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THERAPEUTIC EFFECTS OF *Crataegus azarolus VAR. aronia L.* IN UROLITHIASIS MALE ALBINO RATS

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ABSTRACT:
The study was designed to investigates the therapeutic roles of *Crataegus azarolus* Var. *aronia* **L.** (*C. aronia*) in kidney stone treatment male albino rats' model induced by ethylene glycol (EG). Twenty animals weighing 220–270 g were divided into control group which set as group A, while the rats in B and C groups, received 1% EG for 28 days, but group C also received *C. aronia* (7.5g of plant/100ml water and 10g of plant/ 90 g diet) from day 15 to 28. Kidney function tests, liver function tests, serum electrolytes, serum lipid profiles, and glucose were measured. The obtain body weight, kidneys' weight and kidneys' weight/ body weight were measured, in addition microscopic analysis of formed crystals from urine was studied. *Crataegus aronia* administration showed a marked declination in serum creatinine, urea, cholesterol, triglyceride (TG), very low-density lipoprotein (VLDL), and non-high density lipoprotein cholesterol (non-HDL cholesterol) inverse to rats received EG. Also, the obtain body weight, kidneys' weight and kidneys' weight/ body weight markedly decreased in C group when compared to group B. In conclusion: *C. aronia* has clear therapeutic actions on formed kidney stone might be used employee as natural antiurolithiasis drug.

KEYWORDS: *Crataegus aronia*, ethylene glycol, renal calculi, lipid profiles.

1. INTRODUCTION

Medicinal plants represent an important part in traditional medicine to treat and prevent numerous health disorders (Arshad *et al*., 2020, Mahmud, 2021). Different parts of the herbs such as stem, bark, root, leaf, and fruits have been used to treat a broad range of diseases (Mahmud, 2017a, Mahmud, 2017b), in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders (Saad *et al*., 2005). Since ancient times herbs are used throughout the world and showed a potential therapeutic effects with minimum unwanted effects as documented by patients and doctors in comparison to new chemical drugs (Namdari *et al.*, 2017). Recently, it has been recorded that plants which used in folk medicine are appeared as essential source in development new drug discovery projects (Lu and Lu 2019). Nowadays, the demand on herbal medicines greatly increased for remediation due to their cost, riskless, effectiveness, bioavailability and ect… features as compared with synthetic drugs (Nazhand *et al.*, 2020). Furthermore, concerning to the World Health Organization (WHO) data, close to 80% of populations around the world apply natural rather than synthetic medications mainly medicinal plants for curing of various health disorders (Zhang *et al.*, 2015, Nazhand *et al.*, 2020). In modern pharmacy, near to 50% of medications are derived from natural products particularly plant sources and in new design of drug finding processes depend on conventional medicine plant strategy to convince their safety of uses (Al-Habib *et al*., 2015).

The therapeutic actions of medicinal herbs are related to their secondary metabolites. The plant phytochemical analysis showed the present of several chemical constitutes with different biological effects such as alkaloids, terpenoids, saponins, tannins, flavonoids (Larayetan *et al*., 2019, Mahmud *et al*., 2021), cardiac glycosides (Larayetan *et al*., 2019), essential oils, quinones, steroids and etc (Manzoor *et al*., 2016, Mahmud *et al*., 2021). Some of these plant bioactive compounds possess great pharmacological activities including antioxidant, antimicrobial, immunomodulatory, antitumor, hepato-cardioprotective, neuroprotective (Rjeibi *et al*., 2019, Rjeibi *et al.*, 2020), antiproliferative and anti-inflammatory (Choi *et al.*, 2014, Kallassy *et al.*, 2017). Furthermore, it is also used as sedative, hypnotic, expectorant, cough suppressant, purgative, demulcent, diuretic, antiseptic agent, also used to control bronchitis, asthma, pertussis, gastrointestinal, and various other ailments (Janbaz *et al*., 2013). In the worldwide, day by day the requests on natural products especially medicinal herbs increased for treating and preveting of health disorders due to their safety, minimum negative impacts, higher effectiveness, prominent bioavailability and cheapness in contrast to artificial drugs (Nazhand *et al.*, 2020) due to they could be used for longer duration with fewer risks (Gaire, 2018).

Crataegus genus in Rosaceae family commonly known as hawthorn, comprises over than 200 species (Mahmud *et al*., 2016). The *Crataegus* spp. fruits are commonly eaten as edible food (Bahri-Sahloul *et al.*,2014, Rjeibi *et al.*, 2020). Its more diverse genus belongs to fruit bearing and flowering small trees or shrubs, primarily native to temperate regions of the North Africa and America, India, China, Western Asia, Mediterranean and Europe (Dinesh *et al.*, 2012, Agiel *et al.*, 2019). *Crataegus* genus naturally occurring in wooded and sunny regions in lime rock earth up until 1500 m over sea level (Nazhand *et al.*, 2020). Hawthorn fruits, flowers and leaves are rich in chemicals with nutritional and biological importancy, and they can be used for pharmaceutical gaols in treatment of different medicinal problems (Sagaradze *et al.*, 2019). In phytochemical screening of *Crataegus* species more than 150 chemical constitutes isolated (Wang *et al.*, 2018), like triterpenoids (Al-Habib *et al*., 2015, Nazhand *et al.*, 2020), aromatic amines, essential oils, flavonoids, proanthocyanidins (Al-Habib *et al*., 2015), phenolic acids (Al-Habib *et al*., 2015, Rjeibi *et al.*, 2020), catechins, flavonoids (Moustafa *et al.*, 2019), proteins, saponins, (Mohammed, 2015), glycosides (Kallassy *et al.*, 2017), polyphenols (Hellenbrand *et al.*, 2015, Alirezalu *et al.*, 2020), vitamins, tannins, essential oils (Agiel *et al*., 2019, Rjeibi *et al.*, 2020), cardiotonic amines, purine derivatives (Abu-Gharbieh and Shehab, 2017), minerals (Rjeibi *et al.*, 2020), and others. Concerning to their phytochemical compounds, hawthorn represents as a potential herb with effective biological activities (Moustafa *et al.*, 2019).

In folk medicine, *C. aronia* was tested and appeared as essential medicinal herb which possess a many pharmacological actions (Khiari *et al.*, 2015), such as prevention of cancer, diabetes, sexual weakness, and cardiovascular diseases (Bahri-Sahloul *et*

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al., 2015, Rjeibi *et al.*, 2020), treating renal stones (Ahmed *et al.*, 2016; Ahmed *et al.*, 2017), and also it acts as antimicrobial, antioxidant and antihyperlipidemic activities (Abu-Gharbieh and Shehab, 2017; Rjeibi *et al.*, 2020). The therapeutic actions of *C. aronia* are related to its bioactive compounds including flavonoids, minerals, sugar alcohols, carotenoids, polyphenols, amino acids, tannins (Yahyaoui *et al.*, 2019, Rjeibi *et al.*, 2020) and azarolic acid (Mahmud *et al.*, 2016).

Some new investigations showed that *Crataegus* herb has positive effects on different biochemical variables. It has been found that aqueous extract of *Crataegus azarolus* preserved glutathione level, and prevented the depletion of antioxidant enzyme activities, such as superoxide dismutase, catalase, and glutathione peroxidase, also it protected against elevation of inflammatory markers (alkaline phosphatase and C-reactive protein) (Sammari *et al*., 2021). Furthermore, Al-Mobideen *et al*., (2022) recorded that *C. aronia* has positive effects in reducing creatinine and glucose levels, increasing albumin and total protein levels and improving hyperlipidemia condition, also in the improvement of liver function through the increased antioxidant capacity. In addition, *C. aronia* could normalized some haematological parameters in diabetic rats including red blood cells and platelets number, haemoglobin and hematocrit values.

Urolithiasis is the most common kidney illness, in which a mass of crystals lead to create a hard lump in the kidneys, can vary in size from a few millimeters to several centimeters (Khan *et al.*, 2019). The stones development mostly concerned to decrease of urine volume and increasing release of stone forming constituents including oxalate, calcium, xanthine, urate, cystine and phosphate (Shukla *et al.*, 2017; Al-Snafi, 2018). Some researchers investigated that in kidney stones about 12% of the people affecting, and its recurrence ratio in male is greater than in female (Arya *et al*., 2017). Calcareous (calcium containing) – calcium oxalate (CaOx) and calcium phosphate, and non-calcareous stones- uric acid, struvite, cystine, silica stones are the most common types of renal calculi (Gupta and Shamsher, 2018, Alelign and Petros, 2018), in human calcium stones appeared as the most common type (Al-Bajari *et al*., 2019), nearly 80% of the analyzed kidney stones are belong to CaOx and calcium phosphate types (Garcia *et al*., 2018). Kidney stones usually lead to sever bleeding, heavy pain, disturb the flow of urine and other problems, because of they must be remove or destroy by a surgery (Bahmani *et al.*, 2016). Calcium oxalate urolithiasis model made up by supplementation of EG to find the effect of renal calculi on experimental rats (Mahmud *et al*., 2021). Related to ethnopharmacological application of *C. aronia* for curing of kidney stone at the local area, the current research was designed to investigate the therapeutic actions of *C. aronia* on albino rats' kidney stone.

2. MATERIALS AND METHODS

2.1 Plant materials

The leaves of *C. azarolus* var. *aronia* L. employed during the current study were collected on September, 2019 from the Hasarost Mountains (GPS position 364359.71 N, 44434343.01 E) around 150000 m far from north of Erbil, Iraq. Collected plant was cleaned via applying tape-water and for drying about 20 to 25 days kept in shade room temperature, cutoff into small parts then grounded, by utilizing electrical machine (High speed multi-functional crusher, Model-200A, China). The formed element was kept at 5ºC until use (Mahmud *et al*., 2021).

2.2 Laboratory animals

Twenty adult male albino rats, Rattus norvegicus weighing 220 – 270 g were used in the current study. Animals were bred in the Animal House, Department of Biology, Faculty of Science/ University of Soran and maintained in plastic cages (460 x 30 x 20 cm). They were kept under standard laboratory conditions at 22 $\pm$ 2 oC and exposed to a photoperiod of 12 hrs. light followed by 12 hrs. of darkness (Ahmed and Mahmud, 2021). The rats were fed on standard rat pellets with free access to dechlorinated tap water *ad libitum* (Mahmud and Mahmud, 2013, Abdulla *et al*., 2017).

2.3 Experimental design

This study was planned to discover the therapueutic influences of *C. azarolus* var. *aronia* L. in urolithiatic rats treated with EG. In kidneys CaOx deposition and hyperoxalurea condition was introduced via adding of EG (1%) to drinking water (Mahmud *etal*., 2021), for experimental groups other than the control. Rats randomly were classified into three groups and go on for twenty eight days as follow:

Group; A set as normal rats (n: 7)
Rats were supplemented with normal water and diet for 28 days.

Group; B set as EG treated rats (n: 6)
Rats were supplemented with water contained EG (1%) and normal diet for 28 days.

Group; C set as *C. aronia* treated rats (n: 7)
Rats were supplemented with water contained EG (1%) and normal diet for 28 days, but also they were received dried *C. aronia* leaves powder (7.5 g in 100 ml of drinking water contained EG (1%) and 10 g in 90 g of normal diet) from day 15 to day 28.

2.4 Blood collection

At the 28 days of experiment, the animals were anesthetized with ketamine hydrochloride (80 mg/ kg body weight) and xylazine (12 mg/ kg body weight) intraperitoneally. Blood was drawn from the heart into test tubes without EDTA (4.5mM), then for 15 minute centrifuged at 3000 rpm (Ahmed and Mahmud, 2021) to obtain serum for determination of biochemical variables.

2.4.1 Estimation of obtain body weight/ loss, kidney weight and ratio of kidney weight/ body weight

The body weight, kidney weight and kidney weight/ body weight ratio were determined by measuring the body weight of each rat at the 1st and last days of the experiment and kidney weight at the end of experiment.

2.4.2 Estimation of uric acid, urea and creatinine

Serum uric acid, urea and creatinine were measured by Cobas 6000 C501.

2.4.3 Estimation of serum sodium (Na+), ionized calcium (ICa+), total calcium (TCa+), potassium (K+) and chloride (Cl-) concentration

Serum Na^+, ICa^+, TCa^+, K^+, Cl^- and concentration were measured by Cobas-6000 C501 with their specific test kits.

2.4.4 Estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (T.B.).

Serum ALT, AST and T.B. were measured by Cobas-6000 C501 with their specific test kits.

2.4.5 Estimation of blood glucose and lipid profiles

Glucose, cholesterol, triglyceride (TG), low density lipoprotein (LDL) and high-density lipoprotein (HDL) in blood were measured via Cobas c311, with their specific test kits (Biolab, Japan) (Hitachi, 2009). While VLDL (TG/ 5.), non-HDL cholesterol (cholesterol-HDL) and ratio of LDL to HDL (LDL/HDL) levels were determined.

2.4.6 Collection of Urine Sample

At the end of experiment, the urine samples were collected for urinanalysis to find out CaOX crystals using light microscope in experimental groups.

2.5 Data Analysis

The obtained results were exhibited as means±standard error (SE) and analysis of data was performed via applying GraphPad Software (Version:8).

The comparisons were done among experimental groups using one-way analysis of variance (ANOVA) (Holm-Sidak's Test). P-values less than 0.05 were detected as statistically significant. The symbols, (*, **, ***and ****) in all figures showed that mean differences are notable at P-values less 0.05, 0.01, 0.001and 0.0001 respectively.

3.RESULTS

3.1 Body weight gain / loss

In the rats treated with EG the body weight lost significantly (P<0.01) increased (65.83±2.713) as compared with control rats (83.14±2.040), while, administration of *C. aronia* to EG treated rats non-significantly recovered the body weight toward the normal direction through inhibition of EG's actions (Figure 1, Table (SUPL.)).

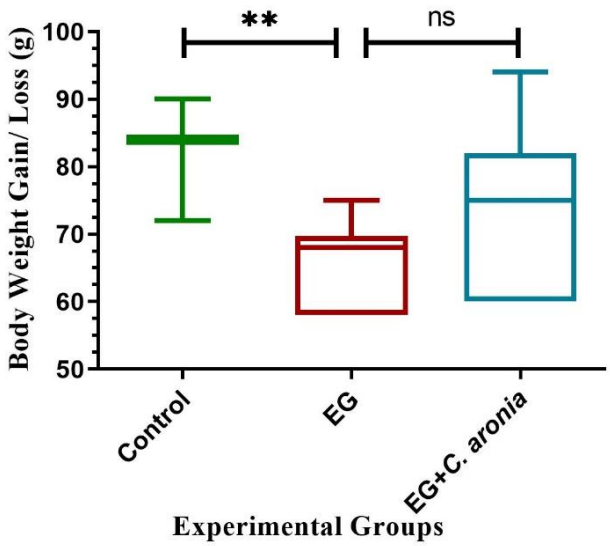


Figure 1. Effect of *C. aronia* on body weight gain/ loss. Keys; Ethylene Glycol, EG; Ethylene Glycol+*Crataegus aronia*, EG+*C. aronia*. Each of *, **, *** and **** mean P< 0.05, 0.01, 0.001 and 0.0001 respectively

3.2 Kidneys weight and kidneys weight/ body weight ratio

Oral administration of EG strongly (P<0.0001) elevated the kidneys weight (1.982±0.068) and kidneys weight/ body weight ratio (0.005±0.0001) as compared with normal rats kidneys weight (1.089±0.132) and kidneys weight/ body weight ratio (0.003±0.0003), but supplementation of *C. aronia* significantly (P<0.001) and (P<0.05) respectively reduced the effects of EG and decreased the kidneys weight (1.379±0.044) and kidneys weight/ body weight ratio (0.004±0.0002) close to the normal rats' value as compared to EG treated rats (Figure 2A, 2B and Table (SUPL.)).

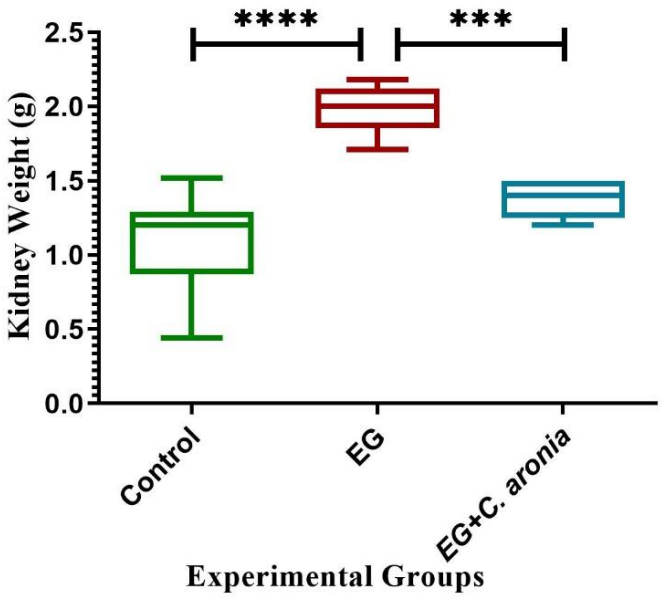


Figure 2A. The effect of *C. aronia* on kidneys weight.

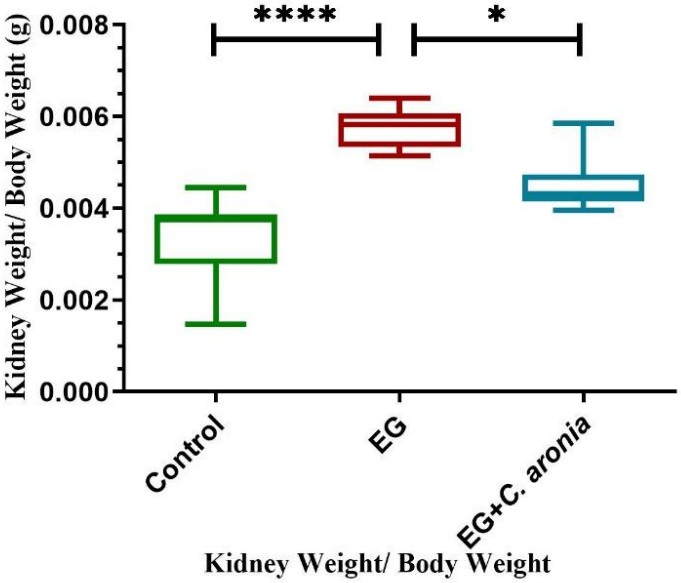


Figure 2B. The effect of *C. aronia* on kidney weight.

3.3 Level of serum uric acid, creatinine and urea

Serum uric acid level in rats treated with EG, markedly (P<0.05) elevated (1.507±0.029) when compared with the rats free from the EG (1.233±0.058). The *C. aronia* couldn't make any significant effects on elevated uric acid concentration in animals received EG inverse to rats received EG alone (Figure3A and Table (SUPL.)).

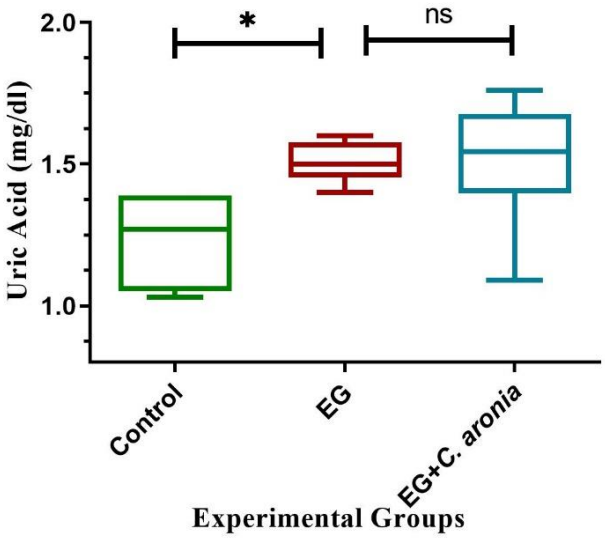


Figure 3A. The effect of *C. aronia* on serum uric acid.

The creatinine level was strongly (P<0.0001) elevated (0.666±0.016) in EG treated rats when compared to control animals (0.428±0.018). Whereas, in rats treated with EG, administration of *C. aronia* significantly (P<0.001) reversed the action of EG on creatinine concentration and decreased its level close to control rats' values (0.528±0.018) (Figure 3B and Table (SUPL.)).

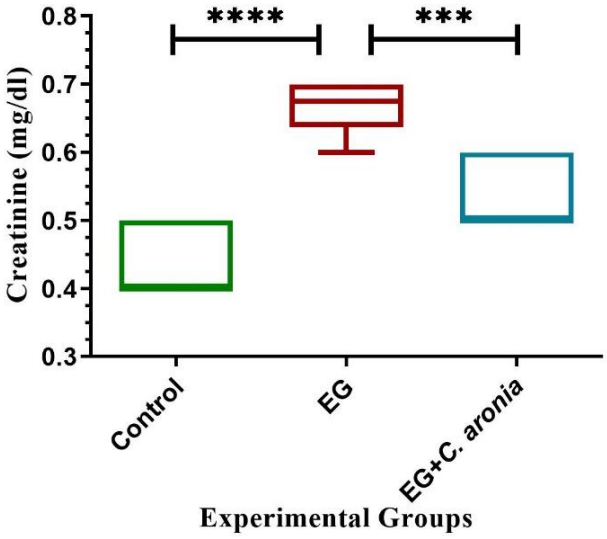


Figure 3B. The effect of *C. aronia* on serum creatinine.

Similar to creatinine, serum urea significantly (P<0.0001) increased (78.50±3.862) due to administration of EG in comparison to normal rats (34.00±1.447), also treatment by *C. aronia* with EG induced urolithiasis rats markedly (P<0.0001) reduced the serum urea toward the control value (47.00±1.528) as compared EG treated rats (Figure 3C and Table (SUPL.)).

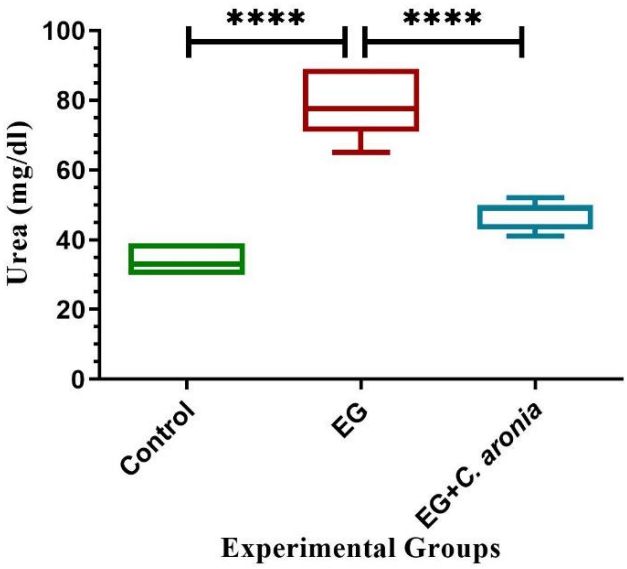


Figure 3C. The effect of *C. aronia* on serum urea.

3.4 Serum sodium, ionized calcium, total calcium, potassium and chloride concentrations

Statistically it has been shown that there were not any significant variation in serum Na^+, ICa^+, TCa^+, K^+ and Cl^- concentration among experimental groups (Figure 4A, 4B, 4C, 4D, 4E respectively and Table (SUPL.)).

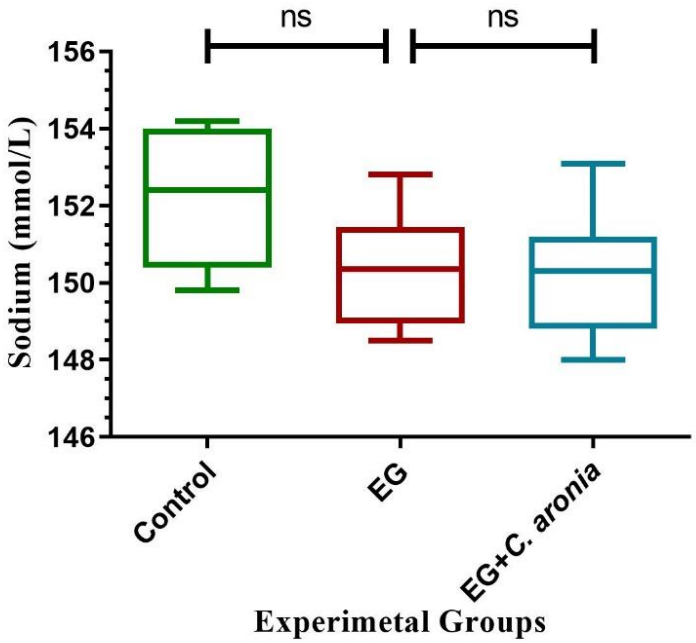


Figure 4A. The effect of *C. aronia* on serum sodium.

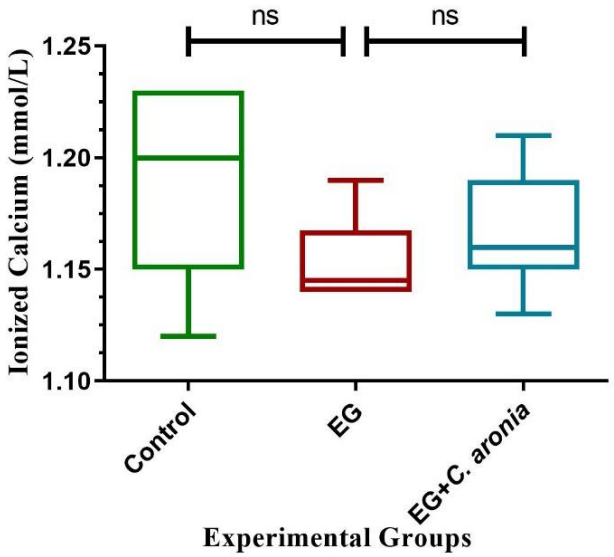


Figure 4B. The effect of *C. aronia* on serum ionized calcium.

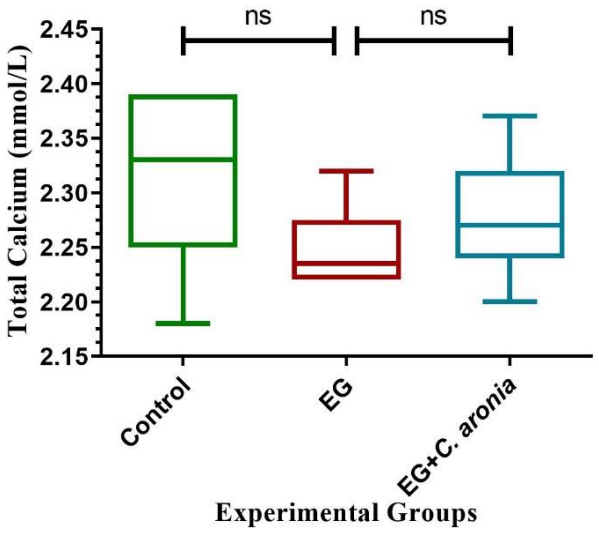


Figure 4C. The effect of *C. aronia* on serum total calcium.

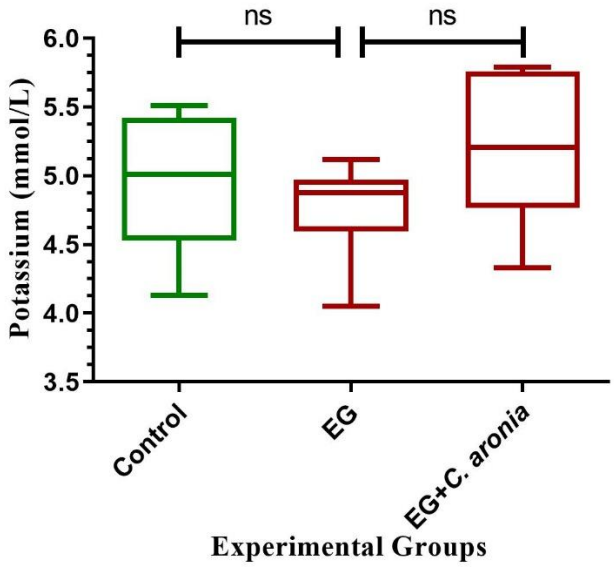


Figure 4D. The effect of *C. aronia* on serum potassium.

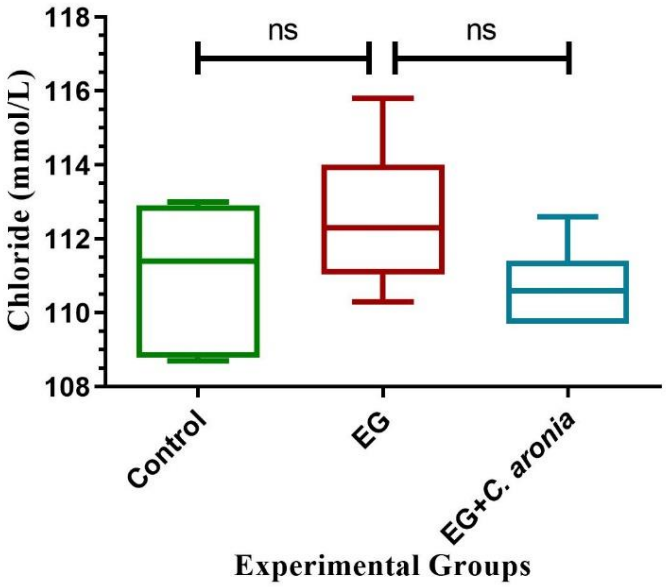


Figure 4E. The effect of *C. aronia* on serum chloride.

3.5 Serum alanine aminotransferase (ALT), asparate aminotransferase (AST) and total bilirubin

In rats administration of EG lead to non-significant reduction in serum ALT and AST concentrations when compared to rats didn't receive of EG, but supplementation of *C. aronia* was reversed the effect of EG on concentration of ALT and AST and return their values close normal concentration (Figure 5 A, 5B and Table (SUPL.)).

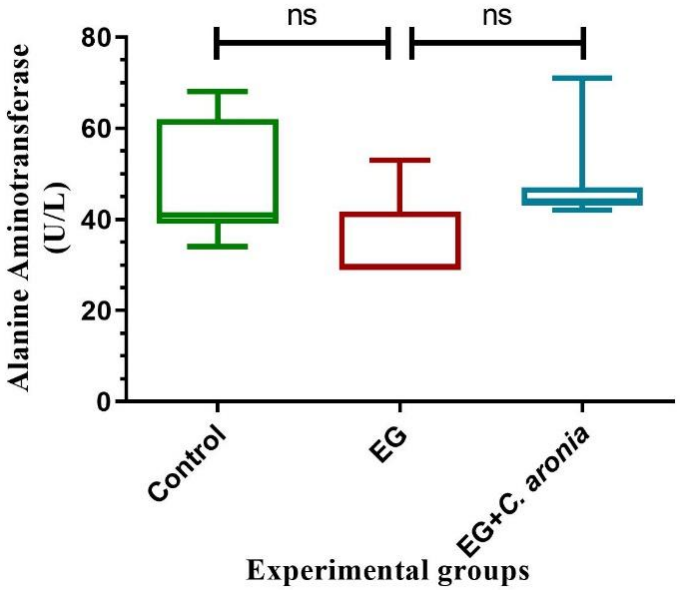


Figure 5A. The effect of *C. aronia* on serum alanine aminotranferase.

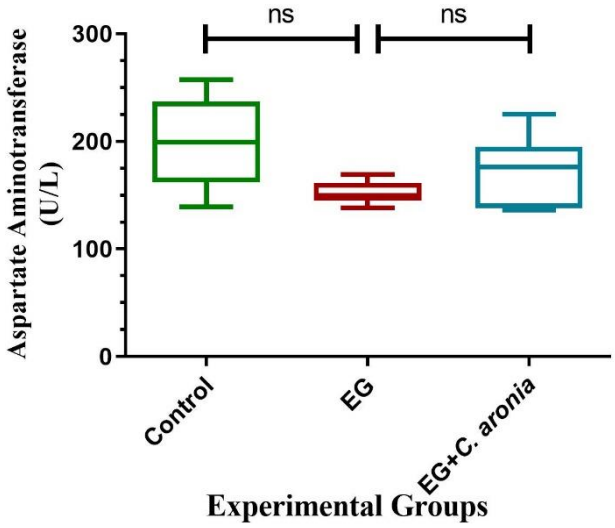


Figure 5B. The effect of *C. aronia* on serum asparate aminotranferase.

In contrast, the serum T.B. concentration non-significantly increased in rats were received EG as compared to rats of control group, but *C. aronia* administration in EG treated animals decreased level of T.B. to near normal concentration when compared to rats treated with EG (Figure 5C and Table (SUPL.)).

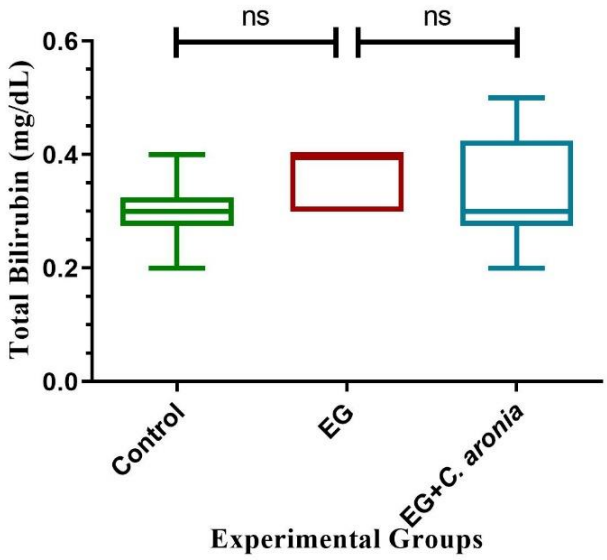


Figure 5C. The effect of *C. aronia* on serum total bilirubin.

3.6 Serum cholesterol, TG, VLDL, non-HDL cholesterol, LDL, ratio of LDL/HDL and HDL levels

In the current investigation, it has been demonstrated that serum cholesterol, TG and VLDL concentration significantly (P<0.01) increased (71.00±1.461), (112.5±3.784) and (22.50±0.756) orderly in animals supplemented with EG compared to control rats (60.43±1.674), (88.43±5.014) and (17.69±1.003) respectively, but in EG treated rats supplementation of *C. aronia* significantly (P<0.01) reduced the level of serum cholesterol (62.86±1.969), TG (92.29±1.997) and VLDL (18.46±0.399) toward the control level (Figure 6 A, 6B, 6C and Table (SUPL.)).

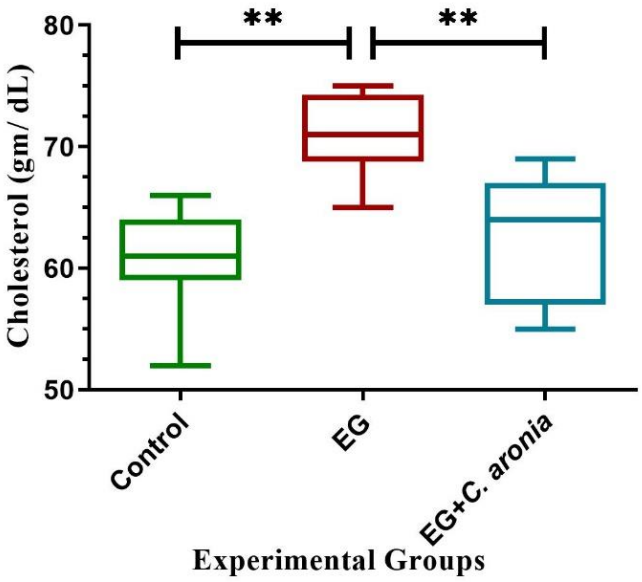


Figure 6A. The effect of *C. aronia* on serum cholesterol.

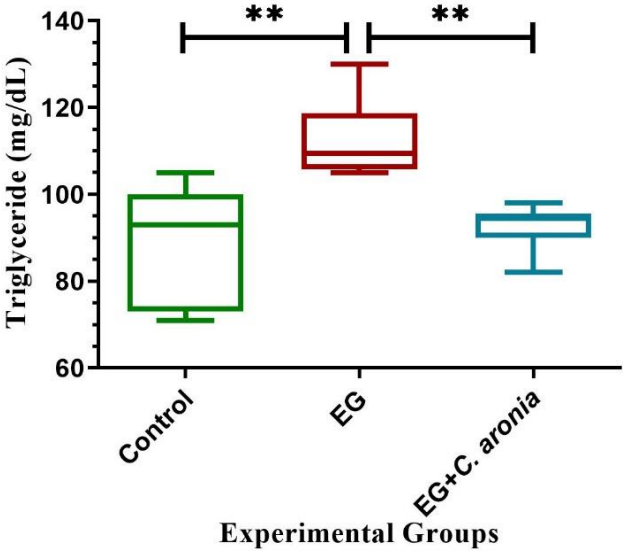


Figure 6B. The effect of *C. aronia* on serum triglyceride.

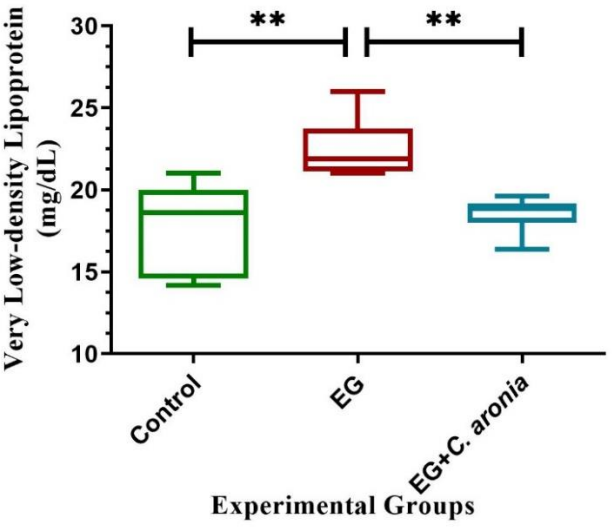


Figure 6C. The effect of *C. aronia* on serum very low-density lipoprotein.

In figure 6D appeared that EG markedly (P<0.001) elevated serum non-HDL cholesterol (35.20±1.241) and LDL (20.00±1.390) as compared to control groups (24.93±1.598) and (13.91±0.234) respectively, but administration of *C. aronia* to EG treated rats significantly (P<0.05) reversed the EG action only on non-HDL cholesterol and reduced its concentration (30.00±1.438) as compared to EG treated rats (Figure 6 D, 6E and Table (SUPL.)).

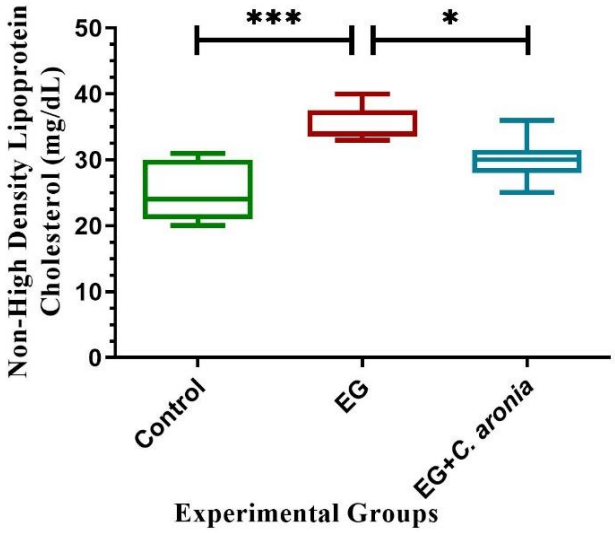


Figure 6D. The effect of *C. aronia* on serum non-high density lipoprotein cholesterol.

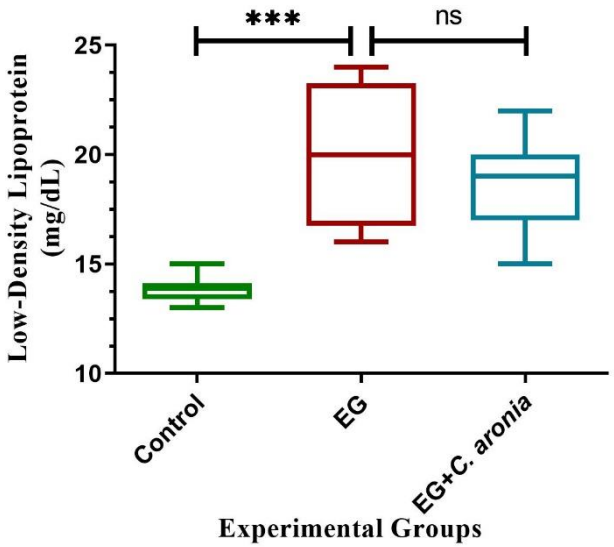


Figure 6E. The effect of *C. aronia* on serum low-density lipoprotein.

In the current investigation the obtained results showed that the serum LDL/ HDL ratio markedly (P<0.05) elevated (0.537±0.047) in rats received EG when compared to normal rats (0.392±0.005), contrast to other lipid measurements the administration of *C. aronia* to EG treated rats didn't reduce the ratio of LDL/ HDL, instead lead to further elevation of serum LDL/ HDL ratio (Figure 6G and Table (SUPL.)).

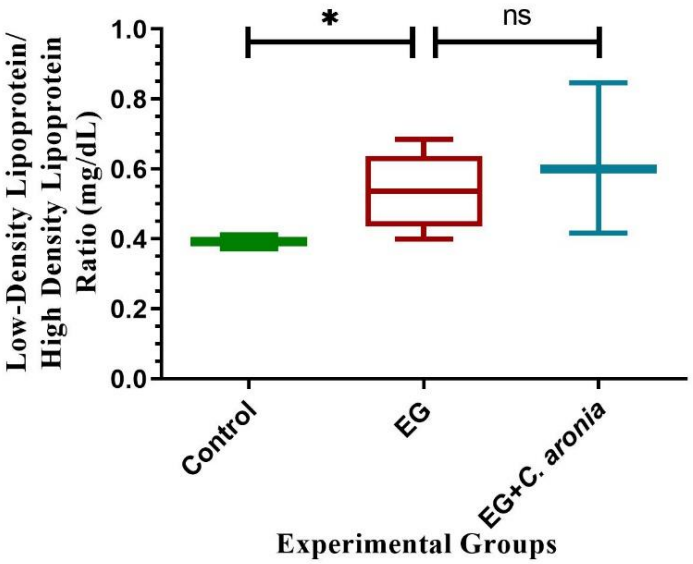


Figure 6G. The effect of *C. aronia* on serum low-density lipoprotein/ high-density lipoprotein ratio.

In data analysis, it has been discovered that value of serum HDL didn't show any significant differences (Figure 6H and Table (SUPL.)).

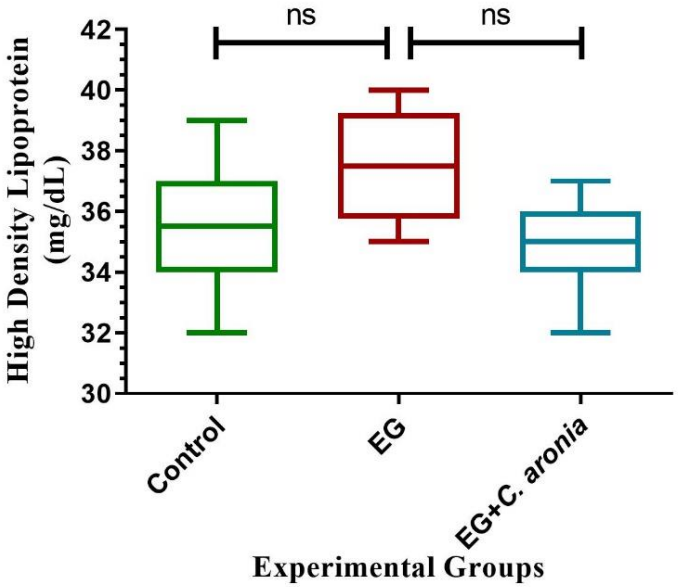


Figure 6H. The effect of *C. aronia* on serum high density lipoprotein.

3.7 Glucose level

Glucose concentration non-significantly decreased in rats supplemented with EG and EG treated rats supplemented with *C. aronia* as compared to control group (Figure 7 and Table (SUPL.)).

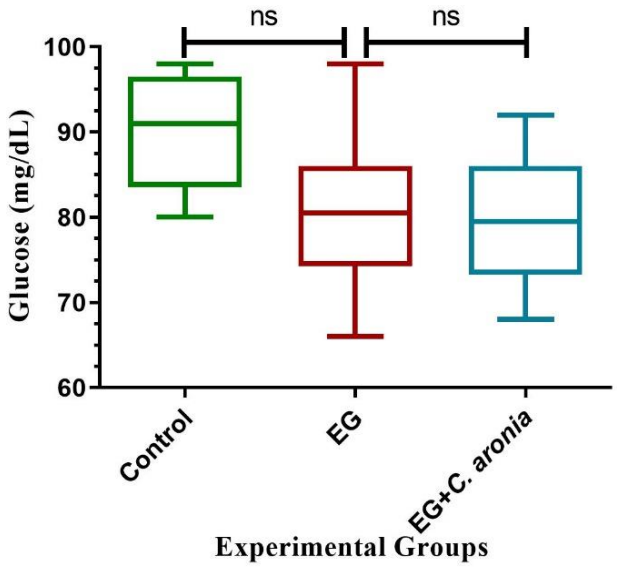
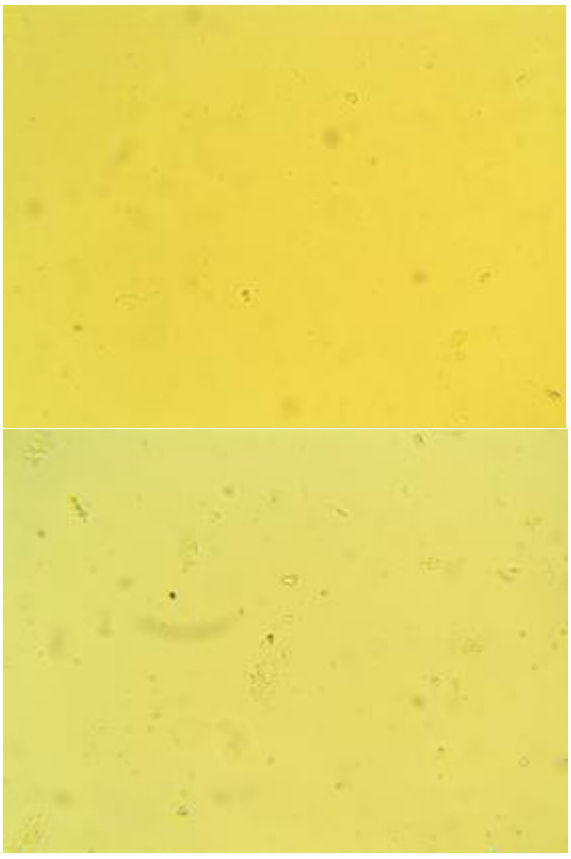


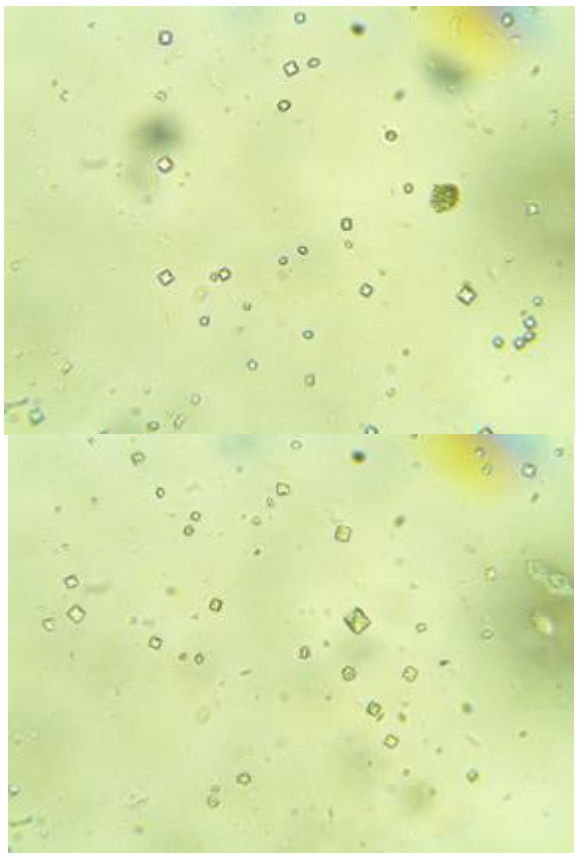
Figure 7. The effect of *C. aronia* on serum glucose.

3.8 Micoscopically urinanalysis

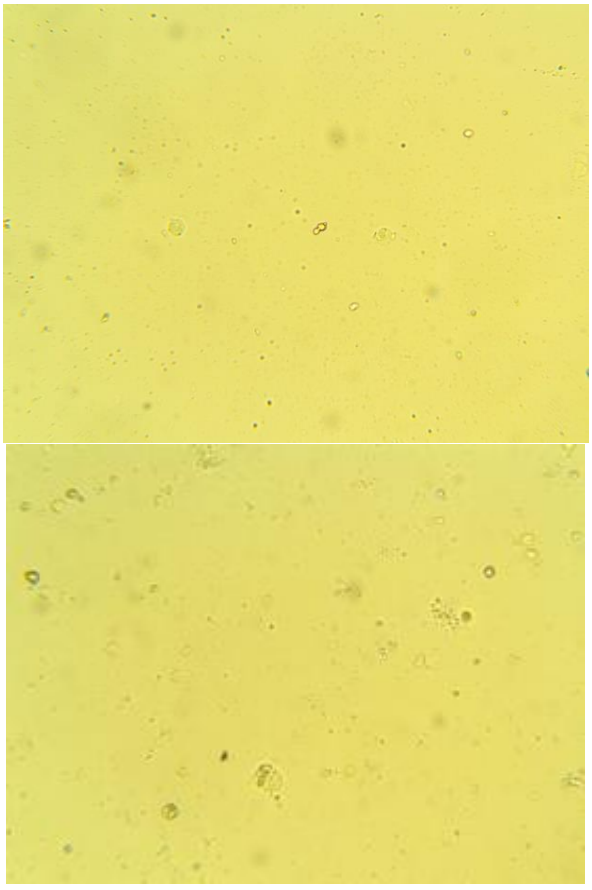
Microscopically urinanalysis in EG treated rats showed that several CaOX crystals were deposited when compared to the control rats (Figure 8A & B). Supplementation of *C. aronia* to the rats previously treated with EG markedly prevented the deposition of CaOX crystals when compared to EG treated group (Figure 8B & C).



A.



B.



C.

Figure 8: In urine, via light microscope the CaOX crystals viewed, **(A)** control rats revealing normal appearance, **(B)** EG treated rats revealing numerous crystals deposition, **(C)** EG+*C. aronia* treated rats revealing prevention crystal deposition.

4. DISCUSSION

In the current study statistical analysis revealed that body weight gain was markedly declined in the rats treated with EG in comparison with untreated rats, while administration of *C. aronia* to rats supplemented with EG non-significantly increased the rate of body weight gain as compared to EG treated animals. These finding is supported by Golshan *et al*., (2017) and Mahmud *et al*., (2021) investigations that body weight gain significantly reduced following EG administration. More recently, Mahmud *et al*., (2021) reported that herbal bioactive ingredients are essential in controlling the kidney stone disorders and similarly to current plant, *Allium siculum* inhibited the action of EG on obtained body weight. In addition, some researchers investigated that in plants, polyphenols via its antioxidant actions in the kidney could prevent CaOx formation (Grases *et al*., 2015; Mahmud *et al*., 2021). Concerning to these investigations, *C. aronia* also may acts through its phenolic compounds like other herbs in cure of kidney stone illness then inhibition reducing in body weight.

In the current study statistical analysis improved that kidney weight and kidney weight/ body weight markedly elevated in rats received EG inversed to control rats, but supplementation of *C. aronia* strongly inhibited the impacts of EG on weight of kidney and kidney weight/ body weight in rats received EG. The obtained results is consistent with (Shekha *et al*., 2015) and Mahmud *et al*., (2021) findings that in rats administration of EG led to tubules distending and kidney regions inflammation. Recently, Mahmud *et al*., (2021) recorded that administration of EG strongly increased the rats' kidney weight as compared to control rats, but supplementation of a herb, *Allium siculum* as in current investigation through decreasing EG's activity declined the kidney weight toward the control status. Several studies are supported the data of the present study that herbal medicine practically applied in curing of urolithiasis (Golshan *et al*., 2017; Mahmud *et al*., 2021), also some herbs are essential in regaining renal from damaging (Karimi *et al*., 2017). Furthermore, *C. aronia* may reduce the impacts of EG on kidney weight and kidney weight/ body weight via its phytochemical components particularly phenolic compounds, because Byahatti *et al*., (2010) and Mahmud *et al*., (2021) showed that renal CaOx and phosphate crystals could dissolve by various phenolic structures. The kidney function measurements particularly serum creatinine and urea levels were notably increased in animals treated with EG as compared to normal rats, while administration of *C. aronia* strongly inverted the effects of EG on serum creatinine and urea concentrations. The therapeutic actions of *C. aronia* are related to its phytochemical constitutes like flavonoids, minerals, sugar alcohols, carotenoids, polyphenols, amino acids, tannins and azarolic acid. In recent investigation Mahmud *et al*., (2021), showed that phenolic acid and flavonoids effectively declined the serum concentrations of creatinine and urea in EG group rats via breaking and dissolving CaOx stones. On the other hand, Ahmed and Mahmud, (2021), reported that *Prosopis farcta* ethanol fruit extract in EG treated rats reduces elevated concentration of creatinine and urea through its polyphenols due to inhibition formation or dissolving renal calculi. Furthermore, it has been recorded that flavonoids prevent CaOx crystals formation by deactivation of an enzyme glycolate oxidase (Shirfule *et al*., 2011; Sharma *et al*., 2017). In previous study, from ethylacetate extract has been investigated that flavonoids and terpenoids are the main phytochemical constitutes of *C. aronia* (Mahmud *et al*., 2016). Antiurolithiatic effects of *C. aronia* may concern to its phytochemicals composition particularly flavonoids and terpenoids, because Arafat *et al*., (2008) and Kaushik *et al*., (2021), concluded that terpenoids and flavonoids have potential antiurolithiatic activity. In addition, the plant bioactive compounds especially polyphenols could bind to the renal cells, due to CaOx crystals fail in binding to the renal cell surfaces (Cheraft-Bahloul *et al*., 2017).

The data of the present study showed that in rats treated with EG serum cholesterol, TG, VLDL, non-HDL cholesterol, LDL and LDL/ HDL ratio markedly increased as compared to control rats, but administration of *C. aronia* to rats given EG strongly inhibited the actions of EG on serum lipid profiles, which leads to declination of the serum concentration of cholesterol, TG, VLDL and non-HDL cholesterol as compared to EG treated rats. Similarly to the current study, Lien *et al*., (2016) and Mahmud *et al*., (2021), discovered that in urolithiasis defect the level of serum TG significantly increased. More recently, Al-Mobideen et al., (2022), investigation support current study that *C. aronia* significantly decreased TG levels in diabetic mice. Furthermore, Hameed *et al*., (2019), investigated that different medicinal plants via their bioactive compounds potentially reduced the level of cholesterol, TG, LDL and VLDL. In recent, Mahmud *et al*., (2021), discovered that in herbs phenolic compounds act as main hypolipidemic agent and they lowering the level of some lipid parameters in EG treated rats. Also, Zeni *et al*., (2017) and Mahmud *et al*., (2021) in their investigations showed that polyphenolics could regulate the hyper-lipidemic condition. In addition, Hameed *et al*., (2019) and Kumar and Pandey (2013), showed that in plants flavonoids play a crucial role in decreasing parameters of lipids. Concerning to previous studies *C. aronia* may act as antiurolithiasis agent through its bioactive chemicals.

5. CONCLUSION

The data obtained by this investigation showed that administration of *C. aronia* to urolithiatic rats has therapeutic actions via recovering kidneys' functions as improved through declination in serum creatinine and urea, also reduction in the rate of crystals in urine due to it can be employee as natural antiurolithiasis drug.

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SUPPLEMENTARY

Table (supplementary (SUPL.)): Effect of *C. aronia* on body weight, kidney weight, kidney weight/ body weight and serum uric acid, creatinine, urea, sodium, ionized calcium, total calcium, potassium, chloride, alanine aminotransferase, Aspartate Aminotransferase, total bilirubin, cholesterol, triglyceride, very low-density lipoprotein, non-high density lipoprotein cholesterol, low-density lipoprotein, low-density lipoprotein/ high density lipoprotein, high density lipoprotein and glucose concentrations in ethylene glycol induced urolithiasis in male albino rats.

Experimental Groups / Measurements	**Control**	**Ethylene Glycol (EG)**	**Ethylene Glycol+*Crataegus aronia* (EG+*C. aronia*)**
Body Weight Gain/ Loss (g)	83.14±2.040 **	65.83±2.713	74.71±4.714 ns
Kidney Weight (g)	1.089±0.132 ****	1.982±0.068	1.379±0.044 ***
Kidney Weight/ Body Weight (g)	0.003±0.0003 ****	0.005±0.0001	0.004±0.0002 *
Uric Acid (mg/dl)	1.233±0.058 *	1.507±0.029	1.515±0.093 ns
Creatinine (mg/dl)	0.428±0.018 ****	0.666±0.016	0.528±0.018 ***
Urea (mg/dl)	34.00±1.447 ****	78.50±3.862	47.00±1.528 ****
Sodium (mmol/L)	152.2±0.675 ns	150.4±0.632	150.2±0.641 ns
Ionized Calcium (mmol/L)	1.189±0.017 ns	1.153±0.008	1.166±0.010 ns
Total Calcium (mmol/L)	2.313±0.031 ns	2.248±0.015	2.274±0.021 ns
Potassium (mmol/L)	4.952±0.205 ns	4.770±0.151	5.198±0.225 ns
Chloride (mmol/L)	111.1±0.706 ns	112.6±0.809	110.7±0.393 ns
Alanine Aminotransferase (U/L)	46.43±4.923 ns	34.67±3.938	48.14±3.863 ns
Aspartate Aminotransferase (U/L)	197.3±16.35 ns	152.0±4.367	174.0±12.31 ns
Total Bilirubin (mg/dL)	0.300±0.025 ns	0.366±0.021	0.333±0.042 ns
Cholesterol (gm/ dL)	60.43±1.674 **	71.00±1.461	62.86±1.969 **
Triglyceride (mg/dL)	88.43±5.014 **	112.5±3.784	92.29±1.997 **
Very Low-density Lipoprotein (mg/dL)	17.69±1.003 **	22.50±0.756	18.46±0.399 **
Non-High Density Lipoprotein Cholesterol (mg/dL)	24.93±1.598 ***	35.20±1.241	30.00±1.438 *
Low-Density Lipoprotein (mg/dL)	13.91±0.234 ***	20.00±1.390	18.57±0.841 ns
Low-Density Lipoprotein/ High Density Lipoprotein Ratio (mg/dL)	0.392±0.005 *	0.537±0.047	0.609±0.047 ns
High Density Lipoprotein (mg/dL)	35.50±0.838 ns	37.50±0.763	34.71±0.606 ns
Glucose (mg/dL)	90.20±3.153 ns	80.67±4.208	79.67±3.313 ns

Data presented as mean ± S.E

The comparison was done between control and ethylene glycol (EG) groups and *Crataegus* (*C.) aronia* and ethylene glycol (EG)

*** =P<0.05 ** =P<0.01 *** =P<0.001 **** =P<0.0001**