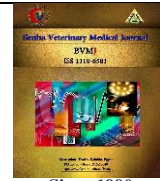




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Biochemical and molecular study of *Lactuca sativa* on diuresis induced experimentally in rats.

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ABSTRACT

This study investigated the biochemical and molecular effects of *Lactuca sativa* (lettuce) on diuresis induced experimentally in rats using furosemide. Diuresis, characterized by excessive urine production, is associated with various factors such as diet, diuretics, diabetes, and temperature. There were four groups of forty male rats (10 in each). Group I, or the normal control group, was given no medication. Group II, or the *L. sativa* group, rats received 2 ml of a suspension of 150 mg/kg b.w of spray dried powder of *L. sativa* orally daily for 30 days. Group III (the furosemide group), rats received I/P injections of 0.4 ml of a dose of 40 mg/kg b.w./ of furosemide once daily for 30 days in order to induce diuresis. Group IV (*L. sativa* treated group) rats received 2 ml of a suspension of 150 mg/kg b.w of spray dried powder of *L. sativa* orally daily for 30 days Plus I/P injections of 0.4 ml of a dose of 40 mg/kg b.w./ of furosemide once daily for 30 days. Results showed that furosemide induced significant changes in serum electrolyte levels, kidney function tests, and hormonal levels. However, *L. sativa* treatment in diuretic rats demonstrated a mitigating effect, normalizing several parameters. The gene expression of angiotensin-converting enzyme and Renin was downregulated in the *L. sativa*-treated group. In conclusion, *L. sativa* exhibited potential benefits in maintaining electrolyte balance and kidney function in diuretic-induced rats. These findings suggest a potential therapeutic role for *L. sativa* in conditions involving electrolyte imbalances and renal dysfunction, warranting further investigation, including clinical studies in human subjects.

1. INTRODUCTION

Over-filtration of body fluid by the kidneys causes a disorder called diuresis. The aforementioned ailment may be the cause of the increased urine production in the patient. Diet, diuretics, diabetes, and temperature (Kodama et al., 2023). Furosemide is the most potent diuretic drug. In addition, it is used for the treatment of hypertension either alone or in conjunction with other drugs. High blood pressure puts more strain on the heart and arteries. It can linger for a long time and cause the heart and arteries to stop functioning properly. This damage to the blood vessels in the brain, heart, and kidneys may result in postural hypotension, dehydration, stroke, heart failure, and renal failure. Heart attacks may also be more likely in people with high blood pressure (Eid et al., 2021).

Lettuce is a great source of vitamins, minerals, lutein, B-carotene, and lycopene, among other carotenoids. In addition, it has little calories and fat and is an excellent source of vitamins and fiber. A diet low in fat and high in fiber may help decrease cholesterol. Moreover, the presence of vitamins C and E in lettuce may contribute to its antioxidant activity (Shi et al., 2014).

The use of traditional drugs as anti-diuretic medicines has increased in recent years. The diuretic effect of various plant extracts in ethnomedicine has been confirmed by animal

research. Despite the plant's widespread use in traditional medicine (Noumedem et al., 2017). According to several studies, wild lettuce leaves exhibit anti-diuretic, antibacterial, anti-arthritis, and hypolipidemic qualities in addition to protecting against kidney and DNA damage (Agunloye and coworkers, 2023). Furthermore, a study has demonstrated the use of wild lettuce leaves in the treatment of diabetes, dyslipidemia, and liver illnesses. Similarly, a fantastic vegetable option for a healthy lifestyle is wild lettuce leaf. This provides both macro- and micronutrient diversity (Kadhim et al., 2020).

The current study aimed to assess the actual and relative value of *L. sativa* aqueous extract's anti-diuretic efficacy versus the widely used diuretic furosemide via investigating some of the biochemical and molecular changes.

2. MATERIAL AND METHODS

The experiment was conducted according to the guide for the care of laboratory animals and approved by the ethical animal committee, Faculty of Veterinary Medicine, Benha University (Approval no. BUFVTM 18-09-23).

2.1 Experimental Animals.

Forty male albino rats, weighing an average of 180–220 g and aged 6–8 weeks, were purchased for use in the

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experimental portion of this study from "The Laboratory Animals Research Center" at Benha University's Faculty of Veterinary Medicine. The rats were housed in separate wire mesh cages with a 12-hour light/dark cycle, adequate ventilation, humidity control, and constant access to a standard pellet diet. Seven days were given to the animals for acclimation before the experiment began.

2.2. Chemicals

2.2.1. Furosemide Dose:

The experimental group received over thirty days furosemide (Lasix) intraperitoneally (40 mg/kg B.W.) purchased from Sanofi Aventis Company. Rats in the untreated group received injections through their peritoneum of the same volume of saline (Sharma et al., 2023).

2.2.2. Preparation of *Lactuca Sativa* administration:

The plant's rhizome, dried leaves, and stems were ground into a fine powder to provide a high surface area for absorption. After that, 150 g of the powder and 250 ml of deionized water were combined in a 500-ml conical flask, sealed, and heated in a thermostatic water bath for 15 minutes. It was filtered to produce the aqueous extract after letting it cool at room temperature. The extract was then refrigerated between 5 and 10 °C for later use (Hussein et al., 2018).

2.3. Experimental design

The present study was carried out on a total of 40 rats divided into 4 experimental groups of 10 rats each:

Group I (normal control group): the rats of this group received no medication.

Group II (L. sativa group): In this group, rats received 2 ml of a suspension of 150 mg/kg b.w. of spray dried powder of L. sativa orally daily for 30 days.

Group III (furosemide group): received I/P injections of 0.4 ml of a dose of 40 mg/kg b.w. of furosemide once daily for 30 days.

Group IV (L. sativa-treated group): in this group, in this group, rats received 2 ml of a suspension of 150 mg/kg b.w. of spray dried powder of L. sativa orally daily for 30 days Plus I/P injections of 0.4 ml of a dose of 40 mg/kg b.w. of furosemide once daily for 30 days.

2.5. Assay methods

2.5.1. Biochemical analysis

The eyes' retroorbital plexus provided blood specimens, which were then centrifuged for 15 minutes at 3000 rpm. Automated micropipettes were used to extract the pure, clear serum, which was then transferred to dry, sterile Eppendorf tubes and kept at -20 degrees Celsius in a deep freezer until it was required for a subsequent biochemical test which be measured by using NS Biotec biochemical analyzer and Tosoh hormonal analyzer, Using Biomed Kit was purchased from EGT-CHEM for Lab technology, Bader city, Industrial area Piece 170, 250 Fadan in East of Elrubaki, Egypt. (Cat Nos.17823- 19829-18125-17684-17364-20149-18670) C reactive protein was measured in each serum specimen. (Thompson et al., 1999), Calcium (Tietz, 1995), Chloride (Molleman et al., 2003), Uric acid (Lieberman et al., 2007), Albumin (Sugio et al., 1999), Total protein

(Gutteridge, and Thornton, 2005), Using an ELISA kit purchased from Siemens healthcare diagnostic Company United kingdom, (Cat Nos.180731- 198321), Cortisol (Litteral et al., 2023), Aldosterone (Palmer et al., 2000), Using an ELISA kit purchased from Siemens healthcare diagnostic Company United kingdom, (Cat Nos.180739-1764523) Anti-diuretic hormone (ADH) (Sukhovet al., 1993) and Parathyroid hormone (PTH) (Coetzee et al., 2004).

2.5.2 Molecular Analysis.

The intestinal tissue was quickly taken out and gently cracked with a scraper. After that, the specimens of tissue were cleaned by being rinsed with ice-cold saline to get rid of any clots and blood cells (Yadav et al., 2020). All colon tissue samples underwent analysis to determine the identities of ACE (Kumari et al., 2023) as well as Renin (Lourenço et al., 2022).

In this RNA extraction procedure, 30 mg of an organ sample was accurately weighed and placed into 2 ml screw-capped tubes. Subsequently, 600 µl of RNA Lysis Buffer (RLT), containing 10 µl β-Mercaptoethanol per ml Buffer RLT, was added to the tubes. The homogenization of the samples was achieved by placing the tubes into adaptor sets fixed in the clamps of the Tissue Lyser, followed by a 2-minute high-speed (30 Hz) shaking step. After homogenization, the lysate underwent centrifugation for 3 minutes at 14000 rpm. To the cleared lysate, one volume of 70% ethanol was added and immediately mixed by pipetting. Up to 700 µl of the sample, along with any formed precipitate, was transferred to a RNeasy spin column in a 2 ml collection tube. Centrifugation for 1 minute at 14000 rpm was performed, and the flow-through was discarded. This step was repeated to ensure efficient sample processing. After the washing steps with Buffer RW1 and Buffer RPE, the RNA was eluted by adding 50 µl of RNase-free water, followed by centrifugation for 1 minute at 10000 rpm. The final eluted RNA product was obtained after this comprehensive extraction protocol (Yuan et al., 2006). Ace and renin were measured by a Real-time PCR machine (Stratagene MX3005P) according to RNeasy Mini Kit instructions (Ismail et al., 2018).

2.6. Statical analysis:

To assess the variations in variable means between groups, the one-way analysis of variance (ANOVA) with the least significant difference (LSD) was employed. The statistical package for social science (SPSS) version 20 for Windows (SPSS® Chicago, IL, USA) program was used to analyze the data, with the results given as mean ± SE. When the probability was less than 0.05, it was considered significant.

3. RESULTS

Table (1) showed that administration of L. sativa to normal rats had a non-significant increase in calcium (Ca), chloride (Cl), sodium (Na), and Potassium (K) levels, a significant increase in Magnesium (Mg) levels, in contrast with the unaltered group, while the furosemide group showed a significant decrease in serum levels of Na, K, and Mg levels and a non-significant decrease in Ca, Cl, levels when compared with the control group. A significant increase in Na, K, and Mg levels was observed in the diuretic group after receiving sativa compared to the furosemide group. Table (2) showed that administration of L. sativa to G II rats caused urea and uric acid levels to remain nearly unchanged.

Injecting rats with furosemide resulted in a significant increase in creatinine, urea, and uric acid, as compared to control rats. Similarly, when contrasted with the furosemide group, the *L. sativa*-treated group showed a significant decrease in creatinine and urea levels.

Table (3) demonstrated that administration of *L. sativa* to normal rats had a non-significant increase in ADH, PTH, and aldosterone levels and a non-significant change in cortisol levels. While injection of rats with furosemide caused a non-significant rise in cortisol levels in the serum and a significant increase in ADH, PTH, and aldosterone levels in

comparison to controls, the treatment diuretic group with *L. sativa* showed a non-significant decrease in PTH, ADH, and PTH levels as contrasted with the furosemide group.

Table (4) declared that when *L. sativa* was given to normal rats, there was no discernible change in ACE and Renin levels. When compared to control rats, furosemide injection significantly elevated renin and ACE levels; however, concurrently, *L. sativa* therapy demonstrated downregulation of renin and ACE gene expression when compared with the furosemide group.

Table 1 Effect of *L. Sativa* treatments on serum calcium, chloride, sodium, potassium, magnesium in diuresis induced experimentally rats

	Calcium (mg/dL)	Chloride (mEq/L)	Na (mEq/L)	K (mEq/L)	Mg (mg/dL)
Normal Control	8.40±0.15 ^a	96.35±3.87 ^{ab}	144.47±2.64 ^a	3.84±0.26 ^a	3.07±0.26 ^b
<i>L. Sativa</i> group	8.55±0.25 ^a	105.97±4.77 ^a	149.00±1.77 ^a	4.91±0.20 ^b	3.84±0.25 ^a
Furosemide group	7.64±0.39 ^a	87.30±2.00 ^b	96.43±4.51 ^c	2.31±0.44 ^c	2.20±0.07 ^c
<i>L. Sativa</i> treated group	8.06±0.43 ^a	91.95±2.02 ^b	124.43±4.51 ^b	4.36±0.60 ^a	2.99±0.19 ^b

Table 2 Effect of *L. Sativa* treatments on serum creatinine, urea and uric acid.

	Creatinine (mg/dL)	Urea (mg/dL)	Uric Acid (mg/dL)
Normal Control	0.75±0.02 ^c	29.37±1.15 ^c	4.22±0.11 ^b
<i>L. Sativa</i> group	0.79±0.03 ^c	29.40±0.86 ^c	4.18±0.22 ^b
Furosemide group	1.29±0.10 ^a	49.00±2.36 ^a	6.86±0.22 ^a
<i>L. Sativa</i> treated group	0.95±0.07 ^b	40.74±1.60 ^b	4.74±0.24 ^b

Information is displayed as (Mean ± S.E.). Values within the same column that have distinct superscript letters differ considerably at (P<0.05).

Table 3 Effect of *L. Sativa* treatments on serum cortisol, aldosterone, ADH and PTH

	Cortisol (nmol/L)	Aldosterone (nmol/L)	ADH (nmol/L)	PTH (nmol/L)
Normal Control	8.15±0.28 ^a	73.60±0.87 ^c	2.46±0.32 ^c	8.94±1.09 ^b
<i>L. Sativa</i> group	8.24±1.46 ^a	79.27±0.60 ^c	3.14±0.15 ^{bc}	9.24±1.09 ^b
Furosemide group	10.12±0.25 ^a	92.43±3.15 ^a	4.15±0.33 ^a	15.36±2.34 ^a
<i>L. Sativa</i> treated group	9.15±1.61 ^a	85.52±1.14 ^b	3.63±0.21 ^{ab}	12.24±0.25 ^{ab}

Table 4 Effects of *L. Sativa* treatments on the expression of the ACE and Renin genes

	ACE (IU/ML)	Renin (IU/ML)
Normal Control	1.01±0.05 ^b	1.01±0.03 ^c
<i>L. Sativa</i> group	0.86±0.02 ^b	0.98±0.04 ^c
Furosemide group	7.48±0.29 ^a	9.42±0.34 ^a
<i>L. Sativa</i> treated group	0.94±0.15 ^b	5.62±0.22 ^b

The data is presented as Mean ± S.E. Significant variation is observed at (P<0.05) for mean values in the same column containing distinct superscript letters.

4. DISCUSSION

Our finding declared that furosemide showed a significant decrease in serum Sodium (Na), Potassium (K), and Magnesium (Mg) levels and a non-significant change in Calcium (Ca), and (Mg) levels. Moreover, these findings concur with those of Muller et al. (2015), who reported that patients receiving furosemide as an anti-hypertensive medication had deficiencies in their levels of calcium, magnesium, Cl, and Na, and also support the findings of Gałęska et al. (2022), who declared how furosemide-treated diabetic cats had down-regulated blood electrolyte levels. These findings are attributable to furosemide's action on the renal tubule, which is to be expected given that it is known to inhibit membrane Na⁺-K⁺-ATPase activity (Iacobelli and Jean-Pierre, 2023).

When *L. sativa* was demonstrated in diuretic rats, the results were interpreted as follows: there was a non-significant increase in Ca and Cl, levels, in addition to a significant increase in Na, K, and Mg whenever relative to the furosemide group. These findings were consistent with those of Collado et al. (2017), who noticed that feeding *L. sativa* to rats increased levels of Na, K, and Mg and slightly increased levels of calcium and chloride. Furthermore, Shasho and Shasho, (2022), declared that consuming green vegetables raises serum electrolyte levels in children.

Our study findings may be because *L. sativa* is a rich vegetable with minerals such as Potassium, sodium, and magnesium, it worked as a compensatory source for the depletion that occurred due to diuresis. These findings coincide with Kiran, (2019), who revealed that *L. sativa*

contains ingredients that assist with diuretic female rats' electrolyte balance.

Our results showed that the creatinine, urea, and uric acid serum levels in the furosemide group were considerably higher than those in the control group. This is probably due to the numerous detrimental effects diuresis has on the kidney. Our findings align with those of Kose et al. (2010), who observed that rats that were over-dehydrated showed elevated renal function tests following intraperitoneal injection of furosemide. Serum osmolality, hemoconcentration, and vasopressin activation rise as body water was lost. When compared to the diuretic-non-treated group, our results indicated that administering *L. sativa* to the diuretic group showed a significant decrease in the levels of creatinine, urea, and uric acid levels. Such results concur with Renna (2018), who stated that low amino acid, protein, and potassium ingredients in lettuce are essential for kidney health in patients with impaired kidney functions. Our results are also in accordance with Zhang et al. (2017), who discovered that the low protein and potassium levels in green vegetables decrease renal impairment in diuretic patients with kidney diseases. Where Healthy kidneys are responsible for managing the amount of potassium in the blood. They manage the potassium eaten through the diet and excreted in the urine (Narasakiet al., 2022).

Our results revealed that the injection of rats with furosemide resulted in a significant increase in serum levels of aldosterone, Antidiuretic hormone (ADH), and Parathyroid hormone (PTH). These results are because furosemide activates the renin-angiotensin-aldosterone system (RAAS). Which is activated by furosemide, which accelerates the development of systolic dysfunction. By

squandering sodium and decreasing circulation volume, furosemide triggers RAAS (Sharma, 2023). Our results corroborate the findings of Clemente-Suárez, et al. (2023), who found that rats given furosemide had considerably increased serum levels of aldosterone, ADH, and PTH.

Our results showed that group IV treated with *L. sativa* showed a non-significant increase in cortisol, ADH, and PTH. While there is a significant decrease in aldosterone when compared with the furosemide group, such findings agree with Seddiki et al. (2022), who claimed that low potassium levels in *L. sativa* led to a downregulation of the renin-angiotensin aldosterone system. The amounts of water, calcium, and sodium in lettuce explained a balance in electrolytes and the renin angiotensin aldosterone system (Dmello et al., 2023).

Our results in Table (4) declared that furosemide injection resulted in significant upregulation of renin and Angiotensin-converting enzyme (ACE) levels when compared with normal control. This is because diuresis impacts and activates RAAS, as discussed previously in Table (4). These results agreed with Thurner et al. (2019), who noticed there was significant gene expression in RAAS-activated mice.

Our findings in this study of *L. sativa* treatment showed a significant decrease in ACE and renin gene expression. These results were in accordance with Zhou et al. (2021), who illustrated that the rehydration in diuretic rats treated with vegetables and nuts and the low cholesterol and fat contents, along with the anti-inflammatory properties found in the green vegetables, these results may be due to that negative feedback occurred between serum electrolytes which improved and renin-angiotensin-aldosterone system. This finding was in accordance with Aptel et al. (1996), who stated that *Lactuca sativa* ingredients (Potassium-enriched diet) administration to rats stimulated Angiotensin II that provoked an intermediate and dramatic drop in aldosterone synthesis, this counter-regulatory mechanism may ensure adequate levels of aldosterone production in vivo, The elevated aldosterone led to decrease in renin and ACE secretion.

5. CONCLUSIONS

In conclusion, the study provides valuable evidence that *L. sativa* may have a beneficial impact on electrolyte balance and kidney function in diuretic-treated rats. These findings contribute to the growing body of literature emphasizing the potential health benefits of incorporating *L. sativa* into diets, particularly in situations where electrolyte imbalances and renal dysfunction may arise. Further research, including clinical studies in human subjects, would be beneficial to validate and extend these findings.

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