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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 flowersof *Hibiscus sabdariffa* onliver and kidneyfunction 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 200mg/kg body weight for 28days.Blood samples were taken for biochemical assaysThe result showed There was no significant difference($P< 0.05$) in the liver and kidney to body weight ratio ,levels of ALP, AST, ALT, Directbilirubin, 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. sabdariifa*was nothepatoxicand 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 glycosidehad been reported (Lin *et al*., 2003) Anthocyanins, flavonols and protocatechoic acid along with otherphytochemicals have been identified as contributors to the observed medicinal effect of *Hibicus sabdariffa* (Seca *et al*., 2001). Scientific research has established thatthe extracts of this flowerhaveantihypertensive properties(Odigie*etal*.,2003 ; Jovene*t al*., 2014;El-Mahmoudy*etal*.,2014) antidiabetes(Rosemary *etal*.,2014), antioxidant properties (Sini *etal*.,2011;Obouayeba*et al*.,2014 ;Ali *et al*., 2005),anti-obesity (Alarcon Aguilar *etal*., 2007;Kim *et al*.,2007)and protects against sperm damage (Idris *et al*., 2012).Inaddition to being a herbal medicinal agent, is use 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

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aqueous extracts of the flowers of*Hibiscussabdariffa* 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 dried for seven days in the room temperature.After drying, they were milledwith a mortar and pestle to get a coarse powderused 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 wasboiled 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 40C 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 freeaccess to food and drinking water. Light/dark period and temperature were controlled at 12/12 hourcycle and 25C, 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

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the result showed (Table 1) that administration of aqueous extract*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(28days).

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

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

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 *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 *H. Sabdariffa* in male albino Rats

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

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 offlowers of *Hibiscus sabdariffa* for 28 days with 25, 50,100 and 200 mg/kg body weight did not significantlychange 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).

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Table 3: kidney function indices of oral administration of aqueous extracts of flowers *H. Sabdariffa* in male albino rats.

Group / Parameters	Control 0mg /kg M±SD	25 mg/kg M±SD	50mg/kg M±SD	100mg/kg M±SD	200mg/kg M±SD	LSD
Sodium Na^+ (mEq/L)	148 ± 5.2	149 ± 2.8	149 ± 5	150 ± 5	150 ± 5	Non sign
Potassium k^+(mEq/L)	4.9 ± 0.4	5.0 ± 0.3	5.11 ± 0.2	5.3 ± 0.2	5.6 ± 0.3	Non sign
BicarbonateHCO3mmol /L	15 ± 2.3	15.2 ± 0.2	15.4 ± 0.3	15.7 ± 0.3	15.7 ± 0.3	Non sign
Calcium Ca +2 mmol/L	180 ± 2.5	179 ± 1.2	178 ± 2.1	180 ± 1.3	180 ± 2.8	Non sign
Chloride (mEq/L)	106 ± 2.8	107 ± 2.5	108 ± 2.3	108 ± 1.4	108 ± 2.8	Non sign
Urea (mg/dl)	28 ± 1.4	28.3 ± 1.4	28.4 ± 1.6	28.4 ± 1.3	28.5 ± 0.7	Non sign
Uric acid (mmol/L)	1.02 ± 0.08	1.04 ± 0.1	1.05 ± 0.2	1.06 ± 0.2	1.06 ± 0.06	Non sign
Creatinine (mg/dL)	0.52 ± 0.03	0.55 ± 0.03	0.57 ± 0.03	0.59± 0.03	0.62 ± 0.02	Non sign
Albumin (g/L)	3.6 ± 0.3	3.5 ± 0.2	3.4 ± 0.2	3.3 ± 0.2	3.2 ± 0.2	Non sign
Total protein (g/dl)	6.6 ± 0.2	6.7 ± 0.2	6.75 ± 0.1	6.6 ± 0.2	6.7 ± 0.1	Non sign

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 assessmentof the activities of markeror diagnostic enzymes plays a significant and well-knownrole 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 (Nyblom*et al*., 2006).AST is normally localized within the cells of the liver, heart, gill, kidney, muscles and other organs. ALT is specific for theliver,concentration of this enzyme is related to the liver tissue and hepatic status (Ghorbani*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*etal*., 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 causes 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,itis 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 admistration of aqueous extact 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*etal*.,2006; Hashemi,2014; Usoh*etal*.,2012). This protective effect appears due toantioxidant 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 Hibiscusrotocatechuric acid (phenol) and Hibiscusanthocyanins which both isolated from the flower had aprotective 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 electrolytesconcentrations : Sodium (Na+)and Potassium (K+) comparable to control groupfollowing oral administration of aqueous extractof plant for 28 days suggestsno effect on thesodiumpump that maintains the constancy of the extracellularconcentration of potassium. Serum chloride and Bicarbonate ions are group of electrolytes that can beused to asses renal functions therefore The nosignificant 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 isimportant in many biological processes such as muscle contraction, servesas 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 needsto 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 calciumhomoeostasis.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 blood stream,. Creatinine level in the serum is proportional to the rate at which it is excreted and is therefore a measure of kidney function (Etenget *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.(Kolawolet *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.

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الخلاصة

هدفت الدراسة الحاليةالى تقصي تأثير للمستخلص المائي لزهور نبات القجرات *Hibiscus sabdariffa* على وظائف الكبد و الكلى في ذكور الجرذان البيضاء. تم تقسيم ثلاثين من ذكور الجرذان البيضاء عشوائيا إلى خمس

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مجموعات، ستة لكل مجموعة. تم تجريعها عن طريق الفم بجرع تصاعدية 0، 25، 50 ،100 و 200 ملغم / كغم من وزن الجسم لمدة 28 يوما. تم أخذ عينات الدم لفحوصات الكيمياء الحيوية. اظهرت النتائج عدم وجود فروقات معنوية في الوزن النسبي للكبد والكلي و مستويات ALP وASTوALTوالبيليروبين المباشر و البيليروبين الكلي و الصوديوم و البوتاسيوم والكالسيوم والبيكاربونات والالبومين و الكلوريد، واليوريا وحمض اليوريك والكرياتينين والبروتين الكلي في الجرذان المعالجة بالمقارنة مع حيوانات السيطرة . ونستنتج من هذه الدراسة ان المستخلص المائي لزهور نبات القجرات لم يكن ذو تاثير سام للكبد والكلى في الجرع التي تم اعطائها.