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Biochemical Effect of Colostrum on Diuresis induced experimentally in Rats

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Abstract

Diuresis, an excessive filtration of body fluids by the kidneys, can lead to various disorders, with furosemide being a potent diuretic commonly used in treatment. This study investigates the impact of colostrum on diuresis-induced rats, focusing on biochemical aspects. Furosemide, a potent loop diuretic, exerts its diuretic effect by inhibiting renal sodium and chloride reabsorption. Widely prescribed for conditions such as heart failure and edema, it provides rapid relief. Administered orally or via injection, its safety and efficacy span various age groups. Electrolyte monitoring, particularly potassium, is essential. Despite general tolerability, cautious use is warranted, considering potential interactions and contraindications, emphasizing the need for close medical supervision. Colostrum is the first milk secretion produced by the mammary glands of mammals, including humans and cows, shortly after giving birth. It is distinct from regular milk in terms of its composition and serves as a crucial source of nutrients and immune factors for newborns. Colostrum is rich in Immunoglobulins, Lactoferrin, Lysozymes, growth factors and minerals. The aim of this study was to investigate the effects of furosemide, a loop diuretic, on various physiological parameters in rats, particularly focusing on electrolyte balance, kidney function, hormonal regulation, inflammation markers, and cardiac stress markers. Additionally, the study aimed to assess the potential mitigating effects of bovine colostrum on these parameters in the context of furosemide-induced diuresis. The research aimed to provide insights into the impact of furosemide and evaluate whether colostrum supplementation could offer protective or compensatory effects on the physiological responses observed during diuresis. Forty rats were used in this investigation, ten in each of the four groups. As normal controls, Group I rats got no medication injections and 40 mg/kg of saline injections every 30 days. Rats in Group II were normal and received an oral dose of 50 mg/kg/day for 30 days. Rats I/P were injected with 40 mg/kg/day of furosemide for 30 days in order to induce diuresis in the furosemide group. Rats in the Colostrum group received 50 mg/kg of colostrum intraperitoneally every day for 30 days, followed by 50 mg/kg of colostrum daily treatment. The findings suggested that rats exhibited furosemide-induced diuresis, which was associated with a considerable increase in creatinine, urea, uric acid, aldosterone albumin, total protein, CRP, LDH, troponin, and CKMB and a marked decrease in serum levels of sodium, magnesium, and chloride. Furosemide also drastically changed the levels of potassium, calcium, and chloride and elevated the expression of the ACE and Renin genes. Treatment with COL in diuretic rats revealed a marked improvement in all previous parameters. This may be due to its potential as a hypotensive effect and to its ingredients.

Keywords: COL, Furosemide, Diuresis, Blood electrolytes, RAAS

Full-length article **Corresponding Author, e-mail:*

1. Introduction

Patients suffering from urinary retention lose so much bodily fluid that their bodies become incapable of operating normally. That happens when a patient loses more fluids than he consumes. Diuresis may happen on a particularly hot day if the patient sweats a lot or is sick with a fever, diarrhea, or vomiting. It can also be induced by loop diuretic therapy, which increases patient urine output [1]. The kidneys are affected by the strong loop diuretic furosemide, which increases the body's water loss. It primarily functions by increasing the body's excretion of water and blocking the kidneys' ability to reabsorb electrolytes. Furosemide has been utilized safely and efficiently in both pediatric and adult patients due to its quick onset and brief duration of action [2]. Furosemide is especially helpful when used in clinical situations where a medication with stronger diuretic

capability is needed [3]. state that, in addition to oral formulations, there is also an intravenous and intramuscular injection solution available. However, these options are usually restricted to patients who cannot take oral medication or who are in urgent clinical situations. BC is the first milk secretion of the cow's mammary gland after calving. Bovine colostrum is high in immunoglobulin, lactoferrin and lysozyme. It also has high levels of growth factors, bioactive peptides and is used as a powerful anti-inflammatory fluid. Compared to milk, cow colostrum has higher levels of proteins, fat, vitamins and minerals. It is used as a compensatory drug for immune system deficiencies and blood electrolyte imbalances [4].

2. Materials and techniques

2.1. Experimental animals

For this investigation, a total of forty male albino rats weighing an average of 180–220 g were employed. The rats were aged between 6 and 8 weeks. The University of Veterinary Medicine in Benha is where the rats were purchased. They were housed in wire-mesh cages that had 12 hours of light and dark cycles, enough ventilation, and humidity. Their usual diet consisted of pellets and copious amounts of clean drinking water.

2.2. Furosemide Dose

The experimental group received furosemide (Lasix) intraperitoneal (40mg/kg B.W) purchased from Safoni Aventis company for a duration of 30 days. The control group of rats was injected with saline of same volume intraperitoneal [5].

2.3. Preparation of COL

Bovine COL (Immu Guard) was purchased from El Ameriea Company at a dose of 50 mg/Kg.b.w /day orally. The mixture was sealed and boiled for 15 minutes in a heated water bath after 50g of powder dissolved in 250 mL of deionized water, which has been squeezed into the 500 ml conical flask. It had been filtered in order to extract an aqueous solution once it had reached room temperature. The extract was kept in a refrigerator between 5 and 10 °C for later use.

2.4. Experimental Design

Bovine COL (Immu Guard) was purchased from El Ameriea Company at a dose of 50 mg/kg/day orally. The mixture was sealed and boiled for 15 minutes in a heated water bath after 50g of powder dissolved in 250 mL of deionized water, which had been squeezed into the 500 mL conical flask. It had been filtered in order to extract an aqueous solution once it had reached room temperature. The extract was kept in a fridge. There were forty rats, divided into four groups (10 each). Group I, the standard control group, received 30 days of saline injections at a dosage of 40 mg/kg and no medication. The Colostrum group (Group II) was made up of typical rats that received an oral dose of 50 mg/kg b.e. of bovine colostrum every 30 days. Rats in Group III, nicknamed the Furosemide group, received an intraperitoneal injection of 40 mg/kg b.w. of furosemide for 30 days in an attempt to cause diuresis. Group IV, which administered bovine colostrum treatment at an oral dose of 50 mg/kg b.w. daily/30, was treated with furosemide at a dose of 40 mg/kg b.w. daily/30 igerator between 5 and 10 °C for later use. (Ethical Approval Number: BUFVTM 18-09-23).

2.5. Sampling

Rats were starved for an entire night and given diethyl ether anesthesia at the conclusion of the thirty-day experiment. Samples of intestinal tissue and blood were taken from both the experimental and control groups of animals.

2.5.1. Blood specimens

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. CRP was measured in each serum specimen. [6], Calcium [7], Chloride [8], Uric acid [9], Albumin [10], Total protein [11], Creatinine [12], Urea [13], Na [14], K [15], Mg [16], PTH [17], LDH [18], Troponin [19], CKMB [20], Cortisol [21], Aldosteron [22], and ADH [23].

2.5.1.1. Apparatuses and equipment employed

Biosystem 20, Ns Piotec SCA 1100 (spectrophotometer), Immulite 1000 elisa equipment, and AXP 77 Electrolytes Analyzer (erma) were utilized.

2.5.2. Tissue Samples

Tissue specimens were placed in a 2-milliliter Eppendorf tube, which was immediately placed in liquid nitrogen and kept at -80 degrees Celsius until RNA extraction. The abdomen was opened, and the colon specimen was gently removed with a scraper. Next, to remove any blood cells or clots, the colon specimen was cleaned by rinsing it with ice-cold isotonic saline. By examining every colon tissue sample, the expression of the genes Renin and ACE [24].

2.5.3. Tools and apparatus for real-time polymerase chain reaction

Biohit Unichannel micropipettes (100-1000, 2-20, 0.5-10) and (20-200) µl, a real-time PCR machine (Stratagene MX3005P), varying-sized filter tips, 0.2 milliliter optical tubes, and optical caps from the Applied Biosystem.

2.6. Statical analysis

The study employed a one-way analysis of variance (ANOVA) with the Least Significant Difference (LSD) method to assess the differences in variable means among the groups. The data, which are shown as the mean ± SE, were analyzed using the Statistical Package for Social Science (SPSS) version 20 for Windows (SPSS® Chicago, IL, USA) program. When $P < 0.05$, the likelihood was considered significant.

3. Results

Table 1: Rats given a furosemide injection experienced a significant drop in serum Na and Mg in contrast to the standard control group. In addition to a non-significant decrease in Ca, Cl, and K, When colostrum was given to diuretic rats, the serum levels of Na and Mg increased significantly when compared to the untreated group, but the Ca,Cl, K, and Mg levels did not significantly

increase. Following colostrum treatment, the parameters of the diuresis group improved to nearly typical values. Table 2 showed that the administration of furosemide to rats resulted in a significant rise in the activity of kidney function tests, including urea, uric acid, and creatinine, in contrast to normal control rats. The colonostrum group, when compared with the normal control, showed a significant increase in creatinine, urea, and uric acid levels. The colonostrtum-treated group, when compared with the normal control, showed a non-significant increase in kidney function. Table 3 demonstrated that giving rats a furosemide injection produced a significant increase in serum levels of PTH, aldosterone, and ADH. The col-treated group showed a non-significant decrease in the previous results. Treatment of the diuretic group with colostrum showed a non-significant increase in serum levels of aldosterone, ADH, PTH, and cortisol when compared with the diuretic-non-treated group.

Table 4 shows that, as compared to a normal control, the induction of diuresis significantly increased the concentrations of albumin, TP, and CRP. A demonstration of colostrum in normal rats showed a significant increase in serum levels of albumin, TP, and CRP when compared with the normal control. Meanwhile, colostrum treatment in the diuretic group showed a significant increase in albumin, TP, and CRP. Table 5 shows that I/P injection of furosemide for induction of diuresis revealed a significant increase in cardiac function tests, including activities of LDH, CKMB, and troponin concentration, when compared with normal rats. The colostrum-treated group showed a significant increase in cardiac function tests in contrast with normal control. A demonstration of colostrum in normal rats revealed a non-significant difference in contrast to the group that did not receive treatment. Table 6 showed that the injection of furosemide produced a significant upregulation of renin and ACE levels. The colostrum-treated group resulted in an upregulation of gene expression of renin and ACE levels when contrasted with typical control rats; however, the upregulation of gene expression was not statistically significant when colostrum was given to ordinary rats.

4. Discussion

Table 6 showed that the injection of furosemide produced a significant upregulation of renin and ACE levels. Colostrum treatment resulted in a downregulation of gene expression of renin and ACE levels when contrasted with typical control rats; however, the downregulation of gene expression was not statistically significant when colostrum was given to ordinary rats. Our findings showed that I/P injection of furosemide into rats resulted in significant rises in serum Na and Mg when contrasted with the untreated group. These findings were consistent with those of [25] and [26]**,** who conducted a study in which patients receiving long-term furosemide therapy frequently experienced both hypernatremia and hypomagnesemia. The water-deprived animals became electrolyte-depleted and water-deficient as a result of diuresis, which caused a significant increase in the daily output of sodium and magnesium in **urine[27].**The increased excretion of sodium, chloride, and potassium in the urine may be due to furosemide binding to the Ca Na-K-2Cl transporter and inhibiting the luminal Ca-Na-K-Cl cotransporter in the thick ascending limb of the loop of Henle [28]. The effect on the distal tubules eliminates the corticomedullary osmotic gradient and inhibits both positive and negative free water clearance without interfering with aldosterone or carbonic anhydrase. Unlike with carbonic anhydrase inhibitors, diuresis is not impeded by the formation of acidosis due to the loop of Henle's strong NaCl absorptive capacity. Furthermore, furosemide inhibits GABA-A receptors in a noncompetitive subtype-specific manner [29].

Rats treated with furosemide-induced diuresis had significantly higher serum levels of calcium, sodium, potassium, and magnesium after receiving colostrum treatment than the diuretic group. These findings concur with those of [30], who discovered that providing rats with bovin colostrum maintained the electrolyte balance necessary to maintain typical plasma volume and rat cell function. Sodium is widely distributed in the ECW and helps maintain plasma volume and osmotic equilibrium between the ECW and the ICW. Based on **[**31]. potassium is an electrolyte found in the interstitial fluid that aids in controlling osmotic pressure and is crucial for nerve stimulation and muscle contraction. Blood calcium levels are maintained within relatively small ranges by this negative feedback mechanism [32]. According to [33], our research on the effects of bovine colostrum indicates that it is a good source of several minerals, particularly calcium, phosphorus, and magnesium, which are present in relatively large amounts along with zinc and selenium. The utilization rate of these minerals is between 92 and 98%, which led to electrolyte compensation in the diuretic rats [34].

Furosemide-induced diuresis has multiple effects on the kidney, as evidenced by the significant rise in serum levels of urea, creatinine, and uric acid in the furosemide group relative to the untreated group. Vasopressin is activated, and serum osmolality, hemoconcentrations, and kidney function tests rise as a result of the body losing water [36]. Our results also corroborate those of [37], who found that an intraperitoneal injection of furosemide increased kidney function tests in rats who were very dehydrated.

When colostrum was given to the diuretic group, compared to the diuretic-non-treated group, renal function tests showed a non-significant decline. This is because the high lactoferrin content of Colostrum shielded human kidney tubular epithelial cells from oxidative stress-induced cell death and apoptosis. Furthermore, lactoferrin inhibited the expression of the profibrogenic genes CTGF, PAI-1, and collagen, preventing kidney fibrosis brought on by TGF-β1. Renal function tests decreased, but not significantly. Additionally, [38] showed that the use of lactoferrin for patients who are critically ill with acute kidney injuries may be able to reduce kidney functions. Our results are also in association with the findings of [39], where it can be very easily shown that lactoferrin reduces the malfunction and corruption that occur due to diuresis. Serum concentrations of PTH, cortisol, aldosterone, and ADH significantly rose in rats given furosemide injections and subsequent deprivation of water and electrolytes.

Table 1. Effect of colostrum treatments on Serum Calcium, chloride, Sodium, Potassium, magnesium in diuresis induced experimentally in rats

	Calcium (mg/dL)	Chloride (mEq/L)	Na (mEq/L)	K (mEq/L)	Mg (mg/dL)
Normal Control	8.40±0.15^{bc}	96.35±3.87^{b}	144.47±2.64^{a}	3.84±0.26^{a}	3.07±0.26^{a}
COL group	10.30±0.31^{a}	131.76±3.67^{a}	152.82±3.86^{a}	4.41±0.27^{a}	2.96±0.16^{a}
Furosemide group	7.64±0.39^{c}	87.30±2.00^{b}	96.43±4.51^{c}	3.30±0.44^{a}	2.20±0.07^{b}
Colostrum treated group	8.85±0.23^{b}	96.45±2.82^{b}	126.47±3.80^{b}	3.85±0.54^{a}	2.62±0.13^{ab}

That data is displayed as the median ± S.E. S.E. is the standard error. There are significant differences at ($P<0.05$) between the mean values in the same column with different superscript letters

Table 2. Impact of colostrum therapy on urea, uric acid, and serum creatinine

	Creatinine (mg/dL)	**Urea (mg/dL)**	**Uric Acid (mg/dL)**
Normal Control	0.75±0.02c	29.37±1.15d	4.22±0.11c
COL group	0.96±0.02b	35.12±1.67c	5.41±0.35b
Furosemide group	1.29±0.10a	49.00±2.36a	6.86±0.22a
Colostrum treated group	1.12±0.04b	42.32±0.64b	6.12±0.43ab

The data is presented as the median ± S.E. S.E. is the standard error. Distinct superscript letters in the mean values of the same column denote a significant difference at ($P<0.05$)

Table 3. Effect of colostrum treatments on Serum Cortisol, Aldosterone, ADH, glucose and Rat weight

	Cortisol (nmol/L)	Aldosterone (nmol/L)	ADH (nmol/L)	PTH (nmol/L)
Normal Control	8.15±0.28^{ab}	73.60±0.87^{b}	2.46±0.32^{b}	8.94±1.09^{b}
COL group	7.10±0.42^{b}	70.13±0.88^{b}	2.16±0.33^{b}	6.17±0.57^{b}
Furosemide group	10.12±0.25^{a}	92.43±3.15^{a}	4.15±0.33^{a}	15.36±2.34^{a}
Colostrum treated group	8.31±1.25^{ab}	76.30±1.47^{b}	2.97±0.32^{b}	10.74±1.02^{ab}

The data is presented as the median ± S.E. S.E. is the standard error. Distinct superscript letters in the mean values of the same column denote a significant difference at ($P<0.05$)

Table 4. Effect of colostrum treatments on Serum Albumin, total proteins and C.R.P.

	Albumin g/dL	**T. protein g/dL**	**CRP mg/dL**
Normal Control	4.24±0.06^{d}	8.19±0.12^{d}	2.80±0.24^{c}
COL group	5.13±0.09^{c}	9.20±0.05^{c}	5.77±0.23^{b}
Furosemide group	6.03±0.10^{a}	10.62±0.19^{a}	6.89±0.41^{a}
Colostrum treated group	5.62±0.13^{b}	9.90±0.10^{b}	4.34±0.36^{b}

The data is shown as (Mean ± S.E.). The standard error is S.E. In the same column, mean values that have distinct superscript letters indicate a significant difference at ($P<0.05$).

Table 5. The impact of colostrum therapy on heart function during experimentally induced diuresis in rats.

	LDH U/L	TROPONIN ng/mL	CKMB IU/l
Normal Control	516.83±37.99^{c}	4.73±0.36^{b}	0.99±0.09^{c}
COL group	564.71±24.47^{c}	4.17±0.33^{b}	1.23±0.13^{c}
Furosemide group	935.85±30.00^{a}	8.18±0.68^{a}	4.20±0.06^{a}
Colostrum treated group	745.74±94.12^{b}	4.61±0.41^{b}	2.77±0.58^{b}

The data is presented as Average ± S.E. Standard error stands for S.E. Mean values in the same column whose whose superscript letters differ indicate a significant variation at ($P<0.05$)

Table 6. Effect of colostrum treatments on Renin and ACE gene expression

	Renin IU/ML	ACE IU/ML
Normal Control	1.01±0.03^{c}	1.01±0.05^{b}
COL group	0.98±0.04^{c}	0.86±0.02^{b}
Furosemide group	9.42±0.34^{a}	7.48±0.29^{a}
Colostrum treated group	5.62±0.22^{b}	4.94±0.15^{b}

The data is presented as Average ± S.E. Standard error stands for S.E. Mean values in the same column whose whose superscript letters differ indicate a significant variation at ($P<0.05$).

Table 7: Bovine Colostrum Ingredients Compared with Milk [35]

Protein (%)	14–16	3.1–3.2
Casein (%)	4.8	2.5–2.6
Albumin (%)	6.0	0.4–0.5
Total immunoglobulin (mg/mL)	42–90	0.4–0.9
Lactose (%)	2–3	4.7–5.0
Minerals		
Calcium (g/kg)	2.6–4.7	1.2–1.3
Phosphorus (g/kg)	4.5	0.9–1.2
Potassium (g/kg)	1.4–2.8	1.5–1.7
Sodium (g/kg)	0.7–1.1	0.4
Magnesium (g/kg)	0.4–0.7	0.1
Zinc (mg/kg)	11.6–38.1	3.0–6.0
Vitamins		
Thiamin (B1) (μg/mL)	0.58–0.90	0.4–0.5
Riboflavin (B2) (μg/mL)	4.55–4.83	1.5–1.7
Niacin (B3) (μg/mL)	0.34–0.96	0.8–0.9
Cobalamin (B12) (μg/mL)	0.05–0.60	0.004–0.006
Vitamin A (μg/100 mL)	25	34
Vitamin D (IU/g fat)	0.89–1.81	0.41
Tocopherol (E) (μg/g)	2.92–5.63	0.06
Immunoglobulins		
IgG1 (g/L)	34.0–87.0	0.31–0.40
IgG2 (g/L)	1.6–6.0	0.03–0.08
IgA (g/L)	3.2–6.2	0.04–0.06
IgM (g/L)	3.7–6.1	0.03–0.06
Antimicrobials		
Lactoferrin (g/L)	1.5–5	0.02–0.75
Lactoperoxidase (mg/L)	11–45	13–30
Lysozyme (mg/L)	0.14–0.7	0.07–0.6

Our findings are in line with those of **[40]**, who discovered that while diuresis did not change plasma levels of adrenocorticotropic hormone (ACTH), renin activity (PRA), plasma Angiotensin II (AII), vasopressin deprivation caused the adrenal capsule containing the zona glomerulosa to rise substantially in absolute weight without changing the density of cells per area unit, suggesting that the hyperplasia of the zona glomerulosa was the cause of the adrenal capsule's growth., Following diuresis, the production of ADH and aldosterone rose. It has been proposed that the ADH triggers aldosterone secretion in addition to its kidney's water-absorbing roles. The colon is then the main organ where aldosterone acts to cause salt and water absorption [41]. When contrasted to the diuretic-non-treated group, the colostrum-treated group's serum levels of aldosterone and ADH were greatly reduced. The minerals lactoferrin and zinc found in colostrum, however, acted as compensatory factors in blood electrolyte hemostasis and as a negative feedback pathway for the pituitary gland to reduce ADH secretion and adrenal gland secretion of aldosterone. This is why PTH and cortisol were not significantly reduced. These outcomes concur with those of [42], who observed that diuretic camels given colostrum and lactoferrin experienced a significant improvement in the hormones that regulate blood minerals and water retention. However, induction of diuresis in rats revealed a significant increase in the concentration of albumin, TP, and CRP. Meanwhile, colostrum treatment showed improvement in these parameters to nearly normal levels. This is in agreement with [43], who revealed that diuresis in female rats led to hemoconcentration and vasoconstriction, which led to increased protein liver production for all serum proteins. In addition, results in line [44] demonstrated that water deprivation increased inflammation and histamine and cortisol secretion as a result of blood acidity, and that the possibility of inflammation increased CRP levels as a result.

Our findings revealed that the colostrum demonstration in normal rats showed a significant decrease in serum levels of albumin, TP, and CRP in contrast to the standard control. This is due to decreasing the inflammatory state because of the lactoferrin and immunoglobulin contents in the colostrum, and this is in agreement with [45], who examined the effect of lactoferrin and immunoglobulins in the colostrum on CRP and inflammation rate in diuretic rats. Injection of furosemide for the induction of diuresis revealed a significant increase in cardiac function tests, including activities of LDH, CKMB, and troponin concentration, when compared with normal. This is in line with the findings of [46]**.** There was a significant increase in cardiac enzyme levels (LDH, troponin, and CKMB) that could be brought on by those diuretics. Activation of the renin-angiotensin-aldosterone system (RAAS) is a critical step in the development of heart failure. The inability of the heart to supply enough blood flow to essential organ systems initially triggers changes in the RAAS. In an attempt to make up for the reduced blood flow, the RAAS specifically releases more angiotensin II. Angiotensin II overproduction exacerbates cardiac failure [47].

Our findings revealed that the colonostrum demonstration in normal rats showed a significant decrease in the serum level of cardiac enzymes, which is also due to lactoferrin. These findings concur with those of [48], who reported a noteworthy decline in diuretic rats given lactoferrin treatment. It has been suggested that lactoferrin's antihypertensive effects result from NO-dependent vasodilation and restriction of the angiotensin I-converting enzyme (ACE) and endothelin-converting enzyme (ECE) enzyme activities [49]. Moreover, furosemide injection resulted in significant upregulation of renin and ACE levels in contrast to a typical control. This is due to the fact that diuresis impacts and activates RAAS, as discussed previously in tables 4 and 5. These results are in agreement with [50], who noticed there was a significant increase in gene expression of RAAS in rats that were injected with loop diuretic-activated rats. While colostrum treatment in diuretic rats reduced gene expression of renin and ACE levels due to lactoferrin action on the renin angiotensin aldosterone system [51].

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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