The anti-oxidant and DNA protective effects of Cicer arietinum ethanolic extract in comparison to rosuvastatin in Triton X-100 treated female rats

Wahb. K. Kadim[1], Huda F. Hasan[2]
[1, 2]Department of Physiology, Biochemistry and Pharmacology-College of Veterinary Medicine University of Baghdad-Iraq
Email: dr.hudaalqaraghuli@yahoo.com
Email: wahbmosawi@gmail.com

Abstract
The current study aimed to detect of the protective effect of the Cicer arietinum seed extract to enhancing of anti-oxidant capacity and anti-inflammatory activity with DNA protectant effect in comparison with rosuvastatin in animals subjected to oxidative stress by triton X-100. Thirty female rats were equally separated into five groups and treated for (28 days). The first group termed G1 treated with distilled water which was negative control group, the second one G2 treated with triton X-100 at dose (100 mg/kg) I.P positive control group, the third group G3 treated with Cicer arietinum crude extract at dose 349.015 /kg B.W after induction with triton X-100, the fourth group G4 treated with rosuvastatin after induction with triton X-100. And the last group treated with combination of Cicer arietinum extract with rosuvastatin after induction with triton X-100.the result of anti-inflammatory ability measured using interleukin-6 (IL-6). The result of IL-6 of both G3 and G4 treated groups revealed a significant decrease in comparison with G2. while there was no significant differences between G3 and G4 while G5 showed significant decrease comparing to all treated groups. Additionally the result of glutathione peroxidase (GPX) and super oxide dismutase (SOD) revealed concentrations in G3 and G4 were significantly higher than in G2.While G5 showed significant decrease during comparing with all treated group, with no significant differences with G1.Furthermore the result of DNA-fragmentation commit assay inG2 showed significant increase in DNA damage comparing to G1.While both of G3 and G4 showed significant decrease in DNA damage comparing to G2, but the G3 decrease in DNA damage when matched to G4.the result of G5 revealed significant decrease comparing to G2and G4.
Keywords: triton X100, Cicer Arietinum, rosuvastatin, hyperlipidemia and rat.

1. Introduction

Triton X-100 (C14H22O (C2H4O) n is a surfactant with a hydrophilic polyethylene oxide chain (9.5 ethylene oxide units on average) and an aromatic hydrocarbon lipophilic or hydrophobic group. A 4-(1, 1, 3, 3-tetramethylbutyl)-phenyl hydrocarbon group makes up the hydrocarbon group (Chang, 2021). Triton X-100 is a non - ionic surfactants detergent that is completely active and soluble in water detergent (Jho et al., 2021). is commonly used to lyse cells in order to remove protein or organelles, or to permeabilize live cell membranes (Zhou et al., 2021).for that Triton can also experimentally increase oxidative stress by increasing lipids that the body's free radicals use as targets for peroxidations, which result in oxidative stress (Tijjani et al., 2020).While rosuvastatin is completely synthetic HMG- coenzyme reductase inhibitor that success to reduce low-density lipoprotein and the cholesterol levels. (Kim et al., 2018). It is characterized by it is high level of selectivity effect inside liver cells in comparative with another of non-liver cells, such as skeletal muscle cells (McTaggart, 2003).The rosuvastatin like other drugs that linked with many side effect like liver dysfunction, muscular side effects, acute pancreatitis and other for that recently try to find alternative safe source of drugs like plants extract .Cicer arietinum is a rich source of energy, proteins, minerals, vitamins, and fiber, as well as phytochemicals that may be helpful for health. (Venkidasamy et al., 2019). Provided small amounts of known antioxidants such as vitamin C, vitamin E and beta-carotene, but on another hand the Cicer arietinum contained secondary plant substances with high antioxidant potential. These included the flavonoids quercetin, kaempferol and myricetin as well as various phenolic acids protecting the body from attacks by free radicals and reactive oxygen compounds (Al-Okaily, 2019)

2. Materials and Methods

Animals: This study was carried out at the College of Veterinary Medicine/University of Baghdad Department of Physiology, Biochemistry and Pharmacology. Thirty mature female rats, 3 months old and weighing 197-210g, were housed in an appropriate habitat with a temperature range of (22-25 °C). For acclimatization, the rats were kept in plastic cages measuring 45x20 x15 cm in an animal

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house around 3 weeks before the experiment started. They were fed with normal pellets with tap water

Ethanolic extraction: One kilogram of Cicer arietinum, carefully rinsed with tap water to eliminate any foreign bodies might be suspended in the Cicer arietinum and then soaked in two liters of distilled water to ensure that no foreign bodies are present, then allowed to dry for three days at room temperature (Olika, and Fikre, 2019). Cicer arietinum seed crushed into a fine powder. The extraction of Cicer arietinum seeds performed by the Soxhlet apparatus using 70 percent ethanol and 50 centigrade temp. Cicer arietinum seeds, dried powder, placed in a thimble portion in weight (70 gm) and placed in the extraction unit of the apparatus, in a round bottom flask, 500 mL of 70 percent ethanol was added, and extraction began at 50 c temperature. The extraction process ended until clear and colorless solvent appeared in the extraction unit. The extract was then filtered and evaporated to dryness for 3 hours in a rotary evaporator at 40 degrees Celsius and 200 spins per minute. The extract was then placed in an incubator at 40 degrees Celsius for 12 hours until the alcohol evaporated and the extract became a thick semi-solid mass with a dark yellow color, after which it was stored in a glass container with a plug-in refrigeration. (Oo et al., 2017).

Experimental design: thirty adult female rats were separated into five groups equally and medicated orally by stomach tube for a period twenty eight days as following:

First group (G1): six adult female rats were administrated with distilled water only, (negative control group).

Second group (G2): six adult female rats were medicated with triton X-100 (100mg/kg/B.W.) (Positive control group).

Third group (G3): six adult female rats were medicated with triton X-100 followed by Cicer arietinum extract at dose (349.015mg/kg).

Fourth group (G4 six adult female rats were medicated with triton X-100 followed by Rosuvastatin at dose (1.77mg/kg).

Fifth group (G5) six adult female rats were medicated with triton X-100 followed by Cicer arietinum extract at dose (349.015mg/kg) and Rosuvastatin at dose (1.77mg/kg) combination.

Preparation of Stock Solutions, Concentrations, and Doses: Stock solutions of Cicer arietinum seeds crude extract at doses (100, 200, 300, 400 and 500) mg/ kg, the concentrations of solutions were (50, 100, 150,200 and 250) mg/ml respectively and the dose then adjusted according to body weight of female rats for that each 0.2 ml of extract stock solution given to each 100 gm of rat body weight by stomach tube.

Rosuvastatin: The recommended human dose of rosuvastatin 20 mg/day. daily dose for treating hyperlidimic disorder according to (Abdeen, 2019; Niedzielski et al., 2021).The rosuvastatin was given orally at dose 1.77mg/kg to the rats after converted human dose 0.28 mg/kg to animal dose by multiplying the human dose by rat factor 6.2 (Janhavi et al., 2020), the concentration of solution was 0.885mg/ml, furthermore, 0.2ml of stock solution of rosuvastatin was given orally to each 100gm of rat body weight by stomach tube.

Interleukin-6 measurement: Serum IL-6 concentration was measured colorimetrically using Rat IL-6 Kit (biosecure), according to (Ouyang et al., 2011).

Serum glutathione peroxidase (GPX) measurement: the test was performed according to abcam kit instruction (Dokic et al., 2012).

Super oxide dismutase (SOD) measurement: the test was performed according to abcam kit instructions (Liu et al., 2011)

DNA- fragmentation commit assay: the test was done according to Trevigen kit (USA). (Al-Ani and Hasan, 2022)

3. Statistical Analysis

Data were statistically analyzed using one way analysis of variance (ANOVA) with a significant threshold of (p0.05). Least Significant Differences were used to determine differences between a certain groups (LSD)

4. Results and Discussion Interlukin-6

The results in a table (1) revealed that the concentrations of interleukin 6. The concentration of G2 showed significant increase (P< 0.05) with the mean value (67.72±1.25) when matched to all other groups. While both G3 and G4 treated groups revealed a significant decrease (P< 0.05) in mean value (64.74±0.90) for G3 treated animals and (62.46±1.08) for G4 treated animals when matched to G2 group with no significant differences between G3 and G4. While the G5 group showed significant decrease (P< 0.05) when the treated animals matched with all groups except when matched with G1 group

Table (1) effect of Cicer arietinum extract, rosuvastatin drug and Cicer arietinum with rosuvastatin combination on interleukin-6 (pg/ml) levels of female rat for 28 days of treatment.

Groups	IL6 (pg./ml)
G1	51.86±1.37d
G2	67.72±1.25a
G3	64.74±0.90ab
G4	62.46±1.08b
G5	58.26±0.77c
LSD	3.24

*Means with a different small letter in the same column are significantly different (P<0.05), *G: control negative group, G: control positive group, G: Cicer arietinum extract treated group, G: rosuvastatin treated group, G: Cicer arietinum and rosuvastatin treated group. *n=6

Super oxide dismutase (SOD) and glutathione peroxidase (GPX): The results for Glutathione peroxidase (GPX) and super oxide dismutase (SOD) concentrations were elucidated in the table (2). The levels of GPX and SOD enzymes for female rats of the G2 showed a significant decrease (P<0.05) with mean values ranges (33.26±1.39, 62.69±3.72) respectively, during matching with G1 and other treated groups. But the G3 and G4 treated groups showed significant elevation (P<0.05) respectively in mean value (36.46±0.89, 70.76±2.3) for G3 and (34.26±1.01, 69.26±0.84) for G4 when compared to control G2 with a significant decrease (P<0.05) as compared with G1 group. While the G5 treated group have significant increase (P< 0.05) when compared with all treated group with no significant difference (P< 0.05) as compared with G1 group.

Table (2) effect of Cicer arietinum extract, rosuvastatin drug and Cicer arietinum with rosuvastatin combination on glutathione peroxidase (mmol/mg) and super oxide dismutase (u/ml) levels of female rat for 28 days of treatment.

Table (1) effect of Cicer arietinum extract, rosuvastatin drug and Cicer arietinum with rosuvastatin combination on interleukin-6 (pg/ml) levels of female rat for 28 days of treatment.		
Groups	GPX (mmol/mg	SOD (u/ml)
G1	41.16±0.37a	78.89±2.83a
G2	33.26±1.39c	62.69±3.72c
G3	36.46±0.89b	70.76±2.36 b
G4	34.26±1.01bc	69.26±0.84 b
G5	39.80±0.44a	76.89±1.16a
LSD	2.67	5.12

*Means with a different small letter in the same column are significantly different (P<0.05), *G: control negative group, G: control positive triton x-100 group, G: Cicer arietinum extract treated group, G: rosuvastatin treated group, G: Cicer arietinum and rosuvastatin treated group. *n=6

DNA- fragmentation comet assay

The results present in table (3) revealed the levels of DNA –fragmentation commit assay of control positive showed significant increase (p≤0.05) in levels of low damage, medium damaged and highly damaged DNA with means value (46.60±0.35, 18.80±0.40, 12.90±0.40) respectively when matched with all treated groups and G1. While undamaged DNA showed significant decrease (p≤0.05) when compared with negative control and all treated groups. Cicer arietinum treated group showed significant decrease (p≤0.05) in levels of low damage .medium damaged and in high% of DNA damage (p≤0.05) with means value (23.10±0.25, 13.60±0.10, 6.10±0.10) respectively during matched with all treated groups with a significant decrease (p≤0.05) when compared with negative control group, in another hand there was significant increase (p≤0.05) in normal undamaged DNA of Cicer arietinum treated group as compared with control negative group. there was significant decrease (p≤0.05) in levels of low damage. medium damaged and highly damaged DNA of rosuvastatin treated group with means values (34.50±0.50, 15.7±0.35, 7.50±0.20) respectively when compared to positive control group but low significant (p≤0.05) than Cicer arietinum treated group. The levels of low damage, medium damage and high % damage DNA of the Cicer arietinum and rosuvastatin combination treated group showed a significant decrease (p≤0.05) in means values (27.40±0.50, 12.8±0.35, 6.30±0.25) when matched with rosuvastatin and control positive group. While undamaged cells of the Cicer arietinum and rosuvastatin combination treated group showed significant elevation (p≤0.05) with mean value (53.50±0.40) as compared to rosuvastatin and control positive group. As well as the No damage% of Cicer arietinum, rosuvastatin and Cicer arietinum with rosuvastatin treated groups showed a significant increase (p≤0.05) as compared with Low%, Medium% and High% of DNA damage, in addition the Low%, Medium% and High% DNA damage, of Cicer arietinum, rosuvastatin and Cicer arietinum with rosuvastatin treated groups showed a significant decrease (p≤0.05) as compared with positive control group.

Table (3) effect of Cicer arietinum extract, rosuvastatin drug and Cicer arietinum with rosuvastatin combination on DNA- fragmentation commit assay (%) levels of female rat for 28 days of treatment.				
Groups	No damage	Low	Medium	High
A	A98.40±0.20a	B1.20±0.20e	C0.30±0.05e	C0.10±0.05d
B	B21.70±0.35e	A46.60±0.35a	C18.80±0.40a	D12.90±0.40a
C	A57.20±0.25b	B23.10±0.25d	C13.60±0.10c	D6.10±0.10c
D	A42.30±0.50d	B34.50±0.50b	C15.7±0.35b	D7.50±0.20b
E	A53.50±0.40c	B27.40±0.50c	C12.8±0.35d	D6.30±0.25c
LSD	0.86			

Means with a different small letter in the same column are significantly different (P<0.05) Means with a different capital letter in the same row are significantly different (P<0.05)

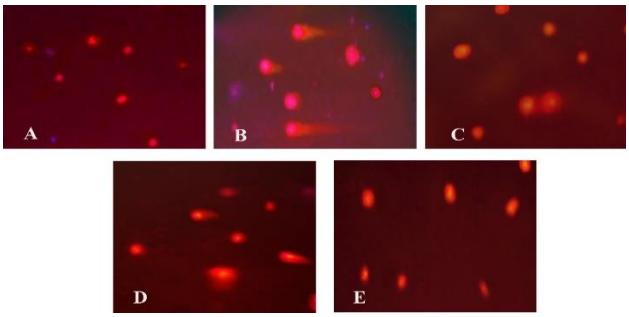

Fig (1): The DNA-fragmentation comet assay A: control negative B: control positive C: Cicer arietinum extract D: Rosuvastatin E: Cicer arietinum and rosuvastatin combination

5. Discussion

Elevated IL-6 caused by (T.X-100) to induce hyperlipidemia and oxidative stress (Tijjani et al., 2020). Both of them were seen as ideal precursors for raising of interleukin-6 levels inside the body (Cetin et al., 2019). In G3 Cicer arietinum showed the ability to inhibit inflammation by its antioxidant element especially biochanin A and biochanin A-7-Ob-D-glycoside which can reduce inflammation leading to inhibit of interlukin-6 (Faridy et al., 2020). Rosuvastatin can be lowering C- reactive protein levels (Omoigui, 2007). By stabilizing the plaque or reducing the inflammatory response led to decrease IL-6, there is also link between inflammation and lipid metabolism rosuvastatin improve lipids led to decrease levels of, IL-6, TNF- (Çetin et al., 2019). While GPX and SOD depletion of antioxidant enzymes in G2 resulted by triton oxidative stress inducing ability by decrease antioxidant enzymes activity like (GSH-Px, GST, CAT, and SOD) deu to exhaust by tissue (Abbas,2012). I also cause hyperlipidemia result in highly lipid peroxidation (Abdou et al., 2018). Cicer arietinum has vitamin E, flavonoids, and phenolic compounds which boost the antioxidant activity and enhancing of scavenging of ROS activity (Al-Snafi, 2016). In G4 the formation of ROS by NADPH oxidase, xanthine oxidase and uncoupled nitric oxide synthase is promoted by the presence of hypercholesterolemia (Zinellu and Mangoni, 2021). Because of this, using statin medications helped to keep ROS levels low and antioxidant enzyme levels high by lowering high levels of lipids (Kattoor et al., 2017). additionally, the increase in DNA fragmentation may be attributed to the ability of Triton X-100 in inducing hyperlipidemia and oxidative stress in animal models by elevating lipids absorbtion which are targets of free radical in the body for peroxidation's, leading to the generation of oxidative stress. Oxidative stress exacerbate the damage of cells (Al-Okaily, 2016). Furthermore triton X-100 can be used in applications to solubilize proteins (Tijjani et al., 2020).the extract of Cicer arietinum reduced the damaged DNA levels and this resulted by presencr of phenolic and flavonoid compounds addition to levels of flavonoid there is also chlorogenic, Xanthophyll, cryptoxanthin, and beta carotene which can be anti-oxidant substances protect the body, and strongly fight against ROS which is one of DNA damage causes (Matallana et al., 2020).the rosuvastatin drug also tend to reduce DNA damage by enhancing of apoptosis in many different types of tumor cells(Amin et al., 2022). Statins also showed enhance for creation of ROS the mutated cell which can lead to remove of damaged DNA. (Li, et al., 2019).

6. Conclusion

Triton x-100 surfactant can cause oxidative stress lead to DNA damage while *Cicer arietinum* extract has good amount of anti- oxidant substances which able to protect the Dna against ROS attacks and rise of anti-oxidant enzymes, rosuvastatin can reduce the oxidative stress by inhibiting of lipids which is main target for ROS

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