group were considered obese. Obese rats were divided into two subgroups: the HFD group (n=6) and the HFD fed diet containing OC (HFD-OC) group (n=6) for four weeks. The composition of the standard diet and the HFD based on mutton fat were previously reported.[10] During the last three days of treatment, animals were housed into metabolism cages and urine samples were collected.
On day 28, the rats were anesthetized by intra-abdominal injection of chloral 10% (w/v). Blood samples were collected from the abdominal aorta and centrifuged at 3000 g for 15 minutes using a Hettich centrifuge (D78532 Tuttlingen, Germany). The kidney was removed and weighed, then divided into two portions, one for histological studies and the other homogenized in phosphate buffer saline (50 Mm, pH 7.2) for the estimation of redox status biomarkers.

Measurement of Lee index, body fat content and kidney index
Lee's index was calculated according to equation (1),[13] body fat content according to equation (2),[14] and kidney index according to equation (3).[13]

$$Lee\ index = \frac{\sqrt[3]{\text{Bodyweight(g)}}}{\text{lenght(cm)}} \text{ X } 1000 \quad \text{---------- Eq. (1)}$$

$$Body\ fat\ content = \frac{\text{Total body fat}}{Body\ weight} \text{ X } 100 \quad \text{---------- Eq. (2)}$$

$$\text{Kidney index} = \frac{\text{Kidney weight}}{\text{Body weight}} \text{X } 100 \quad \text{---------- Eq. (3)}$$

Blood pressure measurement
Before the sacrifice, the CODA^{TM} tail-cuff blood pressure system (Kent Scientific Corporation, USA) was used to measure the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) in conscious rats.

Biochemical analysis
A commercial kit (Biolabo, France) was used to measure creatinine, urea, uric acid, and glucose levels. The glomerular filtration rate (GFR) was calculated by creatinine clearance using the following equation (Eq 4).

$$GFR = \frac{(Urine\ creatine * \text{urine volum})/(\text{serum creatinine})}{1440} \quad \text{---------- Eq. (4)}$$

Serum tumor necrosis factor α (TNF-α) and IL-6 levels (ELISA kits, Sigma-Aldrich, USA), and DPP4 activity (Elisa assay kit, MyBioSource, CA, USA) were estimated following the manufacturer's protocol.

Determination of kidney lipid profile
Kidney total lipids were extracted[16] and their triglyceride (TG) and total cholesterol (TC) contents were calculated (Cypress Diagnostic kit, Belgium).

Measurements of oxidative stress markers
The contents of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), and advanced oxidation protein products (AOPP) were estimated according to the methods described by Quintanilha *et al*.,[17] Kurien *et al*.,[18] and Kayali *et al*.,[19] respectively. The activities of superoxide dismutase (SOD) were determined by the method of Marklund and Marklund,[20] glutathione peroxidase (GSH-Px) by the method of Flohé and Günzler[21] and catalase (CAT) by the method described by Aebi *et al*.[22]

Histopathological studies
The kidney was fixed in formalin (10%), dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 µM sections, and stained with Hematoxylin and Eosin (H&E).[23] The stained sections were photographed under an optic microscope (x40 magnification) coupled to a microscope camera (DN-107T, Japan), and Image J software (National Institute of Health, USA) was used for kidney histomorphometric analysis.

Molecular docking analysis
After evaluating the pharmacokinetic and toxicity profiles of molecules extracted from OC using the online Protox II server (https://tox-new.charite.de/protox_II/) (results not provided), ferulic acid (FA) and vanillic acid (VA) were chosen for their favorable profiles. They have high gastrointestinal absorption, oral bioavailability over 80%, comply with the Lipinski rule without any violations, and show no organic toxicity. These criteria justified their choice for molecular docking analysis.
Three-dimensional (3D) crystal structures of target proteins DPP-4 (PDB ID: 3VJM) and p38 MAPK (PDB ID: 1OVE) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), while the chemical structures of vanillic acid were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). A molecular docking analysis was performed using AutoDock Vina software, and potential interactions between ferulic acid and target proteins were examined with Discovery Studio Visualizer, (BIOVIA, Dassault Systems, Discovery Studio Visualizer, v21.1.0.20298).

Statistical analysis
Data was presented as the mean ± standard deviation (n = 6). Mean differences among the groups were analyzed by a two-way analysis of variances (ANOVA), followed by Tukey's post-hoc test. P-value < 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS)

software version 25. *P<0.05, vs Control (C), #P<0.05, high-fat diet (HFD) vs high-fat diet supplemented with olive cake (HFD-OC).

Results and Discussion

Phenolic compounds in OC identified by HPLC-DAD

Figure 1 shows the chromatogram of OC extract. Sixty-two peaks were detected, of which 12 were identified. The peaks identified correspond to tyrosol (11.3%), hydroxytyrosol (10%), oleacein (6.9%), oleuropein (4.7%), gallic acid (2.6%), caffeic acid (2.5%), vanillic acid (2.18%), ferulic acid (2.09%), and verbascoside (2.02%). The compounds obtained in the OC were consistent with those described in the literature.[24] Tyrosol, the most abundant phenolic compound (11%) in the OC, contrasts with previous studies.[25] Variations in olive fruit maturity, processing methods, and climate conditions may be the reason for the observed differences. Furthermore, this difference can also be explained by the hydrolysis of certain compounds into tyrosol during storage of the OC, under the action of enzymes and/or micro-organisms naturally present in the OC, which enriches the OC in tyrosol. In support of this hypothesis, Kiai et al[26] reported a higher value of tyrosol in olive brine after fermentation, attributed to the hydrolysis of tyrosol glycoside to tyrosol.

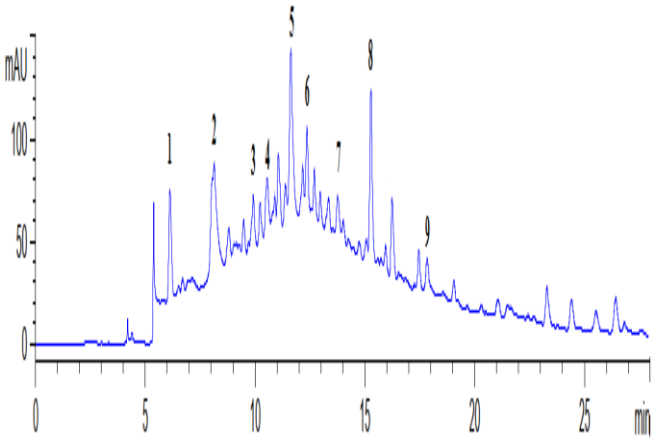

Figure 1: HPLC chromatogram of olive cake extract. Oleuropein (RT = 6.12 min; peak 1), tyrosol (RT = 8.14 min; peak 2) gallic acid (RT = 9.91 min; peak 3), caffeic acid (RT = 10.55 min; peak 4), hydroxytyrosol (RT = 11.62 min; peak 5), vanillic acid (RT = 12.35 min; peak 6), verbascoside (RT = 13.76 min; peak 7), oleacein (RT = 15.27 min; peak 8) and ferulic acid (RT = 17.83 min; peak 9).

Effect of OC on body weight, body fat content, Lee index and food intake

Previous studies have reported that chronic exposure to a high-calorie diet from infancy leads to obesity development in adulthood.[27] To investigate the effect of OC on obesity, post-weaned rats were exposed to a HFD until adulthood, and then treated with OC for 4 weeks. As shown in Figure 1A-C, feeding weaned rats with a HFD led to an increase in body weight and body fat content and resulted in obesity according to the Lee index[28] in adulthood than the control group rats, which was in line with other reports.[29] The increase in body weight probably results from the storage of excess calories from HFD consumption. OC treatment reduced body weight (7%), Lee index (6%), and body fat content (30%) at the end of the study without affecting food intake (Figure 1D) compared to the HFD group. For obese individuals, a reduction of 5% to 10% in body weight is recommended as a therapeutic target,[30] suggesting that OC has an anti-obesity effect, which can probably be attributed to its constituents. Indeed, the phenolic compounds released during OC digestion in the gastrointestinal tract inhibit lipid digestion by suppressing pancreatic lipase activity,[10,31] thus reducing lipid absorption and weight gain. In addition, olive seeds have been reported to inhibit adipocyte differentiation in mice.[14]

Effects of OC on kidney index and renal function markers

Obesity is a major risk factor for the development of kidney disease, in particular childhood obesity.[5] Previous studies reported that feeding an HFD to rats induces obesity and obesity-related kidney disease (ORG).[32] The present study showed that the kidney indices were significantly higher in the HFD rats than the control rats ($p < 0.05$). Additionally, creatinine and urea, as well as uric acid concentrations were increased in the HFD group than in the C group ($p < 0.05$) (Table 1). These parameters are used as biochemical markers of renal function, and their increased serum levels indicate kidney damage.[33] The alterations of renal function biomarkers were also accompanied by a reduction in glomerular filtration and an increase in urinary protein excretion. Furthermore, the elevated proteinuria observed in the HFD group was found to be an indicator of obesity-related kidney disease[31] as well as glomerular injury.[34] These findings indicate the presence of kidney disease in adult obese rats. This aligns with Muller et al.[35] findings, who indicated that post-weaning exposure to HFD induces kidney injury in adult rats. OC treatment decreased creatinine, urea, uric acid, and proteinuria levels, improved EGF and kidney function, and illustrated a reduction in the development of obesity-related kidney disease by OC. These results are consistent with our previous findings, where we reported that OC consumption improves renal function. This beneficial effect of OC on the kidneys can be related to one or all of its constituents. Polyphenols are the most abundant compounds in OC, and there is growing evidence that polyphenols offer great potential for protection against kidney disease.[36] In experimental studies, many phenolic compounds found in OC have shown similar beneficial effects on kidney function.

Histopathological effect of OC

Exposure of rats to an HFD diet results in obesity-associated glomerulopathy, an ORG. This condition is characterized by glomerulomegaly, which can be diagnosed by histopathological studies.[37] We therefore examined histological sections of kidneys from the different groups. As shown in Figure 3a, the H&E section of the HFD group shows glomerular hypertrophy and tubular lumen distortion than the C group. These findings are consistent with the histopathological changes observed in experimental ORG models.[38,39] These changes were improved in the rats fed the high-fat diet supplemented with olive cake (Figure 2C) than the HFD group, suggesting that OC may reduce obesity-related glomerulopathy. The HFD group showed lipid accumulation in glomeruli, similar to previous studies.[38] However, OC treatment reduced lipid accumulation, which may reduce lipotoxicity as well as glomerular hypertrophy and tubular damage.[40] Histomorphometric analyses showed significant differences in the glomerular tuft area and tubule size among the groups ($p < 0.05$). The glomerular tuft area and tubule diameter were higher in the HFD rats in comparison with the C rats ($p < 0.05$); however, the glomerular tuft area was significantly decreased in the HFD-OC group than the HFD group (Figures 3b). These results demonstrate the effective protective effects of OC on obesity-related glomerulopathy. Further studies are needed to understand the mechanisms by which OC protects against ORG.

Table 1: Effect of OC on kidney functional markers

	C	HFD	HFD-OC
Kidney index	0.30 ± 0.05	0.43 ± 0.20*	0.40 ± 0.01#
Creatinine (mg/dL)	0.31 ± 0.02	0.54 ± 0.04*	0.35 ± 0.017#
Urea (mg/dL)	27.70 ± 1.45	48.82 ± 2.66*	34.64 ± 1.70#

Uric acid (mg/dL)	4.10 ± 0.66	6.30 ± 0.98*	5.97 ± 0.32*
Urinary protein (mg/24h)	0.38 ± 0.03	1.18 ± 0.16*	0.68 ± 0.06*#
Glomerular filtration rate (mL/min)	0.95 ± 0.10	0.45 ± 0.07*	0.88 ± 0.05#

C: Rats were fed a standard diet; **HFD:** rats fed the high-fat diet; **HFD-OC:** rats fed the high-fat diet supplemented with olive cake (OC) (7.5%). Data represent mean ± standard deviation (n = 6). *P < 0.05 compared to the control group. #P < 0.05 compared to the HFD group.

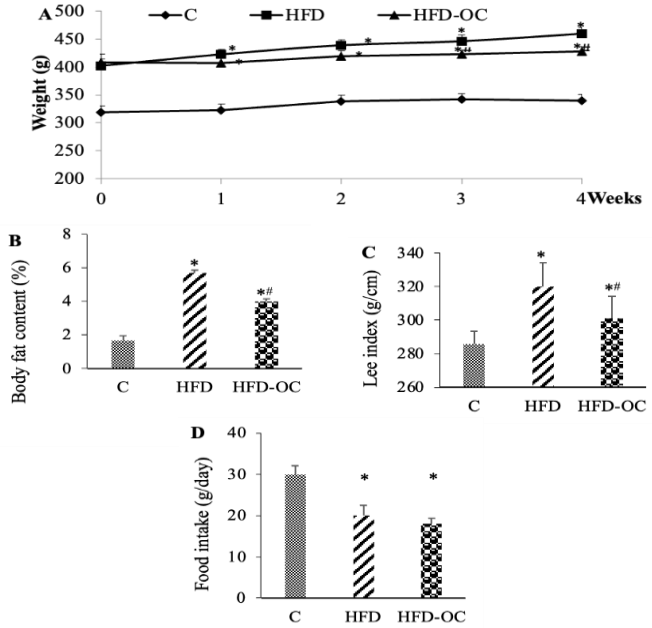

Figure 2: Effect of OC on body weight (A), food intake (B), body fat content (C) and Lee index (D).
C: Rats were fed a standard diet; **HFD:** Rats fed the high-fat diet; **HFD-OC:** rats fed the high-fat diet supplemented with olive cake (OC) (7.5%). Data represent mean ± standard deviation (n = 6). *P < 0.05 compared to the control group. #P < 0.05 compared to the HFD group.

Effects of OC on blood pressure, blood glucose, oxidative stress, inflammatory biomarkers and kidney lipid profile.

To understand how OC prevents obesity-related glomerulopathy (ORG) in rats made obese since weaning, we assessed its effect on blood glucose, oxidative stress, inflammatory biomarkers, and renal lipotoxicity (lipid profile), which are involved in the development of ORG.[6] The results are presented in Table 2. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and blood glucose were similar between the different groups. On the other hand, there was a significant increase in serum levels of TNF-α, IL-6, renal triglycerides (TG), and total cholesterol (TC) in the HFD group than the C group ($p < 0.05$). In addition, an increase in lipid peroxidation end products (TBARS, CD and LOOH), protein oxidation (PC and AOPP), and lower values in antioxidant enzyme activities (SOD, CAT and GSH-Px) in renal tissues were observed in the HFD group ($p < 0.05$). These results suggest that, in this study, HFD induces obesity-related glomerulopathy in rats through lipotoxicity, inflammation, and oxidative stress, rather than through increased blood pressure and blood glucose, as reported by Yan et al.[41] In this study, unlike the previous one, rats were fed a HFD from weaning, which potentially makes them resistant to hypertension and hyperglycemia induced by the HFD. OC treatment has no impact on glycemia or blood pressure. However, it reduced IL6 levels, lipotoxicity, and oxidative stress. These results illustrated the anti-inflammatory, anti-lipotoxic, and antioxidant properties of OC. Several studies have reported that certain drugs and bioactive compounds mitigate ORG by reducing kidney lipid deposition,[37] inflammation[42] and oxidative stress.[43] Thus, we consider that the effect of OC on these three factors constitutes one of these mechanisms of ORG prevention.

Olive cake is composed of olive pulp, skin, seed, and fragments of stones, as well as a small amount of residual oil. Studies reported that feeding pulp or oil down-regulates the expression of genes (SREBP-2, HMG-CoAR and CYP7A1) involved in de novo cholesterol biosynthesis and increases the expression of genes (ABCA1 and LDLR, CD-36) involved in cholesterol efflux.[44] OC also contains several minerals including potassium, calcium, magnesium, sodium and phosphorus.[45] A recent study reported that calcium consumption reduces renal triglyceride and cholesterol accumulation and thus protects the kidneys from obesity-related kidney damage via inhibition of renal lipogenesis activity through increased AMPK expression and decreased fatty acid synthase and acetyl coenzyme carboxylase activity.[46] P38 MAPK is a member of the MAPKs family that plays a crucial role in proinflammatory cytokine production, ORG development and progression.[41] activation of P38 MAPKs, resulting in renal cell proliferation, glomerular hypertrophy and tubular damage.[29] It has been reported that supplementation with zinc inhibit P38 MAPK activity and reduces IL6 and TNF-α levels in post-weanling mice fed the HFD.[39] The reduction in oxidative stress in HFD-OC groups can be attributed to the ability of OC's bioactive compounds to neutralize free radicals, as demonstrated by Samba and Bouderbala.[47] In addition, OC consumption has been shown to reduce ROS production and increase superoxide dismutase (SOD) activity in the kidney.[11] Furthermore, recent studies also show that vanillic acid can regulate redox homeostasis by activating the Nrf2/HO-1 pathway, thereby increasing SOD and GPX activity to maintain intracellular redox balance.[48]

DPP-4 is a new adipokine secreted from the adipose tissue, which plays an important role in the regulation of inflammation in obesity-related glomerulopathies.[37] Evidence showed that DPP4 activity increases in obesity-induced kidney diseases.[49] It has recently been reported that DPP4 inhibitors have anti-obesity[50] and renoprotective activity.[51] In the present study, an increase in DPP4 activity was noted in the HFD group than the C group; this increase was attenuated by OC supplementation, suggesting that OC inhibits DPP4 activity, which may contribute to its beneficial effects against ORG. Various studies have shown that DPP4 inhibitors can protect against kidney damage. For example, linagliptin improves the glomerular filtration barrier, oxidative stress and attenuates obesity-related glomerulomegaly in obese Zucker rats.[50]

Molecular docking

Molecular docking (MD) is a simulation technique used to predict the interaction between a bioactive molecule and the receptor of a target protein. It is essential for drug development.[51] MD explores different ligand conformations in the receptor binding site and evaluates interactions using scoring functions. We utilized molecular docking to study the interaction of two bioactive compounds identified in OC (ferulic acid and vanillic acid) with mitogen-activated protein kinases P38 (MAPK p38) and DPP4. Empagliflozin is employed as a p38 MAPK inhibitor, while Linagliptin is used as a DPP4 inhibitor. Binding energy was used to assess these interactions, a value below -5.0 kcal/mol indicated good binding empaglifozin activity.[52] Results showed that the binding energy between FA and VA with p38 MAPK and DPP4 was less than -5 kcal/mol (Table 3), signifying strong binding interactions between these two and p38 MAPK and DPP4. Furthermore, the binding energy of FA with these target proteins was comparable to that of empagliflozin and linagliptin, anti-diabetic drugs used in the treatment of obesity-related kidney disease.[50,53] This result indicates that FA and VA bind to MAPK p38 and DPP4 and inhibit their activity.

Figures 3 and 4 show the interaction between FA and VA and MAPK p38 and DPP4 and demonstrated the formation of hydrogen bonds, alkyl and pi-alkyl interactions, as well as other interactions. Like empagliflozin, FA and VA interacted with p38 MAPK via a hydrogen bond with ARG 57. In addition, VA and empagliflozin also formed a hydrogen bond with GLU 61 (Figure 4). FU interacts with DPP4 amino acid residues ARG 358, ARG 356 and ILE 407, while VA forms a

hydrogen bond with DPP4 residue GLU 61. Linagliptin forms hydrogen bonds with Ser 630, HIS 740, Ser 209 and GLU 205 ARG 553 in the catalytic pocket of DPP4 (Figure 5). Previous studies showed that residues SER 630, HIS, 740 ARG 125, PHE 357, ARG 358, TYR 631, VAL 656 and ASN 710 were involved in the DPP4 inhibition.[54] Consequently, we hypothesize that the protective effect of OC against ORG may be linked to its modulation of p38 MAPK and DPP4 activity. However, further studies are needed to confirm this hypothesis.

Conclusion

Results of this study showed that feeding rats with high-fat diet during the post-weaning period led to obesity and glomerulopathy in adulthood. Treatment of obese rats with OC reversed this obesity and prevented obesity-related glomerulopathy in adult rats by reducing oxidative stress, renal lipid accumulation, inflammatory response, and dpp4 activity. Molecular docking data revealed that VA and FA could inhibit DPP4 and p38 MAPK to prevent obesity-related glomerulopathy. Future *in vivo* trials could be conducted to explore the potential efficacy of these two active OC compounds in the treatment of ORG.

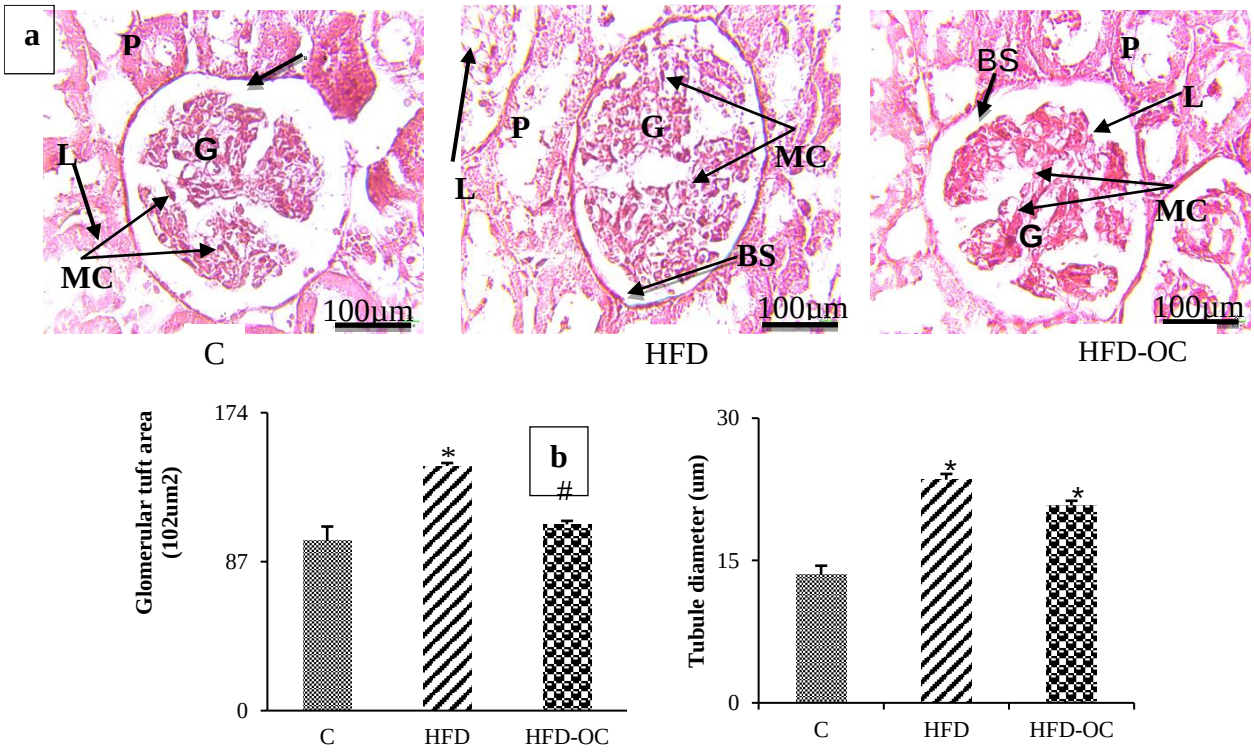

Figure 3: Glomerular and tubular structures in the H & E staining (x40; scale bar = 100 µm) (a) and morphometric parameters of renal histology (b)

C: Rats were fed a standard diet; **HFD:** Rats fed the high-fat diet; **HFD-OC:** rats fed the high-fat diet supplemented with olive cake (OC) (7.5%). Data represent mean ± standard deviation (n = 6). $^{*}P < 0.05$ compared to the control group. $^{\#}P < 0.05$ compared to the HFD group.

BS: Bowman space; **G:** Glomerulus; **L:** Lipid accumulation; **MC:** Mesangial cell; **P:** Proximal convoluted tubules.

Table 2: Effect of OC on blood pressure, glycemia, oxidative stress, inflammatory biomarkers and kidney lipid profile

	C	HFD	HFD-OC
Systolic blood pressure (mmHg)	114.33 ± 4.93	126.75 ± 5.82	123.35 ± 5.83$^{\#}$
Diastolic blood pressure (mmHg)	83.00 ± 5.00	97.00 ± 2.16	94.75 ± 4.03
Blood glucose (mmol/L)	6.36 ± 0.56	7.97 ± 0.30	6.69 ± 0.26
DPP-4 activity (ng/mL)	0.62 ± 0.62	1.25 ± 0.08*	0.68 ± 0.04$^{\#}$
Inflammation			
IL-6 (ng/mL)	37.22 ± 1.74	58.81 ±1.10*	46.31 ± 0.86$^{*\#}$
TNF-α (ng/mL)	2.88 ± 0.61	7.26 ± 0.80*	7.21 ± 0.28*
Renal lipid levels			
Total cholesterol (mg/g tissue)	45.30 ± 4.87	98.29 ± 2.33*	67.83 ± 0.74$^{*\#}$
Triglyceride (mg/g tissue)	35.20 ± 8.87	346.86 ± 19.02*	73.01 ± 1.03$^{*\#}$
Renal oxidative stress markers			
TBARS (mmol/g tissue)	45.30 ± 4.87	98.29 ± 2.33*	67.83 ± 0.74$^{*\#}$
CD (nmol/ mg protein)	0.13 ± 0.03	0.33 ± 0.02*	0.16 ± 0.03$^{\#}$

AOPPs (nmol/ mg protein)	217.30 ± 25.40	322.40 ± 3.8*	198.90 ± 6.10#
SOD (U/ mg protein)	25.56 ± 3.81	12.27 ± 1.85*	20.98 ± 0.61#
CAT (umol/min/g)	22.93 ± 0.47	13.45 ± 0.12*	25.49 ± 0.76#
GSH-Px (U/min/ mg protein)	18.02 ± 2.13	7.91 ± 0.64*	11.88 ± 0.13*#

C: Rats were fed a standard diet; **HFD:** Rats fed the high-fat diet; **HFD-OC:** rats fed the high-fat diet supplemented with olive cake (OC) (7.5%). Data represent mean ± standard deviation (n = 6). *P < 0.05 compared to the control group. #P < 0.05 compared to the HFD group.
AOPPs: Advanced oxidation protein products; **CAT:** Catalase; **GSH-Px:** Glutathione peroxidase; **IL-6:** Interleukin 6; **SOD:** Superoxide dismutase; **TBARS:** Thiobarbituric acid reactive substance; **TNF-*α*:** tumor necrosis factor *α*.

Table 3: Binding affinity, H-bonds and interacting amino acids of bioactive compounds of OC

	DPP4			p38 MAPK		
	Affinity (kcal/mol)	**N° H Binding**	**Interacting amino acids**	**Affinity (kcal/mol)**	**N° H Binding**	**Binding site interacting amino acid residues**
Ferulic acid	-7,6	3	ARG 358, ARG 356, ILE 407, TRP 201	-7,2	2	ARG (57; 67), HIS 64, LEU 55, PRO 58; GLN 60
Vanillic acid	-5,3	2	ASN 710, ARG 125, HIS 740,	-6,0	4	SER 56, GLU 71 ARG 57, GNL 60, LEU 55, ARG 67, HIS 64
Empaglifozin				-7.4	2	GLU 71 ARG 57, THR 68, LEU 174, ALA 34
Linagliptin	-8	4	SER 630, HIS 740, SER 209, GLU 205 ARG 125, TYR 547, PHE 357			

DPP4: Dipeptidyl peptidase-4; p38 MAPK: p38 mitogen-activated protein kinases.

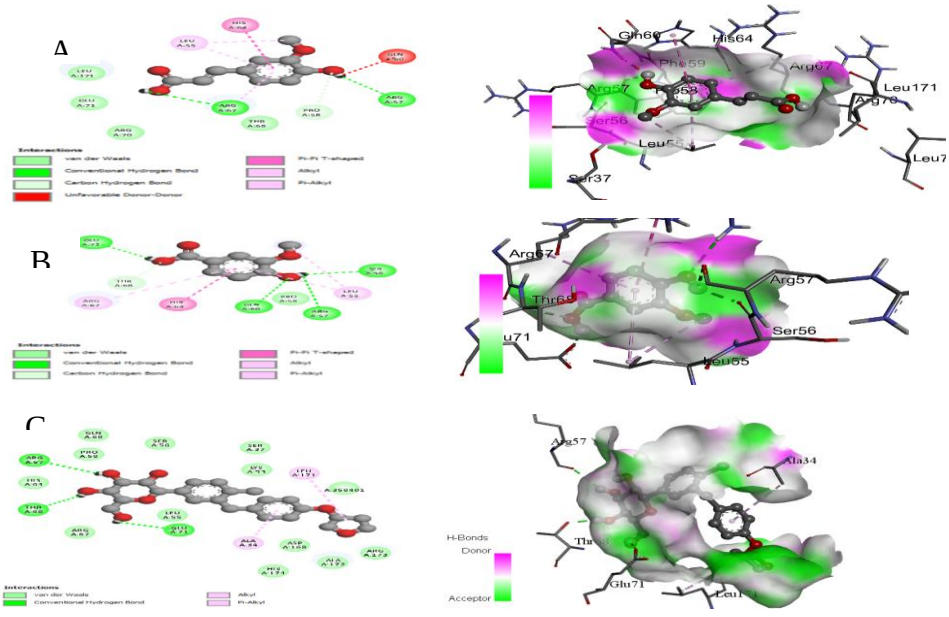

Figure 4: 2D (right) and 3D (left) interaction complexes of ferulic acid (A), vanillic (B) and empaglifozin (C) with p38 mitogen-activated protein kinases (p38 MAPK).

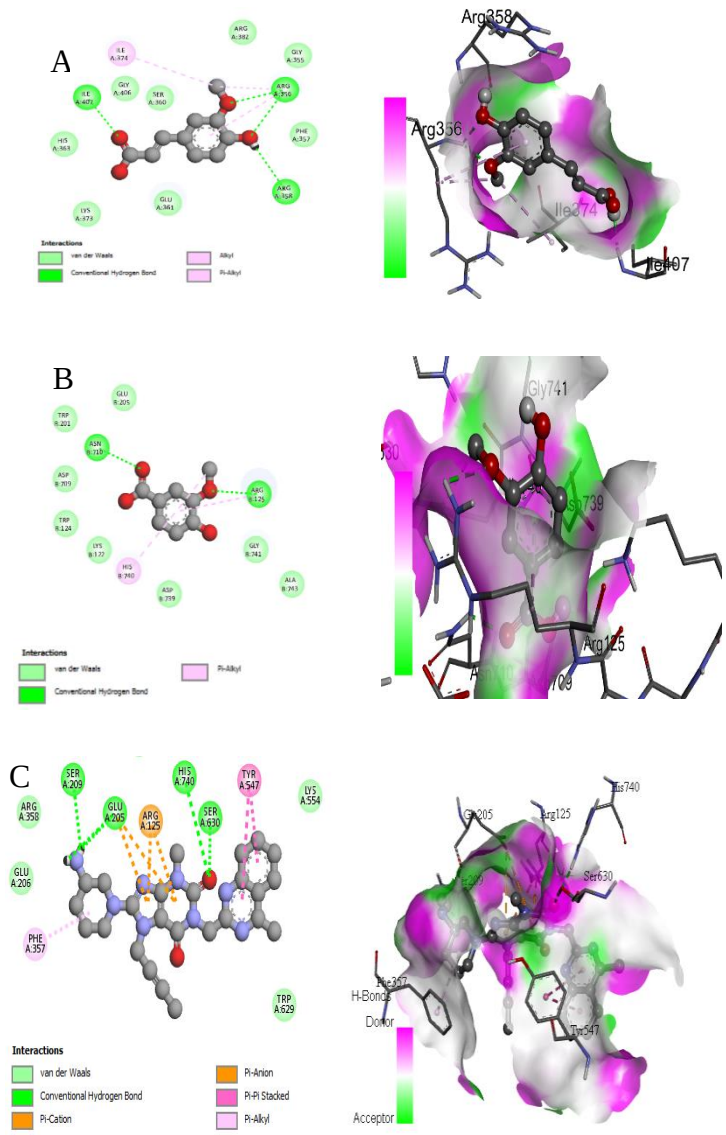

Figure 5: 2D (right) and 3D (left) interaction complexes of ferulic acid (A), vanillic acid (B) and linagliptin (C) with dipeptidyl peptidase-4 (DPP4

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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