**Evaluation The Effect Of Extract Of Phoenix Dactylifera Palm Leaves On Some Blood Serum Electrolytes In Alloxan Treated Female Rats**

* Nabeel M. N. Al-Sharafi  
Department of Physiology and Pharmacology/Faculty of Veterinary Medicine/University of Kufa/Iraq.  Corresponding author: Nabeel M. N. Al-Sharafi.

**Abstract**

This study was conducted to evaluate the effect of ethanolic extract of Palm Leaves (Phoenix Dactylifera) on blood serum electrolytes in alloxan treated female rats. Eighteen albino adult female rats of wistar strain average body weight between 150g to 200g were randomly divided into three experimental groups (n = 6). Group I (serves as control group), Group II (injected intraperitoneally (i.p.) with single dose alloxan 100 mg kg/B.W), Group III (injected intraperitonealy (i.p.) with single dose alloxan 100 mg kg/B.W and daily orally gavages with 200mg/kg B.W palm leaves extract). Serum sodium, potassium, magnesium, chloride and phosphor were estimated. Alloxan injection induced decrease in serum sodium concentration and increase in serum potassium, magnesium, chloride and phosphor concentration. Oral administration of palm leaves extract resulted in a significant elevation (p < 0.05) in serum sodium concentration near the normal value and significant decrease (p < 0.05) in serum potassium and chloride concentrations, while non significant reduces in serum magnesium and phosphor concentrations. palm leaves extract may be useful in improving the clinical benefits for serum electrolyte changes in diabetes...

**INTRODUCTION**

The management of diabetes without any side effects is still a challenge to the medical system as the treatment for diabetes is relatively limited with significant side effects. There is growing interest in the use of natural health products as an alternative approach to current medications. Plant sources has become a target to explore new drugs and in searching biologically active compounds.

Alloxan (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetron) is an oxygenated pyrimidine derivative which is present as alloxan hydrate in aqueous solution. Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus.

The interrelationship between diabetes and various minerals is characterized by a high degree of reciprocity. Chronic uncontrolled hyperglycemia can cause significant alterations in the status of these nutrients, and conversely, some of these substances, especially those that have been characterized as micronutrients, can directly modulate glucose homeostasis. However, minerals play diverse roles in the body. They most commonly function as essential coenzymes and cofactors for metabolic reactions and thus help support basic cellular reactions (glycolysis, the citric acid cycle, lipid and amino acid metabolism) required to maintain energy production and life.

According to World Health Organization, medicinal plants can be a good source of variety of drugs. Various societies across the world have shown great interest in curing diseases using plants/ plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As a preventive and curative measure, plants and their products have been used in the treatment of infections for...
centuries. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare (6).

Date palm (Phoenix dactylifera L) as long been one of the most important fruit crops in the arid regions of the Arabian Peninsula, North Africa and Middle East. During the past three centuries, date were also introduced to new production areas in Australia, India, Pakistan, Mexico, Southern Africa, South America and the United States (7). The palm leave extract was worked as anti-diabetic in the alloxan induce diabetic rats model for minimize the complication associated with the diabetic and related disorder (8).

MATERIAL AND METHODS

Animals and Experimental Design:
Adult female Wistar albino rats weighing (150-200g) were purchased from animal house of faculty of Veterinary Medicine-Kufa University-Iraq. Rats were housed individually with constant environment in controlled stainless steel cages, temperature (25°C ± 5°C) and light cycle were held constant 12/12 hr. The experimental period was 4 weeks on which food and water were provided ad libitum.

Animal's Diet:
Pellet diet (commercial diet).

Preparation of palm leaves Extract:
Fresh palm leaves of Phoenix Dactylifera were collected from Kufa area in Najaf province in Iraq. The leaves were air dried on laboratory bench top and the leafy exudates homogenized in an electric blender.

Extraction
Extraction of palm leaves was performed according to Markham (9), in two steps as following:

Step one
200 g of palm leaves were crushed with 400ml of mixture methanol 95% and distilled water (9:1), mixed for 18h in magnetic stirrer at room temperature, and then filtered under vacuum using Whitman No. (1).

Step two
The filtrate residues from step one was mixed again with 200ml of mixture methanol 95% and distill water (1:1) for 18h in magnetic stirrer at room temperature and the filtered was collected as described in step one. Then, the filtrate collected in step 1 and 2 was evaporated in the incubator (42°C) to reach one-third of original volumes. The concentrated extract was separated from low organic materials by addition of chloroform 20:100 (extract:chloroform) in separator funnel, then the mixture was left for one hour to separate in two layers: lower layer contain chloroform and upper layer contain (total polyphenol). The upper layer was separated with chloroform 10:100 (extract:chloroform), from the upper layer, total polyphenol was collected and dried in incubator at (40ºC), and then collected as powder.

Alloxan injection:
A single intraperitoneal injection with alloxan (100 mg / Kg body weight) (10). Rats were fast overnight before injection with alloxan.

Experimental Design:
The animals were divided into 3 groups (each of 6 rats). The experimental groups illustrated as follow:
Group 1: Healthy rats served as normal controls.
Group 2: single intraperitoneal (i.p.) injection of alloxan100mg/kg (B.W).
Group 3 a single intraperitoneal (i.p.) injection of alloxan100mg/kg (B.W)+ gavages orally with 200mg/kg B.W. palm leaves extract.

Fasting blood samples were drawn from heart puncture of rat at days 15 and 30 of experiment for measurement serum sodium, potassium, chloride, magnesium and phosphor concentration mg/dL using semi-automatic chemistry analyzer Belgium using kit Cyan com./Belgium). The blood samples left for 15 minutes at 37°C for serum separation, then centrifuged at 3000 rpm for 20 minutes, then sera were separated to used in analyses.

STATISTICAL ANALYSIS
The results are expressed as the mean values with their standard error. One-way ANOVA followed by Duncan’s variance was performed to compare between treatment groups. Significance was set at p<0.05 by used Statistical Package for Social Science (SPSS 20) Ready statistic program 20.

RESULT
The results in Table 1 revealed that alloxan injection led to a significant decrease in serum sodium concentration in T2 group compared with the corresponding control group (P< 0.05). Following oral gavages of palm leaves extract
serum sodium concentration reverted back to near normal level. This increase in serum sodium concentration in alloxan injection group was accompanied by a significant increase in serum potassium, magnesium, chloride and phosphor concentration in Tables 2, 3, 4 and 5 respectively in comparison with control group (P<0.05). Oral administration of palm leaves extract (T3) caused a significant decreases in these elevated of serum potassium and chloride concentrations, while non significant reduces in serum magnesium and phosphor concentrations in day 15 and 30 of experiment.

Table (1) Effect of palm leaves extract on serum sodium concentration (mEq/L) in alloxan injected female rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>138.01±0.90aB</td>
<td>136.27±1.28aC</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>113.65±2.12bA</td>
<td>105.50±1.67aA</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>114.44±2.01aA</td>
<td>126.73±1.18bB</td>
</tr>
</tbody>
</table>

-C= control.
- T1=Animal intraperitoneally injected with alloxan (100mg/kg B.W.)
- T2=Animal intraperitoneally injected with alloxan (100mg/kg B.W.)+Palm leaves extract (200mg/kg orally).
- Capital letter denote difference between groups, P>0.05.
- Small letter denote difference within groups, P>0.05.

± SE.

Table (2) Effect of palm leaves extract on serum potassium concentration (mEq/L) in alloxan injected female rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>5.72±0.19aA</td>
<td>5.92±0.20aA</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>6.71±0.19aAB</td>
<td>8.29±1.18AB</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>7.89±0.19bB</td>
<td>5.52±0.18aA</td>
</tr>
</tbody>
</table>

-C= control.
- T1=Animal intraperitoneally injected with alloxan (100mg/kg B.W.)
- T2=Animal intraperitoneally injected with alloxan (100mg/kg B.W.)+Palm leaves extract (200mg/kg orally).
- Capital letter denote difference between groups, P>0.05.
- Small letter denote difference within groups, P>0.05.

± SE.

Table (3) Effect of palm leaves extract on serum magnesium concentration (mg/dL) in alloxan injected female rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>1.898±0.54aA</td>
<td>2.27±0.27aA</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>3.90±0.11aB</td>
<td>3.52±0.02aB</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>3.72±0.04aB</td>
<td>3.88±0.22aB</td>
</tr>
</tbody>
</table>

± SE.
- C= control.
- T1=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) .
- T2=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + Palm leaves extract (200mg/kg orally).
- Capital letter denote difference between groups, P> 0.05.
- Small letter denote difference within groups, P> 0.05.
± SE.

Table (4) Effect of palm leaves extract on serum chloride concentration (mmol/L) in alloxan injected female rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>192.95±5.60aA</td>
<td>203.87±6.93aA</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>378.24±2.75aB</td>
<td>381.79±5.62aC</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>380.72±3.97bB</td>
<td>337.85±5.41aB</td>
</tr>
</tbody>
</table>

- C= control.
- T1=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) .
- T2=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + Palm leaves extract (200mg/kg orally).
- Capital letter denote difference between groups, P> 0.05.
- Small letter denote difference within groups, P> 0.05.
± SE.

Table (5) Effect of palm leaves extract on serum phosphorus concentration (mg/dL) in alloxan injected female rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
<td>0.275±0.03aA</td>
<td>0.351±0.09aA</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>0.347±0.02aA</td>
<td>0.624±0.03bB</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>0.279±0.05aA</td>
<td>0.591±0.01bB</td>
</tr>
</tbody>
</table>

- C= control.
- T1=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) .
- T2=Animal intraperitonealy injected with alloxan (100mg/kg B.W.) + Palm leaves extract (200mg/kg orally).
- Capital letter denote difference between groups, P> 0.05.
- Small letter denote difference within groups, P> 0.05.

DISCUSSION

The pancreatic β cells were destroyed using alloxan, a toxic glucose analogue that accumulate in pancreatic beta cells via GLUT 2 glucose transporter. In the presence of thiols, especially glutathione (GSH), alloxan generates reactive oxygen species (ROS) in cyclic redox reactions. The reduction product of alloxan is dialuric acid. Auto-oxidation of dialuric acid generates ROS, which are responsible for the death of the β cells (11).

Serum electrolyte concentrations are among the most commonly used laboratory tests by clinicians for assessment of a patient’s clinical conditions and disease states. Sodium, potassium, and chloride are among the most commonly monitored electrolytes in clinical practice. Magnesium, calcium, and phosphate are also monitored as determined by the patient’s disease states and/or clinical indication (12). Serum sodium level was observed to decrease significantly following diabetes induction glucose excretion in urine by diabetics imposes an osmotic diuresis or dehydration (13), with the consequence of electrolyte lost with dehydration in hyperglycemia, the elevated serum glucose concentration results in high serum osmolarity, thus creating an osmolar gradient between the plasma compartment.
and the extracellular fluid leading to a shift of water into the intravascular space the net effect is a dilution of the serum sodium concentration resulting in hyponatremia \(^{(14)}\).

Patients with hyponatremia associated with low total body sodium often exhibit signs and symptoms of dehydration. These manifestations include thirst, dry mucous membranes, weight loss, sunken eyes, diminished urine output, and diminished skin turgor \(^{(14)}\). The most common medical conditions associated with hyporeninemic hypoaldosteronism include diabetes \(^{(15)}\), the low levels of aldosterone or tubular unresponsiveness to this hormone are present in the majority of patients with hyperkalemia and impaired renal function \(^{(16)}\) many patients with renal impairment can, therefore, maintain a near normal, serum potassium concentration. They are still prone to developing hyperkalemia if excessive potassium is consumed and when renal function deteriorates \(^{(17)}\).

Oh et al \(^{(18)}\) noted a somewhat different pattern, however. In 35 patients with diabetic ketoacidosis, the increase in anion gap exactly paralleled the fall in bicarbonate. However, during recovery, these patients all developed a hyperchloremic type of metabolic acidosis. The mechanism of hyperchloremic acidosis in diabetic ketoacidosis caused by considering the consequences of adding a large quantity of beta-hydroxybutyric acid to the extracellular fluid, as occurs early in the development of diabetic ketoacidosis. Initially, each hydrogen ion combines with a bicarbonate, “destroying” it to produce CO\(_2\) and water. The accompanying anion (in this case beta-hydroxybutyrate) is retained in the plasma and is an “unmeasured” anion. However, because the clearance of ketoacid anions by the kidney is relatively high, as long as volume depletion is avoided and the glomerular filtration rate is adequate, many of these unmeasured anions will be excreted in the urine along with accompanying cations (sodium). This wasting of ketone salts produces a contraction of extracellular fluid volume and signals the kidney to retain dietary or infused sodium chloride. As a result, the bicarbonate in the extracellular fluid remains at a reduced level while the anion gap is diminished, due to a relative hyperchloremia \(^{(19)}\).

Hypermagnesemia has an impact on vital organs, which is usually seen in diabetic end stage renal disease due to decreased renal excretion, Shashidhar, t al \(^{(20)}\) recording in 60 diabetic hypermagnesemia in diabetic end stage renal disease patients, before and after hemodialysis. Was found the serum magnesium level to be significantly higher before dialysis in most of the patients which can be attributed to associated cardiovascular and central nervous system complications.

The hyperphosphatemia may be resulting due to renal dysfunction, phosphate excretion is further reduced to cause an even greater increase of serum phosphate concentration \(^{(21)}\). The increase in serum phosphate concentration increases the risk for deposition of insoluble calcium-phosphate complex in soft tissues (metastatic calcification). This deposition may further reduce the serum concentration of ionized calcium and lead to increased parathyroid hormone production and release. A sustained period of high parathyroid hormone level leads to excessive bone resorption, which will severely weaken its structural integrity \(^{(22)}\). Finally, it is concluded that the serum electrolyte changes in alloxan diabetic rats which including hyponatremia, hyperkalemia, hyperchloremia, hypermagnesemia and hyperphosphatemia, palm leaves extract may be useful in improving the clinical benefits for serum electrolyte changes in alloxan diabetic rats.

ACKNOWLEDGMENT

The author sincerely thank to lecturer Dr. Mohammed T. Nagi, Nadia K. J. Al-Dawah and Dr. Sadia S. Mahdi (Kufa university- faculty of Veterinary medicine/department of Physiology and pharmacology) for their efforts and help in the laboratory works.

REFERENCES