Effects of aqueous extract of Hibiscus sabdariffa L. on some biochemical indices of liver and kidney function in male albino rats.

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Abstract
The present study aimed to investigate the effects of aqueous extract of flowers of *Hibiscus sabdariffa* on liver and kidney function in male albino rats. Thirty male albino rats were randomly divided into five groups, six rats for each group. The extract was administered orally in doses of 0, 25, 50, 100, and 200 mg/kg body weight for 28 days. Blood samples were taken for biochemical assays. The result showed there was no significant difference (P < 0.05) in the liver and kidney to body weight ratio, levels of ALP, AST, ALT, Direct bilirubin, Total bilirubin, Sodium, Potassium, Calcium, Bicarbonate, Albumin, Chloride, Urea, Uric acid, creatinine and total protein of the treated rats when compared with the control. This study suggested that the aqueous extract of *H. sabdariffa* was not the hepatotoxic and nephrotoxic effects at the doses administered.

Introduction
Hibiscus plant (Malvaceae) includes more than 300 species, Among them is *Hibiscus sabdariffa* L. which is a valuable source of traditional medicine (Ubani et al., 2010). The dried flower contain the flavonoids, gossypetin, sabdaretin, hibiscetin (Pietta, 2000) also The presence of saponin, tannins, cyanogenic glycoside had been reported (Lin et al., 2003) Anthocyanins, flavonols and protocatechuic acid along with other phytochemicals have been identified as contributors to the observed medicinal effect of *Hibiscus sabdariffa* (Seca et al., 2001). Scientific research has established that the extracts of this flower have antihypertensive properties (Odigie et al., 2003; Jovenet et al., 2014; El-Mahmoudy et al., 2014) antidiabetes (Rosemary et al., 2014), antioxidant properties (Sini et al., 2011; Obouayeba et al., 2014; Ali et al., 2005), anti-obesity (Alarcon Aguilar et al., 2007; Kim et al., 2007) and protects against sperm damage (Idris et al., 2012). In addition to being a herbal medicinal agent, it is used as a local drink material in many countries, including Iraq, where it is commonly called Cajarat.

Liver is the largest gland and one of the vital organs of the body. It performs many vital metabolic and homeostatic functions. Drugs and other foreign substances are metabolized and inactivated in the liver and is therefore susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the liver (Emelike et al., 2014). On the other hand, the kidneys play a very important role in the regulation of electrolytes, intracellular fluid volume, the PH buffer system, endocrine processes such as RBC synthesis, Vitamin D secretion, blood pressure maintenance, and in the elimination of waste products. As such, overall body homeostasis is dependent on the functional integrity of the kidneys (Kamal, 2010). Any substance that is toxic to the kidney would adversely affect the total body metabolism. It is therefore important to establish the safety of food, drink and drugs before they are ingested. The current study was therefore, aimed to determine the effects of...
aqueous extracts of the flowers of *Hibiscus sabdariffa* on liver and kidney function in male albino rats.

**Materials and method**

**Preparation of Hibiscus sabdariffa aqueous extract.**

Fresh flowers of *Hibiscus sabdariffa* were bought from local market in Najaf city, Iraq. They were washed with tap water to remove debris and dust particles, and left to dry for seven days in the room temperature. After drying, they were milled with a mortar and pestle to get a coarse powder used for the extraction. Aqueous extract of the plant was prepared according to the method of Lyare and Adegoke (2011). Two hundred grams (200 g) of dried *Hibiscus sabdariffa* flowers was boiled in 1000 mL of distilled water for 15 min. The boiled sample was allowed to cool and then filtered. The filtrate was evaporated to dryness in an oven at 40°C to produce a dark red residue. The extract was administered orally using a 2 ml syringe modified for this purpose.

**Experimental design**

In this study a total of 30 male albino rats were obtained from the animal house of the College of medicine, University of kufa. All animals were allowed free access to food and drinking water. Light/dark period and temperature were controlled at 12/12 hour cycle and 25°C, respectively. The rats were divided into 5 groups of 6 rats each. One Group rats were normal and untreated. The four group were normal rats treated with aqueous extract of flower of *Hibiscus sabdariffa* orally with 25, 50, 100, 200 mg/kg bodyweight. At the end of experimental period, on the twenty ninth (29) day of the extract administration, rats were weighed and anaesthetized with chloroform. The liver and kidney from both control and test animals were removed and weighed to calculate the liver and kidney to body weight ratio.

**Blood collection**

Blood was collected from all the treated and control rats by cardiac puncture under chloroform anaesthesia and collected into two sample test tubes for each rat. Plane sterile test tubes were used to collect blood samples for serum electrolytes, preceded by centrifuging and subsequent separation of the blood plasma with a standard pipette.

**Blood analysis**

Alkaline phosphatase (ALP) activity was assayed in the liver according to the method of Wright *et al.* (1972) while the activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined in the liver following the method of Reitman and Frankel (1952). The concentrations of creatinine, urea, uric acid, bilirubin, total protein, albumin and electrolytes, were determined in the serum following standard procedures as described in the respective assay kits.

**Statistical analysis**

All values were expressed as mean ± SEM. Differences between groups were evaluated by one-way ANOVA followed by Tukey multiple comparison tests. Results were considered significant at P < 0.05.

**Results**

liver and kidney to body weight ratio
the result showed (Table 1) that administration of aqueous extract \textit{hibiscus sabdariffa} at all doses (25, 50, 100, 200 mg/kg body weight) investigated did not significantly \((P<0.05)\) affect liver and kidney to body weight ratio compared with control throughout the experimental period (28 days).

Table 1: Liver and kidney -body weight ratio of oral administration of Aqueous Extracts Of flowers of H. Sabdariffa in Rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control 0mg/kg M±SD</th>
<th>25 mg/kg M±SD</th>
<th>50mg/kg M±SD</th>
<th>100mg/kg M±SD</th>
<th>200mg/kg M±SD</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver-body weight ratio (%)</td>
<td>3.09 ± 0.06</td>
<td>3.1 ± 0.03</td>
<td>3.2 ± 0.1</td>
<td>3.1 ± 0.06</td>
<td>3.1 ± 0.08</td>
<td>Non sign</td>
</tr>
<tr>
<td>Kidney-body weight ratio (%)</td>
<td>1.14 ± 0.05</td>
<td>1.14 ± 0.03</td>
<td>1.13 ± 0.01</td>
<td>1.14 ± 0.02</td>
<td>1.12 ± 0.02</td>
<td>Non sign</td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard deviation \((n = 6)\), values are statistically different from control at \(p>0.05\).

Liver function indices

Results showed (Table 2) there are no significant \((P<0.05)\) changes in liver parameters (ALP, AST, ALT, Total bilirubin, Direct bilirubin) in four groups that orally administrated with 25, 50, 100 and 200 mg/kg body weight of aqueous extract of flowers of \textit{Hibiscus sabdariffa} for 28 days compared to control group. Although the rate of (ALP, AST, ALT, Total bilirubin, Direct bilirubin were changed between groups but these changes were not significant \((P<0.05)\).

Table 2: Liver function indices of oral administration of aqueous extracts of flowers \textit{H. Sabdariffa} in male albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Control 0mg/kg M±SD</th>
<th>25 mg/kg M±SD</th>
<th>50mg/kg M±SD</th>
<th>100mg/kg M±SD</th>
<th>200mg/kg M±SD</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>110 ± 7.1</td>
<td>111 ± 7.2</td>
<td>114 ± 8.9</td>
<td>116 ± 2.3</td>
<td>117 ± 4.6</td>
<td>Non sign</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>94 ± 3.6</td>
<td>95 ± 8.8</td>
<td>100 ± 7.1</td>
<td>101 ± 3.1</td>
<td>108 ± 5.7</td>
<td>Non sign</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>44.3 ± 0.2</td>
<td>44.5 ± 0.3</td>
<td>44.7 ± 0.3</td>
<td>45.1 ± 0.1</td>
<td>45.3 ± 0.2</td>
<td>Non sign</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>1.11 ± 0.4</td>
<td>1.13 ± 0.03</td>
<td>1.15 ± 0.14</td>
<td>1.16 ± 0.3</td>
<td>1.17 ± 0.3</td>
<td>Non sign</td>
<td></td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.2 ± 0.01</td>
<td>0.22 ± 0.03</td>
<td>0.22 ± 0.01</td>
<td>0.23 ± 0.03</td>
<td>0.23 ± 0.03</td>
<td>Non sign</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard deviation \((n = 6)\), values are statistically different from control at \(p>0.05\).

Kidney function indices

Administration of aqueous extract of flowers of \textit{Hibiscus sabdariffa} for 28 days with 25, 50, 100 and 200 mg/kg body weight did not significantly change the serum levels of sodium, potassium, bicarbonate, calcium and chloride ions; as well as urea, creatinine, uric acid, albumin and total portion concentrations compared with the control (Table 3).
Table 3: kidney function indices of oral administration of aqueous extracts of flowers H. Sabdariffa in male albino rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Control 0mg/kg M±SD</th>
<th>25mg/kg M±SD</th>
<th>50mg/kg M±SD</th>
<th>100mg/kg M±SD</th>
<th>200mg/kg M±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Na⁺ (mEq/L)</td>
<td>148 ± 5.2</td>
<td>149 ± 2.8</td>
<td>149 ± 5</td>
<td>150 ± 5</td>
<td>150 ± 5</td>
<td>Non sign</td>
</tr>
<tr>
<td>Potassium K⁺(mEq/L)</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.3</td>
<td>5.11 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>5.6 ± 0.3</td>
<td>Non sign</td>
</tr>
<tr>
<td>Bicarbonate HCO₃mmol/L</td>
<td>15 ± 2.3</td>
<td>15.2 ± 0.2</td>
<td>15.4 ± 0.3</td>
<td>15.7 ± 0.3</td>
<td>Non sign</td>
<td></td>
</tr>
<tr>
<td>Calcium Ca +2 mmol/L</td>
<td>180 ± 2.5</td>
<td>179 ± 1.2</td>
<td>178 ± 2.1</td>
<td>180 ± 1.3</td>
<td>180 ± 2.8</td>
<td>Non sign</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>106 ± 2.8</td>
<td>107 ± 2.5</td>
<td>108 ± 2.3</td>
<td>108 ± 1.4</td>
<td>108 ± 2.8</td>
<td>Non sign</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>28 ± 1.4</td>
<td>28.3 ± 1.4</td>
<td>28.4 ± 1.6</td>
<td>28.4 ± 1.3</td>
<td>28.5 ± 0.7</td>
<td>Non sign</td>
</tr>
<tr>
<td>Uric acid (mmol/L)</td>
<td>1.02 ± 0.08</td>
<td>1.04 ± 0.1</td>
<td>1.05 ± 0.2</td>
<td>1.06 ± 0.2</td>
<td>1.06 ± 0.06</td>
<td>Non sign</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.52 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>0.57 ± 0.03</td>
<td>0.59± 0.03</td>
<td>0.62 ± 0.02</td>
<td>Non sign</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>3.6 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>Non sign</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.6 ± 0.2</td>
<td>6.7 ± 0.2</td>
<td>6.75 ± 0.1</td>
<td>6.6 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>Non sign</td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard deviation (n = 6), values are statistically different from control at p>0.05.

**Discussion**

Alteration in weight is an indication of impairment in the normal functioning of body organs (Amresh et al., 2008). Organ to body weight ratio may indicate organ swelling, atrophy or hypertrophy, also Organ-body weight ratio is a marker of cell constriction and inflammation (Moore and Dalley, 1999). The non-significant effect on the rat kidney-body weight ratio following the administration of various doses of the plant extract (Table 1) suggests that the extract did not induce inflammation or constriction of the liver and kidney cells (Moore and Dalley, 1999). The assessment of the activities of marker diagnostic enzymes plays a significant and well-known role in diagnosis, disease investigation and in the assessment of drug or plant extract for safety/toxicity risk.

ALP is located in the biliary duct of the liver, is consider one of the biomarkers of the hepatocytes (Nyblomet et al., 2006). AST is normally localized within the cells of the liver, heart, gill, kidney, muscles and other organs. ALT is specific for the liver, concentration of this enzyme is related to the liver tissue and hepatic status (Ghorbaniet et al., 2013), the change in the value of this enzyme is known as a sign of liver damage or too much pressure on liver (Burger-Mendonca et al., 2008). These
enzymes (ALP, AST, ALT) are major importance in assessing and monitoring liver cytolysis (Nyblom et al., 2004). In this study, all doses of the extract administered did not cause any significant change in the level of these enzymes, that mean the extract did not cause liver damaged also unaffected in the activities AST suggests that the functions of vital organs like liver, heart and kidney are not impaired. The plasma concentration of Bilirubin is one of the indices that reflect the functionality and cellular integrity of the liver (Shivaraj et al., 2009). Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cells, its transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile (Nelson and Cox, 2000). The administration of aqueous extract of this plant did not cause any significant change in levels of direct and total bilirubin. This suggests that the secretory function of the liver was not impaired. Some studies have observed that the plant has been traditionally used to protect and promote liver functions, which demonstrate the beneficial effects of the plant extract on liver functions (Lin et al., 2003; Dahiru and Umaru, 2003; Essa et al., 2006; Hashemi, 2014; Usos et al., 2012). This protective effect appears due to antioxidant properties of flower of this plant (Liu et al., 2006) that could be due to the rich Vitamin C content of the extract, it has been found the aqueous extract of Hibiscus sabdariffa is enriched in high antioxidant constituents, mainly flavonoids and vitamin C (Hirunpanich et al., 2006), which serves as an antioxidant and a reductant (Wang et al., 2000) and due to the presence of Hibiscus rotocatechuric acid (phenol) and Hibiscus anthocyanins which both isolated from the flower had a protective effect.

Assessing the levels of excretory metabolites like electrolyte, urea, and creatinine can be used to evaluate renal function (Adebayo et al., 2003; Yakubu et al., 2003). No significant change in the selected serum electrolyte concentrations: Sodium (Na+) and Potassium (K+) comparable to control group following oral administration of aqueous extract of plant for 28 days suggest no effect on the sodium pump that maintains the constancy of the extracellular concentration of potassium. Serum chloride and bicarbonate ions are group of electrolytes that can be used to assess renal functions therefore. The nonsignificant increase in the serum chloride and bicarbonate ions following administration of aqueous extract of H. sabdariffa flowers at various doses may be an indication of did not affect tubular and glomerular function (Kayode et al., 2012). Calcium ion is one of the most important elements in the body. It is important in many biological processes such as muscle contraction, serves as an intracellular second messenger for hormones. It is important in nerve cells for effective transfer of nerve impulses and for blood clotting (Guyton and Hall, 2000). It is also known to activate a number of enzymes. Despite all these functions, its intracellular concentration need to be kept essentially low by the calcium pump (Borle, 1972). The unchanged level of serum Ca^{2+} following the administration of the plant extract at all doses reflect the maintain calcium homeostasis. It thus means that unchanged in the selected serum electrolyte concentrations may suggest no impairment in the renal function.

Urea is a waste product of protein metabolism. It is formed in the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the blood by the kidneys, it can be used as a test of renal function (Adedapo, et al., 2009). The urea level shows no significant change (P<0.05) compared to control group. This finding suggests that renal function was not compromised following the administration of the extract.
Another parameter for determining kidney function is Creatinine, a protein produced by muscle and released into the bloodstream. Creatinine level in the serum is proportional to the rate at which it is excreted and is therefore a measure of kidney function (Eteng et al., 2009). In rats treated with the extract of *Hibiscus sabdariffa*, there was a slight reduction in the level of serum creatinine which was not significant (P>0.05) compared with control. Also there is no significant change in Uric acid, which is the major product of the catabolism of purine nucleotides, however, the bulk of purines ultimately excreted as uric acid come from degradation of endogenous nucleic acids. This again indicates that the extract did not affect kidney function after oral administration of extract.

Total protein is a measure of all plasma proteins in the blood, The level of total protein may be affected by alteration in hepatic synthesis, protein distribution, dehydration or over hydration, and protein breakdown or excretion. (Kolawole et al., 2011). Since albumin is the chief protein of the plasma and other serous fluids, any effect that negatively affects albumin content would be expected to have a deleterious impact on total plasma proteins. An increase in total protein is usually the result of tissue damage (Kolawole, et al., 2014). In this study, all doses of the extract administered did not cause any significant change in the level of total protein and albumin. That suggests the extract did not cause significant tissue damage in the rats. It is also an indication that excretion of protein via the kidney was not altered.

**CONCLUSION**

The results of this study suggest that aqueous extract of *flower of Hibiscus sabdariffa* does not impair liver and kidney function in male albino rats. Clinical study is necessary to confirm its safety in human.

**References**


الخلاصة

هدف الدراسة الحالية إلى تقصي تأثير المستخلص المائي لزهور نبات الفجرات على وظائف الكبد و الكلى في ذكور الجرذان البضاء. تم تقسيم ثلاثين من ذكور الجرذان البضاء عشوائيا إلى خمس
مجموعات، ستة لكل مجموعة. تم تجريعها عن طريق الفم بجرع تصاعدية 0، 25، 50، 100، و 200 ملمغ / كغم من الوزن الجسم لمدة 28 يوما. تم أخذ عينات الدم لفحوصات الكيمياء الحيوية. أظهرت النتائج عدم وجود فروقات معنوية في الوزن النسبي للكبد والكلي ومستويات ALT وAST و ALP والبيروبين المباشر والبيروبين الكلي والبوتاسيوم والكالسيوم والصوديوم والبوتاسيوم والكالسيوم والسيانيد والبوتاسيوم ومستويات ALT وAST والبيروبين المباشر والبيروبين الكلي في الجرذان المعالجة بالمقارنة مع حيوانات السيطرة. ونستنتج من هذه الدراسة أن المستخلص المائي لزهر نبات الفجرات لم يكن ذو تأثير سام للكبد والكلي في الجرع التي تم إعطائها.