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Effect of Acarbose and olive leaf extract (Oleuropein) on Glycemic index and Antioxidant Status in High Fructose and H_2O_2 Exposed Rats (Parts-II)

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Abstract

This study was carried out to investigate the effect of acarbose or combination of acarbose and Oleuropein (OLEU) on glycemic index and antioxidant status of male rats exposed to high fructose(40%) and 0.05% hydrogen peroxide H_2O_2 Thirty-two adult male rats were randomly divided into four equal groups: control group (CC); T1(HFr-H_2O_2) group were exposed to high fructose (40%) in drinking water and given orally 0.05% H_2O_2; T2: 30mg/kg of acarbose were given orally to HF-H_2O_2 treated rats, while combination of acarbose and Oleuropein (50mg/kg. B.W) were given orally to (HFr-H_2O_2) treated rats in group T3. Blood samples were taken at zero time and after two months of the experiment for measuring some parameters related to glycemic index and antioxidant status. The results declared an elevation in the glycemic index (insulin and glucose concentrations and insulin resistance (IR)) and malondialdehyde (MDA) and depression in glutathione (GSH) concentrations in T1 group at the end of experiment comparing to other treated groups. Antioxidant status and glycemic index were restored after acarbose or combination of acarbose and Oleuropein treatment characterized by significant depression of serum glucose, insulin concentrations and IR, and alleviation of oxidative stress clarified by and hyperglycemic effect of fructose and H_2O_2 that can be ameliorating by oral administration of acarbose. Besides, combination of OLUE supplement with acarbose possessed additive effects.

Keywords: Acarbose, fructose, glycemic index, H_2O_2, MDA, Oleuropein,

Introduction

Fructose is a monosaccharide found in fruits, vegetables and honeys. It is commercially utilized in beverages and foods, because of its high relative saccharinity, low price, palatability and taste enhancement [1]. Oxidative stress and inflammation that are thought to be associated with development of obesity, dyslipidemia, has been associated with high fat/high fructose diet [2,3]. Over intake of Fructose is lead to formation of Toxic Advanced Glycation End-Products [4, 5], and oxidative stress [6,7]. that are implicated in many chronic disease [8, 9]. Hydrogen peroxide (H_2O_2) is a non-polar molecule with relatively long half-life able to diffuse across biological membranes, Hydrogen peroxide has been considered a major species of biological reactive oxygen, generated as by product of respiration and end product of several metabolic reactions [10]. Majority H_2O_2 is produced by dismutation of superoxide anion using superoxide dismutase (SOD) [11, 12, 13]. It is considered as an oxidizing agent, has

the ability to prevent plague and gingival inflammation [14], the damaging effect of H_2O_2 is attributed to generation hydroxyl radicals, and then oxidative stress, claimed to be responsible for many diseased condition [15, 16, 17, 18, 19]. Induction of atherosclerosis [20], dyslipidemia and DNA damage [21] were reported after H_2O_2 exposure in rats.

Acarbose, a class of oral medications known as alpha glucosidase inhibitors is used only to treat or prevent type 2 diabetes mellitus [22,23]. Acarbose and insulin therapy together are linked to a reduction in oxidative stress and inflammation in type 2 diabetes patients [24]. The favorable effects of ACA on intra-tumoral immunity, specifically on T cell immunity within the tumor microenvironment, highlight the possibility for future combination therapy with ACA and immunotherapies [25]. Combination of acarbose and metformin was associated with decrease mortality of Covid-19 in patient with type 2 diabetes [26].

Olive leaf extract (Oleuropein) is a free radical scavenger and antioxidant polyphenol with a variety of pharmacological actions, including anti-inflammatory, vasodilatory, hypotensive, antioxidant, anti-apoptotic, and neuroprotective activities [27, 28]. It has been shown that OLE has cardioprotective activity, could lessen myocardial damage after an acute myocardial infarction through its anti-oxidative and anti-inflammatory capacities [29].

According to available literature, several studies concerning beneficial and damaging effect of H_2O_2 have been well studied in vitro. yet very limited studies have concerned the detrimental effect of H_2O_2 in vivo system, likewise, its deleterious effect on its combination with fructose have not been elucidate. The beneficial effect of ACA and OLEU or their combination on some parameters correlated with anti-oxidant status and glycemic index in (HFr-H_2O_2) exposed rats were the aim of this study.

Materials and Methods

All procedures used in this study were reviewed and approved by The Scientific Committee of the College of Veterinary Medicine, University of Baghdad in compliance with the ethical principles of animal welfare.

Thirty-two adult male rats were randomly divided into four equal groups (eight rat/group), and handled for two months as follows: group one animals served as the control group (CC), group two (T1) animals were given high fructose (40%) in drinking water and 0.05% Hydrogen Peroxide (H_2O_2) orally [30], group three (T2) Rats in this group were handled as in group T1 in addition to given orally acarbose (30mg/kg) [31], group four (T3) Rats in this group were handled as in group T2 in addition to given orally olive leaf extract (oleuropein) (50mg/kg) B.W.[32]. Heart puncture technique was used to obtain blood samples at the beginning and end of the study. Serum samples were used for measuring the following parameters: Glycemic index: (Insulin and glucose concentrations using glucose and insulin hormone kit (Biosystem/ Spine) and insulin resistance (IR) according to [33], serum reduced glutathione (GSH) concentration according to [34,35] and malondialdehyde (MDA) concentration as described by [36]. Two-way analysis of variance was used to statistically examine differences between experimental groups (ANOVA) [37].

Results and Discussion

The result in table 1, 2 and 3 showed that exposure of rat to 40% fructose in drinking water and 0.05% H_2O_2 (T3 group) orally for two month caused significant increase ($P < 0.05$) in serum concentration of glucose, insulin hormones and IR comparing to the value in control and other treated groups. While oral intubation of acarbose (T2 group) or acarbose plus oleuropein to (HF/ H_2O_2) exposed rats showed hypoglycemic activity and improved of glycemic index clarified by significant ($P < 0.05$) decrease in previous mentioned parameters comparing to T1, Significant ($P < 0.05$) difference between T2 and T3 were also present. It seems that combination of ACA and OLEU (T3 groups) showed significant decrease ($P<0.05$) comparing to (T2). Besides the values of IR and insulin hormone concentration in T3 group

tends to return to normal at the end of the experiment for the control., The result also showed significant (P<0.05) increase in serum MDA and elevation in GSH concentrations in T1 (HFr/ H_2O_2) group comparing to another treated group (in table 4 and table 5 respectively). Besides, significant (P<0.05) decrease in serum MDA and elevation in GSH concentrations after treatment with acarbose (T2 group) or combination of ACA and OLEU (T3 group) comparing to T1 indicating improvement in the antioxidant status, it seems that combination of ACA and OLEU, ameliorate oxidative status better than using ACA alone.

Table 1. Effect of acarbose or acarbose- olive leaf extract (Oleuropein) combination on serum glucose concentration (mg/dl) in high fructose / H_2O_2 exposed adult male rats.

Groups Time	CC	T-1	T- 2	T- 3
Zero time	89.70±2.17 Aa	89.39±1.72 Ab	89.15±1.55 Ab	86.69±1.94 Ab
After two months	87.16±1.64 Da	236.06±17.35 Aa	145.39±1.38 Ba	109.86±3.13 Ca

The values are shown as Mean ± SE, where n=8.

CC: control group, **T1:** Rats were given drinking water containing 40% fructose and given orally 0.05% of H_2O_2., **T2:** Rats were handled as in group T1 in addition to given orally acarbose 30mg/kg. B.W, **T3:** Rats were handled as in group T2 in addition to given orally Oleuropein 50mg/kg B.W.

*Significant difference between groups is horizontally represented by different capital letters (p<0.05).

*Significant differences in small letters shown vertically within group (p<0.05).

Table 2. Effect of acarbose or acarbose- olive leaf extract (Oleuropein) combination on serum insulin hormone concentration (ulU/ml) in high fructose / H_2O_2 exposed adult male rats.

group Time	CC	T-1	T- 2	T- 3
Zero time	4.68±0.18 Aa	5.10±0.35 Ab	4.61±0.24 Ab	5.03±0.26 Aa
After two months	5.16±0.26 Ca	11.98±0.30 Aa	7.79±0.40 Ba	5.67±0.21 Ca

The values are shown as Mean ± SE, where n=8.

CC: control group, **T1:** Rats were given drinking water containing 40% fructose and given orally 0.05% of H_2O_2., **T2:** Rats were handled as in group T1 in addition to given orally acarbose 30mg/kg. B.W, **T3:** Rats were handled as in group T2 in addition to given orally Oleuropein 50mg/kg B.W.

*Significant difference between groups is horizontally represented by different capital letters (p<0.05).

*Significant differences in small letters shown vertically within group (p<0.05).

Table 3. Effect of acarbose or acarbose- olive leaf extract (Oleuropein) combination on insulin resistance (IR) in high fructose / H_2O_2 exposed adult male rats.

group Time	CC	T-1	T- 2	T- 3
Zero time	1.038±0.055 Aa	1.125±0.080 Ab	1.018±0.070 Ab	1.074±0.053 Ab
After two months	1.110±0.054 Ca	6.954±0.461 Aa	2.797±0.154 Ba	1.536±0.067 Ca

The values are shown as Mean ± SE, where n=8.

CC: control group, **T1:** Rats were given drinking water containing 40% fructose and given orally 0.05% of H_2O_2., **T2:** Rats were handled as in group T1 in addition to given orally acarbose 30mg/kg. B.W, **T3:** Rats were handled as in group T2 in addition to given orally Oleuropein 50mg/kg B.W.

*Significant difference between groups is horizontally represented by different capital letters ($p<0.05$).

*Significant differences in small letters shown vertically within group ($p<0.05$).

Table 4. Effect of acarbose or acarbose- olive leaf extract (oleuropein) combination on serum rat malondialdehyde (MDA) concentration (Umol/l) in high fructose / H_2O_2 exposed adult male rats.

group Time	CC	T-1	T- 2	T- 3
Zero time	4.18±0.13 Aa	4.13±0.16 Ab	4.22±0.19 Ab	4.49±0.12 Ab
After two months	4.33±0.13 Da	14.24±0.30 Aa	8.09±0.18 Ba	6.54±0.56 Ca

The values are shown as Mean ± SE, where n=8.

CC: control group, **T1:** Rats were given drinking water containing 40% fructose and given orally 0.05% of H_2O_2, **T2:** Rats were handled as in group T1 in addition to given orally acarbose 30mg/kg. B.W, **T3:** Rats were handled as in group T2 in addition to given orally Oleuropein 50mg/kg B.W.

*Significant difference between groups is horizontally represented by different capital letters ($p<0.05$).

*Significant differences in small letters shown vertically within group ($p<0.05$).

Table 5. Effect of acarbose or acarbose- olive leaf extract (oleuropein) combination on serum levels of reduced glutathione (GSH) in rats (Umol/l) In high fructose / H_2O_2 exposed adult male rats.

group Time	CC	T-1	T- 2	T- 3
Zero time	7.48±0.66 Aa	7.91±0.26 Aa	7.73±0.38 Ab	7.72±0.33 Ab
After two months	7.65±0.27 Ca	2.81±0.18 Db	8.99±0.42 Ba	11.61±0.62 Aa

The values are shown as Mean ± SE, where n=8.

CC: control group, **T1:** Rats were given drinking water containing 40% fructose and given orally 0.05% of H2O2., **T2:** Rats were handled as in group T1 in addition to given orally acarbose 30mg/kg. B.W, **T3:** Rats were handled as in group T2 in addition to given orally Oleuropein 50mg/kg B.W.

*Significant difference between groups is horizontally represented by different capital letters ($p<0.05$).

*Significant differences in small letters shown vertically within group ($p<0.05$).

The result in the present study pointed to beneficial effect of acarbose in improving glycemic index of rat exposed to (HFr/ H_2O_2). The hypoglycemic effect of ACA has been documented by many researchers [38,39,40] In the small intestine, it can also competitively bind to -glucosidase and prevent the breakdown of oligosaccharides and polysaccharides, delaying the absorption of intestinal glucose and regulating blood sugar levels [41,42,43]. Combination of antidiabetic plant such as Artemisia roxburghiana with acarbose caused hypoglycemia by inhibition of glucosidase and a-amylase enzymes [40]. Acarbose can lower post-prandial hyperglycemia by inhibiting a-amylase and -glucosidase activity [44,45].

An increase in hepatic glucokinase and depression in glycolytic products concentration by acarbose could be a mechanism of its hypoglycemic effect [31]. Acarbose inhibit carbohydrates digestion and produce weight loss by increase glucagon like peptide-1 **[**46]. Acarbose improve insulin sensitivity via activation of peroxisome proliferator–activated receptor γ (PPAR γ), new singling pathway related to glucose metabolism and insulin singling proteins that produce glucose lowering effect and decrease insulin resistance [47].

The result also showed significant increase in GSH and decrease in MDA by acarbose indicated its anti-oxidant effect that ameliorates oxidative stress as documented by [24]. Hyperglycemia could play a role in oxidative stress, it elevated IR, impaired glucose tolerance and regarded as a diabetes's main source of oxidative damage is lipid peroxidation [48]. Actually, through a number of different processes, hyperglycemia alters the redox state of cells, it promotes increased NADPH oxidase activation [49]. changes their regulation of protein tyrosine kinase and of protein kinase C, it leads to sorbitol accumulation and increases NADH/NAD+ ratio and decreases NADPH/NADP+ ratio via hyperactivity of the sorbitol (polyol) pathway **[**50**].** Accordingly, the anti-oxidant effect of acarbose could be explained by its hypoglycemic activity.

The result here in study recorded the hypoglycemic and antioxidant of oleuropein in T3 treated rats. The effectiveness of OLEU and hydroxytyrosol extract in restoring glucose, insulin, and the HOMA-IR was similar with the earlier report of **[**51,52**]**. Olive leaf may reduce hyperglycemia by enhancing peripheral tissue absorption of glucose **[**53], and encourage pancreatic cells to secrete insulin [54], oleuropein can have a significant impact on how diabetes complications are treated and prevented [55]. Oleuropein's hypoglycemic impact is due to increased glucose uptake in peripheral tissues, insulin release, and decrease of intestinal glucose absorption rate [56,57]. In addition, OLUE, a natural antioxidant with potential to slow the course of diabetes by releasing insulin-induced glucose that reduced high blood sugar, is well known for its anti-inflammatory qualities **[**58**].**

The anti-oxidant effect of OLEU has been clarified by many researches **[**32**,**59 **,**60,61**].** OLEU suppresses cytotoxicity induced by H_2O_2 and protect cells against oxidative stress in vitro [62], oleuropein is also reported to possess the highest superoxide dismutase (SOD)-like activity among other fractions of oleuropein [63]. The reduced lipid peroxidation biomarker observed after OLEU treatment may be attributable to the significant antioxidant roles played by hydroxytyrosol, oleuropein, and oleuropein aglycone. This strength may be explained by their capacity to trap radicals before they reach their cellular targets and breakdown free radicals by quenching reactive oxygen species [64, 65**].** The three compounds shared 3,4-dihydroxyphenyl ethanol group may be responsible for the phenolic's activity and double bond in their chemical structure **[**66, 67, 68**].** In addition**,** Oleuropein's ability to chelate metal ions like Cu and Fe, which catalyze free radical generation reactions, as well as its capacity to inhibit a number of inflammatory enzymes like lipoxygenases without affecting the cyclo-oxygenase pathway, may contribute to its ability to prevent the formation of free radicals **[**69**].**

Exposure of rats to fructose in drinking water and H_2O_2 in the current study caused impairment of glycemic index and a case of

oxidative stress. Fructose fed animal display impaired glucose tolerance and decrease insulin sensitivity **[**70**],** decreased hepatic insulin clearance after carbohydrate overfeeding **[**71**].** In HFFD the associated IR and the increased fasting glucose level was due to inhibition phosphorylate p-IRS-1 inhibited/phosphorylate p-IRS-1, which in turn inhibited its downstream molecule Akt by its phosphorylation. The inhibition of this pathway extended to decrease GLUT-4 and consequently increase both serum insulin and fasting glucose levels, as well as disturb insulin sensitivity **[**72]. Disturbed energy balance caused by fructose overload and stress can result in an impaired ability of insulin to suppress gluconeogenesis and/or glycogenolysis and/or to stimulate glycogen synthesis in the liver, possibly leading to hepatic insulin resistance [73, 74]. Through extracellular ATP signaling, long-term fructose stimulation makes pancreatic b-cells more sensitive to the effects of glucose on insulin release [75]. Moreover, in both rodent models and human individuals, it easily causes hyperinsulinemia [76, 77], most probably due to insulin resistance [78]. All of the negative effects of fructose have been linked to an increase in reactive oxygen species (ROS) [79]. Oxidative stress increased as a result of a high fructose diet. reduced levels of the body's enzymatic and non-enzymatic antioxidants [80]. Following fructose ingestion, the ongoing hyperglycemia, hyperinsulinemia, IR, and dyslipidemia promote the generation of free radicals, mainly ROS, which leads to the onset of oxidative stress [81, 82**].** Accordingly, hyperglycemia induced by fructose could be a mechanism of oxidative stress induced by fructose.

Elevation in serum glucose and insulin concentration and IR was recorded after exposure to fructose and H_2O_2. Oxidative stress and free radical's producer ability of H_2O_2 has been documented **[**83, 84, 85**].** H_2O_2 induced Ferroptosis (form of necrosis caused iron induced accumulation lipid hydroperoxide) leading to oxidative stress **[**86]. Nuclear factor erythroid-related factor 2 (Nrf2), mediated heme oxygenase-1 (HO-1) expression, has been implicated in several detoxifying and antioxidant defense processes [87,88], and play a key role against oxidative stress. It can be speculated that H_2O_2 -induced oxidative injury by reducing the generation of intracellular reactive oxygen species and malondialdehyde and down regulate HO-1 and Nrf2 expressions **[**89], could be mechanism of oxidative stress induced by hydrogen peroxide. On the other hand, oxidative stress that could be produced by reserving H_2O_2 may play a role in glycemic index and glucose homeostasis. The resultant oxidative stress caused activation. signally pathway mediators of IR including activation of uncoupling protein -2 causing IR and pancreatic B-cell dysfunction [90, 91**],** has been claimed to be a mechanism for detrimental role of H_2O_2 on glycemic index

Conclusions

On conclusion, the current study documented the oxidative and hyperglycemic effect of fructose and H2O2 that can be ameliorating by oral administration of acarbose. Besides, combination of OLUE supplement with acarbose possessed additive effects.

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Conflict of interest

The researches declare that there is no conflict of interest in the publication of this paper.

References

1. Hirai A. Hariman H. Lunes M. Kikuchi M. Osiro K. and etal., (2009). Dietary fructose, fruits, fruit juices and glucose tolerance status in japaneseBrazilians.Nutr Metab Cardiovasc Dis., 19(2):77-83.

2. Feillet-Coudray, C., Fouret, G., Vigor, C., Bonafos, B., Jover, B., Blachnio-Zabielska, A., ... & Coudray, C. (2019). Long-Term Measures of Dyslipidemia, Inflammation, and Oxidative Stress in Rats Fed a High-Fat/High-Fructose Diet. *Lipids*, *54*(1), 81-97.

3. Todoric, J., Di Caro, G., Reibe, S., Henstridge, D. C., Green, C. R., Vrbanac, A., ... & Karin, M. (2020). Fructose stimulated de novo lipogenesis is promoted by inflammation. *Nature metabolism*, *2*(10), 1034-1045.

4. Gugliucci, A. (2017). Formation of fructose-mediated advanced glycation end products and their roles in metabolic and inflammatory diseases, Adv. Nutr., 8(1): 54–62.

5. Sakasai-Sakai, A., Takata, T., Takino, J. I., & Takeuchi, M. (2019). The relevance of toxic AGEs (TAGE) cytotoxicity to NASH pathogenesis: A mini-review. *Nutrients*, *11*(2), 462.

6. Castro, M.C., Francini, F., Schinella, G., Caldiz, C.I., Zubiría, M.G., Gagliardino, J.J. and Massa, M.L. (2012). Apocynin administration prevents the changes induced by a fructose-rich diet on rat liver metabolism and the antioxidant system. Clin Sci., (123): 681–692.

7. 7.Sreeja, S., Geetha, R., Priyadarshini, E., Bhavani, K., & Anuradha, C. V. (2014). Substitution of soy protein for casein prevents oxidative modification and inflammatory response induced in rats fed high fructose diet. *International Scholarly Research Notices*, *2014*.

8. Shimomura, M., Oyama, J., Takeuchi, M., Shibata, Y., Yamamoto, Y., Kawasaki, T., Komoda, H., Kodama, K., Sakuma, M., Toyoda, S. (2016). Acute effects of statin on reduction of angiopoietin-like 2 and glyceraldehyde-derived advanced glycation end-products levels in patients with acute myocardial infarction: A message from SAMIT (Statin for Acute Myocardial Infarction Trial). Heart Vess., 31: 1583–1589.

9. Takeuchi, M., Takino, J. I., Sakasai-Sakai, A., Takata, T., & Tsutsumi, M. (2017). Toxic AGE (TAGE) theory for the pathophysiology of the onset/progression of NAFLD and ALD. *Nutrients*, *9*(6), 634.

10. .WinterbournC.C. (2017). Biological Production, Detection and Fate of Hydrogen Peroxide. Antioxi. Redox. Signal., 29(6): 1–32.

11. .Hayyan, M., Hashim ,M.A. and AlNashef, I.M. (2016). Superoxide Ion: Generation and Chemical Implications. Chemical Rev., 116 (5): 3029–85

12. Taati, B., Arazi, H., & Suzuki, K. (2020). Oxidative stress and inflammation induced by waterpipe tobacco smoking despite possible protective effects of exercise training: A review of the literature. *Antioxidants*, *9*(9), 777.

13. Fujita, H., Mae, K., Nagatani, H., Horie, M., & Nagamori, E. (2021). Effect of hydrogen peroxide concentration on the maintenance and differentiation of cultured skeletal muscle cells. *Journal of bioscience and bioengineering*, *131*(5), 572-578.

14. Muniz, F. W. M. G., Cavagni, J., Langa, G. P. J., Stewart, B., Malheiros, Z., & Rösing, C. K. (2020). A systematic review of the effect of oral rinsing with H2O2 on clinical and microbiological parameters related to plaque, gingivitis, and microbes. *International journal of dentistry*, *2020*.

15. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., ... & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 694.

16. Kaludercic, N., & Di Lisa, F. (2020). Mitochondrial ROS formation in the pathogenesis of diabetic cardiomyopathy. *Frontiers in Cardiovascular Medicine*, *7*, 12.

17. Kim, J., Choi, J. Y., Seo, J., & Choi, I. S. (2021). Neuroprotective effect of cannabidiol against hydrogen peroxide in hippocampal neuron culture. *Cannabis and Cannabinoid Research*, *6*(1), 40-47.Krentz, A. J., & Bailey, C. J. (2005). Oral antidiabetic agents. *Drugs*, *65*(3), 385-411.

18. Oguma, N., Takahashi, K., Okabe, S., & Ohta, T. (2021). Inhibitory effect of polysulfide, an endogenous sulfur compound, on oxidative stress-induced TRPA1 activation. *Neuroscience Letters*, *757*, 135982.

19. Cheng, X. M., Hu, Y. Y., Yang, T., Wu, N., & Wang, X. N. (2022). Reactive Oxygen Species and Oxidative Stress in Vascular-Related Diseases. *Oxidative Medicine and Cellular Longevity*, *2022*.

20. Abdul-Katum, A.H.and Khudair, K.K.(2008). Comparative study between the prophylactics effects of aqueous extract of Black Current (*Vitis vinferia* L.) and vitamin E on some biological parameters related with heart diseases in oxidative stressed rats. The Iraqi Journal of Veterinary Medicine. 32 (2): 227-252.

21. Ali, Z. S., & Khudair, K. K. (2019). Synthesis, Characterization of Silver Nanoparticles Using Nigella sativa Seeds and Study Their Effects on the Serum Lipid Profile and DNA Damage on the Rats' Blood Treated with Hydrogen Peroxide: Zainab Sattar Ali and Khalisa Khadim Khudair. *The Iraqi Journal of Veterinary Medicine*, *43*(2), 23-37.

22. Zhang, J. P., Wang, N., Xing, X. Y., Yang, Z. J., Wang, X., & Yang, W. Y. (2016). Efficacy of acarbose and metformin in newly diagnosed type 2 diabetes patients stratified by HbA1c levels: *Journal of Diabetes*, *8*(4), 559-567.

23. .Holman, R. R., Coleman, R. L., Chan, J. C., Chiasson, J.-L., Feng, H., Ge, J., Gerstein, H. C., Gray, R., Huo, Y., and Lang, Z. (2017). Effects of acarbose on cardiovascular and diabetes outcomes in patients with coronary heart disease and impaired glucose tolerance (ACE): a 49andomized, double-blind, placebocontrolled trial. The lancet Diabetes & endocrinology, 5(11), 877-886.

24. Li, F. F., Fu, L. Y., Xu, X. H., Su, X. F., Wu, J. D., & Ye, L. (2016). Analysis of the add-on effect of alpha-glucosidase inhibitor, acarbose in insulin therapy: a pilot study. Biomed Rep. 2016; 5 (4): 461–6. *MIDDLE EAST JOURNAL OF INTERNAL MEDICINE*, *2*(3), 3.

25. Turbitt, W. J., Orlandella, R. M., Gibson, J. T., & Norian, L. A. (2019). Acarbose, but not metformin, reduces tumor burden and improves intra-tumoral immune responses in a pre-clinical breast cancer model. *Cancer Research*, *79*(13_Supplement), 509-509.

26. Li, J., Wei, Q., McCowen, K. C., Xiong, W., Liu, J., Jiang, W., ... & Li, W. X. (2022). Inpatient use of metformin and acarbose is associated with reduced mortality of COVID-19

patients with type 2 diabetes mellitus. *Endocrinology, Diabetes & Metabolism*, *5*(1), e00301.

27. Pourkhodadad, S., Alirezaei, M., Moghaddasi, M., Ahmadvand, H., Karami, M., Delfan, B., & Khanipour, Z. (2016). Neuroprotective effects of oleuropein against cognitive dysfunction induced by colchicine in hippocampal CA1 area in rats. *The Journal of Physiological Sciences*, *66*(5), 397-405.

28. Zhao, Q., Bai, Y., Li, C., Yang, K., Wei, W., Li, Z., ... & Zhang, X. (2017). Oleuropein protects cardiomyocyte against apoptosis via activating the reperfusion injury salvage kinase pathway in vitro. Evidence-Based Complementary and Alternative Medicine, 2017.

29. Janahmadi, Z., Nekooeian, A. A., Moaref, A. R., & Emamghoreishi, M. (2015). Oleuropein offers cardioprotection in rats with acute myocardial infarction. *Cardiovascular toxicology*, *15*(1), 61-68.

30. Khudair, N. T., & Al-Okaily, B. N. (2022). Renal ameliorating effect of resveratrol in hydrogen peroxide induced male rats. *Iraqi Journal of Veterinary Sciences*, *36*(3), 571-577.

31. Obaid, Q. A., Khudair, K. K., & Al-Shammari, A. M. (2022). Glucose deprivation using 2-deoxyglucose and acarbose induce metabolic oxidative stress and apoptosis in female mice bearing breast cancer. *Biochimie*, *195*, 59-66.

32. Nasrallah, H., Aissa, I., Slim, C., Boujbiha, M. A., Zaouali, M. A., Bejaoui, M., ... & Abdennebi, H. B. (2020). Effect of oleuropein on oxidative stress, inflammation and apoptosis induced by ischemia-reperfusion injury in rat kidney. *Life Sciences*, *255*, 117833.

33. Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A., Treacher D.F. and Turner R.C.(1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28:412-419.

34. Nasif, Z. N. (2002). Molecular Characterization & Determinating The Level Of Oxidative Stress & Some Antioxidants In Iraqi Women With Breast Tumors.

35. Burtis, C. and Ashwood, E. (1999). Textbook of clinical chemistry. 3d Ed. London, 2(33): 1145-1150.

36. Jetawattana, S. (2005). Malondialdehyde (MDA), a lipid oxidation product. *Free Radicals in Biology and Medicine*, *22*(77/2), 9-18.

37. Snedeccor, GW. And Cochran, WG, (1973). Statistical methods. 6th Ed. The Iowa State University press., pp:238-248.

38. Zhang, B. W., Sang, Y. B., Sun, W. L., Yu, H. S., Ma, B. P., Xiu, Z. L., & Dong, Y. S. (2017a). Combination of flavonoids from Oroxylum indicum seed extracts and acarbose improves the inhibition of postprandial blood glucose: In vivo and in vitro study. *Biomedicine & Pharmacotherapy*, *91*, 890-898.

39. Holmbäck, U., Forslund, A., Grudén, S., Alderborn, G., Söderhäll, A., Hellström, P. M., & Lennernäs, H. (2020). Effects of a novel combination of orlistat and acarbose on tolerability, appetite, and glucose metabolism in persons with obesity. *Obesity science & practice*, *6*(3), 313-323

40. Kumar, A., Aswal, S., Chauhan, A., Semwal, R. B., Singh, R., Andola, H. C., ... & Semwal, D. K. (2022). Antidiabetic effect of aqueous-ethanol

extract from the aerial parts of Artemisia roxburghiana. *Natural Product Research*, *36*(5), 1300-1305.

41. Pishdad, R., Pishdad, P., & Pishdad, G. R. (2020). Acarbose versus Repaglinide in Diabetes Treatment: A New Appraisal of Two Old Rivals. *The American Journal of the Medical Sciences*, *359*(4), 212-217.

42. Ghabi, A., Brahmi, J., Alminderej, F., Messaoudi, S., Vidal, S., Kadri, A., & Aouadi, K. (2020). Multifunctional isoxazolidine derivatives as α-amylase and α-glucosidase inhibitors. *Bioorganic chemistry*, *98*, 103713

43. Gerstein, H. C., Coleman, R. L., Scott, C. A., Xu, S., Tuomilehto, J., Rydén, L., & Holman, R. R. (2020). Impact of Acarbose on incident diabetes and regression to normoglycemia in people with coronary heart disease and impaired glucose tolerance: insights from the ACE Trial. *Diabetes Care*, *43*(9), 2242-2247.

44. Attjioui, M., Ryan, S., Ristic, A. K., Higgins, T., Goni, O., Gibney, E., ... & O'Connell, S. (2020). Kinetics and mechanism of α-glucosidase inhibition by edible brown algae in the management of type 2 diabetes. *Proceedings of the Nutrition Society*, *79*(OCE2).

45. Ji, W., Yang, S., Zhang, W., Sun, Z., Wen, Q., & He, K. (2021). Pharmacodynamic comparison of acarbose tablets in Chinese healthy volunteers under chewing and swallowing conditions. *Journal of Clinical Pharmacy and Therapeutics*, *46*(3), 814-819.

46. Altay, M. (2022). Acarbose is again on the stage. *World Journal of Diabetes*, *13*(1), 1.

47. Chung, Y. C., Chen, Y. I., Lin, C. M., Chang, S. W., Hsu, T. H., Ho, W. J., ... & Tzeng, C. Y. (2020). Electroacupuncture combined with acarbose improves insulin sensitivity via peroxisome proliferator–activated receptor γ activation and produces a stronger glucose-lowering effect than acarbose alone in a rat model of steroid-induced insulin resistance. *Acupuncture in Medicine*, *38*(5), 335-342.

48. Ceriello, A., & Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arteriosclerosis, thrombosis, and vascular biology*, *24*(5), 816-823.

49. Carmiel-Haggai, M., Cederbaum, A. I., & Nieto, N. (2005). A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *The FASEB Journal*, *19*(1), 136-138.

50. Armstrong, A. M., Chestnutt, J. E., Gormley, M. J., & Young, I. S. (1996). The effect of dietary treatment on lipid peroxidation and antioxidant status in newly diagnosed noninsulin dependent diabetes. *Free Radical Biology and Medicine*, *21*(5), 719-726.

51. Wang, N., Liu, Y., Ma, Y., & Wen, D. (2018). Hydroxytyrosol ameliorates insulin resistance by modulating endoplasmic reticulum stress and prevents hepatic steatosis in diet-induced obesity mice. *The Journal of nutritional biochemistry*, *57*, 180-188.

52. Fki, I., Sayadi, S., Mahmoudi, A., Daoued, I., Marrekchi, R., & Ghorbel, H. (2020). Comparative study on beneficial effects of hydroxytyrosol- and oleuropein-rich olive leaf extracts on high-fat diet-induced lipid metabolism disturbance and liver injury in rats. BioMed Research International, 2020.

53. Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition*, *45*(4), 287-306.

54. De Bock, M., Derraik, J. G., Brennan, C. M., Biggs, J. B., Morgan, P. E., Hodgkinson, S. C., ... & Cutfield, W. S. (2013). Olive (Olea europaea L.) leaf polyphenols improve insulin sensitivity in middle-aged overweight men: a randomized, placebo-controlled, crossover trial. *PloS one*, *8*(3), e57622.

55. Abdelkarem, H. M., El-Sherif, M. A., Gomma, S. B., Kassem, S. S., & Abdelkader, M. M. (2022). Olive Leaf Powder Modulate Insulin Production and Circulating Adipokines in Streptozotocin Induced Diabetic Rats. Journal of Dietary Supplements, *19*(4): 550-565.

56. Pari, L., & Saravanan, G. (2002). Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *131*(1), 19-25.

57. Park, J. H., Jung, J. H., Yang, J. Y., & Kim, H. S. (2013). Olive leaf down-regulates the oxidative stress and immune dysregulation in streptozotocin-induced diabetic mice. *Nutrition research*, *33*(11), 942-951.

58. Ahmadvand, H., Noori, A., Dehnoo, M. G., Bagheri, S., & Cheraghi, R. A. (2014). Hypoglycemic, hypolipidemic and antiatherogenic effects of oleuropein in alloxan-induced Type 1 diabetic rats. *Asian Pacific journal of tropical disease*, *4*, S421-S425.

59. Merra, E., Calzaretti, G., Bobba, A., Storelli, M. M., & Casalino, E. (2014). Antioxidant role of hydroxytyrosol on oxidative stress in cadmium-intoxicated rats: different effect in spleen and testes. *Drug and Chemical Toxicology*, *37*(4), 420-426.

60. Borjan, D., Leitgeb, M., Knez, Ž., & Hrnčič, M. K. (2020). Microbiological and antioxidant activity of phenolic compounds in olive leaf extract. *Molecules*, *25*(24), 5946.

61. Çömez, M. S., Cellat, M., Özkan, H., Borazan, Y., Aydın, T., Gökçek, İ., ... & Özsoy, Ş. Y. (2020). Protective effect of oleuropein on ketamine-induced cardiotoxicity in rats. *Naunyn-Schmiedeberg's archives of pharmacology*, *393*(9), 1691-1699.

62. Ha, J. Y., Goo, S. Y., Sung, J. S., & Shin, H. S. (2009). Antioxidant and cytoprotective activity of the olive leaf (Olea europaea L. var. Kalamata) extracts on the mouse embryonic fibroblast cell. *Food Science and Biotechnology*, *18*(4), 965-970.

63. Choi, N. Y., & Shin, H. S. (2007). Physicochemical properties and physiological activities of Olive leaf (Olea europaea L. var. Kalamata) fractions. *Food Engineering Progress*.

64. Prasad, K. (1999). Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. *Circulation*, *99*(10), 1355-1362.

65. Srinivasan, P., Sabitha, K. E., & Shyamaladevi, C. S. (2007). Attenuation of 4-nitroquinoline 1-oxide induced in vitro lipid peroxidation by green tea polyphenols. *Life sciences*, *80*(12), 1080-1086.

66. Jemai, H., Fki, I., Bouaziz, M., Bouallagui, Z., El Feki, A., Isoda, H.,

& Sayadi, S. (2008). Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. *Journal of agricultural and food chemistry*, *56*(8), 2630-2636.

67. Lafka, T. I., Lazou, A. E., Sinanoglou, V. J., & Lazos, E. S. (2013). Phenolic extracts from wild olive leaves and their potential as edible oils antioxidants. *Foods*, *2*(1), 18-31.

68. Hassanzadeh, K., Akhtari, K., Hassanzadeh, H., Zarei, S. A., Fakhraei, N., & Hassanzadeh, K. (2014). The role of structural CH compared with phenolic OH sites on the antioxidant activity of oleuropein and its derivatives as a great non-flavonoid family of the olive components: a DFT study. *Food chemistry*, *164*, 251-258.

69. Visioli F, Galli C, Plasmati E, Viappiani S, Hernandez A, Colombo C, Sala A (2000) Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress. Circulation 102:2169–2171

70. Veličković, N., Teofilović, A., Ilić, D., Djordjevic, A., Vojnović Milutinović, D., Petrović, S., ... & Matić, G. (2019). Modulation of hepatic inflammation and energy-sensing pathways in the rat liver by high-fructose diet and chronic stress. *European journal of nutrition*, *58*(5), 1829-1845.

71. Bojsen-Møller, Kirstine N., Anne-Marie Lundsgaard, Sten Madsbad, Bente Kiens, and Jens Juul Holst. "Hepatic insulin clearance in regulation of systemic insulin concentrations—role of carbohydrate and energy availability." *Diabetes* 67, no. 11 (2018): 2129-2136.

72. Attia, R. T., Abdel-Mottaleb, Y., Abdallah, D. M., El-Abhar, H. S., & El-Maraghy, N. N. (2019). Raspberry ketone and Garcinia Cambogia rebalanced disrupted insulin resistance and leptin signaling in rats fed high fat fructose diet. *Biomedicine & Pharmacotherapy*, *110*, 500-509.

73. Li, L., Li, X., Zhou, W., & Messina, J. L. (2013). Acute psychological stress results in the rapid development of insulin resistance. *The Journal of endocrinology*, *217*(2), 175.

74. Zhang, D.M., Jiao, R.Q. and Kong,L.D. (2017b). High dietary fructose: Direct or indirect dangerous factors disturbance tissue and organ functions. Nutrients, 9(40: 335.

75. Bartley, C., Brun, T., Oberhauser, L., Grimaldi, M., Molica, F., Kwak, B. R., ... & Maechler, P. (2019). Chronic fructose renders pancreatic β-cells hyper-responsive to glucose-stimulated insulin secretion through extracellular ATP signaling. *American Journal of Physiology-Endocrinology and Metabolism*, *317*(1), E25-E41.

76. Ter Horst, K. W., Schene, M. R., Holman, R., Romijn, J. A., & Serlie, M. J. (2016). Effect of fructose consumption on insulin sensitivity in nondiabetic subjects: a systematic review and meta-analysis of diet-intervention trials. *The American journal of clinical nutrition*, *104*(6), 1562-1576.

77. Hannou, S. A., Haslam, D. E., McKeown, N. M., & Herman, M. A. (2018). Fructose metabolism and metabolic disease. *The Journal of clinical investigation*, *128*(2), 545-555.

78. Herman, M. A., & Samuel, V. T. (2016). The sweet path to metabolic demise: fructose and lipid synthesis. *Trends in Endocrinology & Metabolism*, *27*(10), 719-730.

79. Delbosc, S., Paizanis, E., Magous, R., Araiz, C., Dimo, T., Cristol, J. P., ... & Azay, J. (2005). Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. *Atherosclerosis*, *179*(1), 43-49.

80. Putakala, M., Gujjala, S., Nukala, S., & Desireddy, S. (2017). Beneficial effects of Phyllanthus amarus against high fructose diet induced insulin resistance and hepatic oxidative stress in male wistar rats. *Applied biochemistry and biotechnology*, *183*(3), 744-764.

81. Datta, K., Sinha, S., & Chattopadhyay, P. (2000). Reactive oxygen species in health and disease. *National Medical Journal of India*, *13*(6), 304-310.

82. Abolghasemi, J., Sharifi, M. H., Nasiri, K., & Akbari, A. (2020). Thyme oxymel by improving of inflammation, oxidative stress, dyslipidemia and homeostasis of some trace elements ameliorates obesity induced by high-fructose/fat diet in male rat. Biomedicine & Pharmacotherapy, 126 : 110079.

83. Halliwell, B. and Gutteridge, J.M.C. (2015). Free Radicals in Biology and Medicine. Oxford University Press, USA., Pp : 349-353.

84. Khojah, H.M., Ahmed, S., Abdel-Rahman ,M.S. and Hamza ,A.B. (2016). Reactive oxygen and nitrogen species in patients with rheumatoid arthritis as potential biomarkers for disease activity and the role of antioxidants. Free Radic. Biol. Med., 97: 285–291

85. Taqvi, S., Bhat, E. A., Sajjad, N., Sabir, J. S., Qureshi, A., Rather, I. A., & Rehman, S. (2021). Protective effect of vanillic acid in hydrogen peroxide-induced oxidative stress in D. Mel-2 cell line. *Saudi Journal of Biological Sciences*, *28*(3), 1795-1800.

86. Jiang, J., Li, J., Zhu, J., Yu, Y., Duan, G., Zhou, L., & Li, Y. (2020). Synthesis of sandwich-structured magnetic graphene-Zn-MOFs composites for quantitative determination of acarbose in rat plasma. *Talanta*, *209*, 120514.

87. Hu, Y., Liu, W., Huang, R., & Zhang, X. (2010). Postchallenge plasma glucose excursions, carotid intima-media thickness, and risk factors for atherosclerosis in Chinese population with type 2 diabetes. *Atherosclerosis*, *210*(1), 302-306.

88. He, X., Wang, L., Szklarz, G., Bi, Y., & Ma, Q. (2012). Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *Journal of Pharmacology and Experimental Therapeutics*, *342*(1), 81-90

89. Zhang, H., Yuan, B., Huang, H., Qu, S., Yang, S., & Zeng, Z. (2018). Gastrodin induced HO-1 and Nrf2 up-regulation to alleviate H 2 O 2-induced oxidative stress in mouse liver sinusoidal endothelial cells through p38 MAPK phosphorylation. *Brazilian Journal of Medical and Biological Research*, *51*.

90. Evans, J. L. (2003). Gold fi ne ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction. *Diabetes*, *52*(1), 1-8.

91. Krauss, S., Zhang, C. Y., Scorrano, L., Dalgaard, L. T., St-Pierre, J., Grey, S. T., & Lowell, B. B. (2003). Superoxide-mediated activation of uncoupling protein 2 causes pancreatic

β cell dysfunction. *The Journal of clinical investigation*, *112*(12), 1831-1842