Renal and vascular studies of aqueous extract of *Urtica dioica* in rats and rabbits

K.F. Dizaye, B.O. Alberzingi and S.R. Sulaiman

Department of Pharmacology, College of Medicine, Hawler Medical University, Erbil, Iraq

(Received April 1, 2012; Accepted April 7, 2013)

Abstract

Urtica dioica has a variety of uses in traditional medicine for genitourinary ailments kidney disorders, allergies, diabetes, anemia, gastrointestinal tract ailments, musculoskeletal aches and alopecia. However, only a few of these uses have scientific bases that support their clinical uses. This study was done to evaluate some of the in vivo and in vitro pharmacological actions of this plant. Eighteen local domestic rabbits (Oryctolagus cuniculus) were used for in vitro studies (effect of the plant extract on isolated pulmonary arteries and isolated urinary bladder smooth muscle) and in vivo studies (effect of the extract on renal function). Six male albino rats were used for studying the effects of the plant extract on blood pressure and heart rate. *Urtica dioica* extract produced a significant increase in urine volume and urinary Na^+ excretion without significant changes in K^+ excretion rates in experimental rabbits. No changes occurred in Glomerular filtration rate and %Na^+ reabsorption of filtered load. Neither vasodilatation nor vasoconstriction of isolated pulmonary arteries of the rabbit was seen after applying the aqueous extract of *U. dioica*. Besides it could not reverse the vasoconstrictor effect of phenylephrine. *Urtica dioica* has no detectable effects on the isolated bladder; moreover it did not reverse the contraction that was produced by pilocarpine. In experimental rats, the plant extract produced a profound drop in blood pressure associated with decreased heart rate. In conclusion the aqueous extract of *U. dioica* produced diuretic and natriuretic effects with out significant effect on the K^+ excretion rate in rabbits. Moreover it produced a profound drop in blood pressure and heart rate.

Keywords: *Urtica dioica* extract; Hypotensiion; Diuretic; Natriuresis.
Available online at http://www.vetmedmosul.org/ijvs

دراسة تأثير المستخلص المائي لنبات القراص على الكلى والأوعية الدموية في الأرانب و الجرذان

كاوه دزه يي، بيكرد البرزنجي و شيروان رحمن

كلية الطب، جامعة هولير الطبية، اربيل، العراق

الخلاصة

في هذا البحث تم تقيم ودراسة تاثير المستخلص المائي لنبات القراص على الكلى والاوعية الدموية. ان مستخلص النبات (*U. dioica*) ادى الى زيادة معنوية في حجم الادرار وطرح ايونات الصوديوم في البول بدون تغيرات معنوية في طرح ايونات البوتاسيوم في الارانب المختبرية. ولم يحدث اي زيادة معنوية في GFR ونسبة اعادة امتصاص مرشح الصوديوم. لم يظهر هذا النبات اي تقلصات او ارتخاء في الشريان الرئوي المعزول للارنب بعد اضافة المستخلص المائي لنبات (*U. dioica*) ، الى جانب ذلك لم يعكس التقلصات الناتجة من قبل (Phenylephrin). لم يكن لهذا النبات اي تاثير ملحوظ في الانسجة المعزولة لكل من (Bladder وjejunum) و مع ذلك لم تستطيع ان تعكس التقلصات الناتجة من قبل (Pilocarpine). سبّب اعطاء مستخلص هذا النبات في الجرذان المختبرية انخفاضاً بارزاً في ضغط الدم مصاحبة بانخفاض في ضربات القلب. نستنتج من هذه الدراسة ان مستخلص نبات القراص (*U. dioica*) له تاثير ادراري للبول و طرح ايونات الصوديوم في البول بدون وجود تاثير معنوي على طرح ايونات البوتاسيوم، ويعمل على انخفاض ضغط الدم مع تقليل ضربات القلب.

Introduction

Urtica dioica (stinging nettle) is an herbaceous perennial flowering plant, of family Urticaceae and genus Urtica. It is native to Europe, Asia, northern Africa, and North America (1). Nettle contains the many compounds including polysaccharids, vitamin C, carotene, beta-sitosterol, the flavonoids quercetin, rutin, kaempferol, and trans-ferulic acid, dotriacotane, ursolic acid, scopoletin, rutin, and p-hydroxylbenzalcohol (2,3). It is believed that Nettle is a galactagogue (4) can reduce TNF-α and other inflammatory cytokines (5,6). Nettle root extracts have been extensively studied in human clinical trials as a treatment for benign prostatic hyperplasia (7).

Nettle has been used for a variety of diseases including genitourinary ailments (nocturia, frequency, dysuria, urinary retention, irritable bladder, and infections), kidney disorders, allergies, diabetes, internal bleeding (including uterine bleeding, epistaxis, and melena), anemia, GI tract ailments (diarrhea and dysentery, and gastric hyperacidity), musculoskeletal aches, osteoarthritis, and alopecia. However, only few of these uses have scientific bases that

This study was done to investigate some in vivo and in vitro pharmacological actions of *Urtica* dioica.

Materials and methods

Preparation of plant extract

Mature plant was collected from Duhok area and identified in herbarium section in Department of Biology, College of Science University of Salahaddin-Hawler. The extraction process including drying the whole plant by leaving it in room temperature for one week. The dried leaves were crushed into powder. Two g of the powder was placed in 100 ml boiled water and left for l hour then filtered into a conical flask. An equivalent of 20 mg dried material per ml of aqueous infusion was obtained (15). According to the results pilot study doses of 20 mg/kg and 50 µ/1ml were prepared for intravenous injection and application on the organ bath respectively.

Drugs used in the study

Intravenous injection of Furosemide (0.3 mg/kg, Phenyelphrine (20 µ/1ml) solution, Pilocarpine (20 µ/1ml) solution (16).

Animals

Rats

In this study six male albino rats were used. Their weights ranged from 170-200 g. They were kept in the animal house of college of Medicine/Hawler Medical University. The room temperature was maintained at 25ºC. A 12 hr light/ dark cycle was set. Rodent food rich in nutrient and tap water were supplied.

support these clinical uses of nettle. There is evidence that oral or topical use of stinging nettle leaf extract might improve symptoms of pain in patients with osteoarthritis (8). Some clinicians use stinging nettle leaf extract in combination with conventional non-steroidal anti-inflammatory drugs (NSAIDs) or other analgesics. Evidence suggests that adding stinging nettle might allow for a lower analgesic dose in some patients to be used (9). Topically, stinging nettle leaf seems to relieve pain and disability in patients with osteoarthritis of the thumb, according to preliminary research (10). More evidence is needed to rate stinging nettle for these uses.

Aqueous nettle extract exerts a hypotensive action in the rat, although it causes vasoconstriction of the aorta via activation of Alpha adrenergic receptors (11) Its orak use is associated with possible abortive and uterine-stimulant effects when used orally (12). There are few reports about the toxic effects of nettle. Nettle root can cause gastrointestinal complaints, sweating and allergic skin reactions (13). Topically, fresh nettle leaves can cause localized rash, itching, stinging, and tongue edema (14).

Rabbits

Eighteen male domestic rabbits (Oryctolagus cuniculus) were used for in vivo and in vitro studies. In the animal house the rabbits were kept in a suitable room temperature (25 ºC) and were fed barley and vegetables. The weight of the rabbits used in this experiment ranged from 1.2-1.8 kg.

In vitro studies

Isolated tissues

The rabbits were sacrificed and the abdomen was opened rapidly. Bladder identified and completely removed. The pulmonary arteries were taken after the thoracic cage was opened (A spiral strip of 2-3 cm long was prepared), both tissues were put in a petridish containing aerated, freshly prepared Krebs solution (composition in g\L NaCl 5.54, KC1 0.35, $CaC1_2$ 0.23, $NaHCO_3$ 2.1 $MgSO_4.7H_2O$ 0.29, KH_2PO_4 0.16, NaH_2PO_4 0.16 and glucose 2.1) (17).

One end of the isolated tissues was ligatured to a J shaped tube by monofilament nylon; the other end was also ligatured with monofilament nylon to a frontal transducer for tension and magnification. The tissue was put vertically in an organ bath (Organ Bath Bioscience Laboratory supply company Ollimann and Cokg) containing Tyrode or Krebs solution (according to the tissue) which is aerated with oxygen through the J shaped tube. The aeration is for the sake of tissue survival and it also helps in better mixing of the added drugs and chemical substances with the Tyrode solution. The temperature of organ bath is set at 37 C° to obtain optimal activity of the tissue.

After the piece of tissue was set in the organ bath, it was left for 30 min to equilibrate before any recording were made. Meanwhile the physiological solution was washed and replaced every 15 minutes. A resting tension of 0.5 g was recorded in all preparations. The tension variations were recorded with a two channel oscilograph (Washington 400 MD2, Bioscience England) recorder and isometric transducer (Washington transducer type D Bioscience Searle). The mechanical activity of the isolated tissue was recorded at a speed of 0.25 mm/second.

The studied drugs were added to the physiological solution in the organ bath followed by a wash whenever the effects were recorded.

In Vivo studies

Anesthesia

The rabbits were anesthetized by a combination of ketamine and xylazine. Both ketamine and xylazine were injected intraperitoneally together in a dose of 35mg/kg, 5mg/kg respectively (18).

Collection of blood sample from rabbits

Blood sample was collected from one of the rabbit's marginal ear veins which were made clearly obvious and prominent by application of a piece of cotton slightly wetted with xylene with rapid frictional movement on the vein, until the vein appeared very clearly. A needle was carefully inserted and blood was withdrawn. In each collection 1-1.2 ml of venous blood was obtained in plastic test tube without anticoagulant, and was send to the lab for different tests.

Collection of urine samples

The urine samples were collected by applying firm pressure on the lower abdomen and above the bladder by using the thumb and index finger. This maneuver is repeated until the bladder is emptied. Care should be taken to avoid injury to the bladder and hematuria (19).

Na^+ and K^+ concentrations in urine were measured by flame photometry. Urine osmolality was measured by using semielectronic osmometer. The total solute excretion (TSE) was estimated by multiplying urine osmolality by urine flow rate.

Measurement of glomerular filtration rate

GFR was determined by measuring the renal clearance of endogenous creatinine. During experiments, Chemical analysis of diluted urine and blood samples for creatinine was performed using the methods quoted by Varley *et al.* (20). The following equation was used to calculate the clearance values (21).

$$\text{Creatinine clearance} = \frac{UV}{P.t}$$

Where, U= the concentration of creatinine in the urine.
V= the volume of urine excreted in (t) minute.
P= the plasma concentration of creatinine.
t= time for urine collection.

Measurement of blood pressure and heart rate in conscious rats

Systolic blood pressure and heart rate were measured by a tail-cuff device that used a pneumatic sensor to detect the tail arterial pulse and an aneroid sphygmomanometer for measurement of the blood pressure. Animals were placed in a Plexiglas restraining cage at an ambient air temperature of 30-31C° for at least 20 minutes before each blood pressure determination. Animals were adapted to the blood pressure measurement procedure three times before the first pressure recording was made.

Statistical analysis

All data are expressed as means ± standard error of means (M ± SEM) and statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variance (ANOVA). Comparisons among groups were done using Duncan test and paired student t-test. $P<0.05$ was considered as statistically significant.

Results

In vitro studies

Effect of *U. dioica* (50 µ/1ml) on the isolated tissues (urinary bladder smooth muscle and pulmonary artery) of the rabbit

Aqueous extract of U. dioica (50 µ/1ml) produced neither contraction nor relaxation on isolated bladder smooth muscle, and the aqueous extract could not reverse the contraction effect of pilocarpine (20 µ/1ml) (figure 1).

Urtica dioica (50 µ/1ml) aqueous extract did not produce any vasodilator or vasoconstrictor effect on isolated pulmonary artery, and could not block the vasoconstrictor effect of (20 µ/1ml) phenylephrine as seen in (figure 2).

In vivo studies

Effect of furosemide on the renal function of the rabbit

The intravenous injection of 0.3 mg/kg of furosemide produced a significant increase in urine flow that reached maximum after 30 min and its effect progressively decreased, but its diuretic effect still remained after 90 min, as seen in (Table 1).

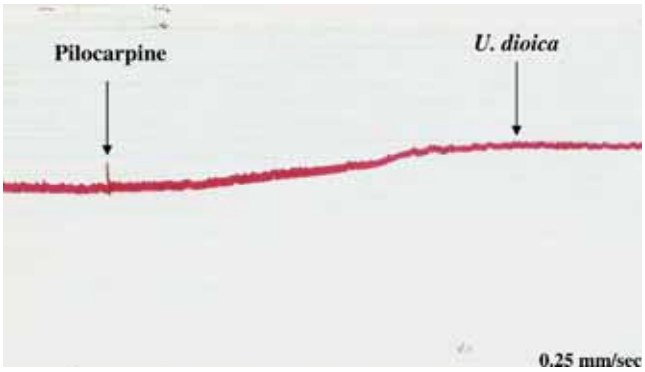

Figure 1: Effect of *U. dioica* (50 µ/1ml) aqueous extract on the isolated bladder smooth muscle of rabbit.

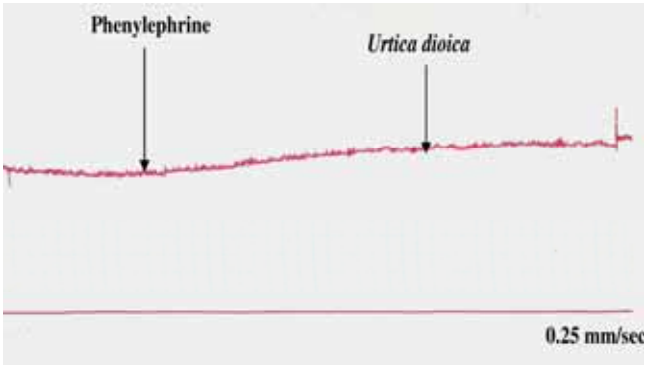

Figure 2: Effect of *U. dioica* (50 µ/1ml) aqueous extract and phenylephrine (20 µ/1ml) on the Isolated pulmonary artery of rabbit.

The intravenous injection of 0.3 mg/kg of furosemide produced a maximum increase in Na^+ excretion after 30min then declined obviously after one and half hour, as shown in (table 1). There was a maximum increase in K^+ excretion after 30 min of intravenous injection of 0.3 mg/kg of furosemide which followed by a decrease in K^+ excretion and reached minimum after 90 minutes, as seen in (Table 1).

The intravenous injection of 20mg/kg of stinging nettle U. dioica aqueous extract produced significant increases in urine volume, sodium excretion and total solute excretion, after 30min and its effect still remained after one and half hour (90min) which was maximum effect, as seen in (Table 2).

Meanwhile, the injection of this dose of the plant extract induced a non significant increase in K^+ excretion and GFR.

Effect of *U. dioica* aqueous extract on systolic blood pressure and heart rate of rats

Intraperitoneal injection of 20 mg/kg of stinging nettle *U. dioica* aqueous extract produced a significant drop in systolic blood pressure and this hypotensive effect was accompanied by a decrease in the heart rate after 30min, as shown in (Table 3).

Discussion

In vitro studies

Effect of *U. dioica* aqueous extract on isolated tissues (jejunum, urinary bladder smooth muscle and pulmonary artery) of the rabbit

From results it's obvious extract of U. dioica does not show any effect on contraction patterns of the isolated jejunum and bladder smooth muscles of the rabbits. Moreover the plant extract has totally failed in reversing (or enhancing) the smooth muscle contraction that's been induced by pilocarpine.

This is not agreed with other reports which indicated that the nettle sting contain acetylcholine among its constituent (22). U. dioica could not produce any effects on isolated pulmonary artery and did not possessed any vasodilator effect or inhibitory activity on the vasoconstrictor effect of *phenylephrine; therefore it has no Alpha1* and Beta2 adrenoceptor agonistic and antagonistic effect. Although there is contradictory evidence about the effect of *U. dioica* on the vascular smooth muscle. The results of a study done by Testai et al (2002) (23). showed that the hypotensive responses of *U. dioica* root extract, through a vasorelaxing effect on isolated rat thoracic aorta mediated by the release of endothelial nitric oxide and the opening of potassium channels, and through a negative inotropic action. But this is in contrast with the present study, which used the root of the nettle. Also the nettle contains polyphenols and tannins (24) which are well known for their vasorelaxant effects (25). In another study, aqueous extract of nettle (0.1- 5g/l) produced a dose-dependent increase in basal tone of isolated rat aorta (cause vascular contractility). This effect was endothelium independent and was abolished by 1 µM prazosine (an Alpha 1-adrenergic antagonist) (26).

In vivo studies

Effect of U. dioica aqueous extract on the renal function of rabbit

Intravenous infusion of 20 mg/ml of U. dioica aqueous extract produced a significant rise ($P<0.05$) in urine volume and marked and significant increase in Na+ excretion rate ($P<0.05$), these natriuretic and diuretic effects of U. dioica aqueous extract were similar to furosemide (a loop diuretic) which caused an increase in urine volume and Na+ excretion. This is in agreement with a study performed on anesthetized male Wister rats that received a continuous intravenous perfusion during 1.25 hour of an aqueous extract of aerial parts of U. dioica that resulted in increase in diuresis and natriuresis (27).

Table 1: Effect of intravenous injection of 0.3 mg/kg of furosemide on the renal function of the rabbit (n=6).

Parameters	Control	T1	T2	T3
Urine flow ml/min./kg	8.40 ± 1.86 a	26.76 ± 4.61 c	19.02 ± 1.99 bc	17.61 ± 1.74 b
Na^+ excretion rate µEq/min./kg	155.46 ± 50.78 a	942.03 ± 194.12 b	574.45 ± 125.02 bc	268.93 ± 74.55 ab
K^+ excretion rate µEq/min./kg	11.48 ± 4.87 a	46.55 ± 14.24 b	21.31 ± 9.37 ab	7.14 ± 1.89 a
U. Osmolality mOsm/l	405.28 ± 84.49 a	272.48 ± 25.17 a	316.8 ± 35.34 a	401.05 ± 18.86 a
TSE mOsm/min./kg	3.236 ± 1.013 a	6.971 ± 0. 802 b	6.021 ± 0. 881 b	7.054 ± 0.814 b

The different letters mean there is a significant difference at $P< 0.05$.
T1=measurements after 30 minutes, T2= measurements after 1 hour, T3= measurements after 1:30 hour.

Table 2: Effect of intravenous injection 20 mg/kg of *U. dioica* aqueous extract on the renal function of the rabbit (n=6).

Parameters	Control	T1	T2	T3
Urine flow ml/min./kg	4.04 ± 0.70 **a**	17.08 ± 2.68 **b**	13.67 ± 2.79 **b**	18.13 ± 1.76 **b**
Na^+ excretion rate µEq/min./kg	169.59 ± 56.98 **a**	988.35 ± 334.12 **b**	1335.50 ± 339.06 **b**	1331.38 ± 215.46 **b**
K^+ excretion rate µEq/min./kg	19.71 ± 2.91 **a**	76.42 ± 31.90 **a**	68.30 ± 25.85 **a**	56.76 ± 21.53 **a**
%Na^+ reabsorption of filtered load	89.40 ± 6.10 **a**	91.70 ± 6.06 **a**	76.65 ± 14.40 **a**	88.70 ± 6.10 **a**
U. Osmolality mOsm/l	698.60 ± 344.32 **a**	388.00 ± 61.53 **a**	552.50 ± 125.10 **a**	345.50 ± 60.81 **a**
TSE mOsm/min./kg	2.251 ± 0. 797 **a**	5.748 ± 0.700 **b**	5.794 ± 0. 626 **b**	5.712 ± 0. 621 **b**
Plasma Na^+ concentration µEq/l	162 **a**	–	–	152 **a**
GFR (ml/min. /kg)	5.5 **a**	–	–	6.9 **a**

The different letters mean there is a significant difference at $P< 0.05$.
T1=measurements after 30 minutes, T2= measurements after 1 hour, T3= measurements after 1:30 hour.

Table 3: Effect of intravenous injection of 20 mg/kg of *U. dioica* aqueous extract on the blood pressure and heart rate of rat (n= 5).

Parameters	Control	T1
Blood pressure mmHg	129 ± 1.8	112 ± 5.8*
Heart rate Beats/minute	399 ± 15.2	351 ± 14.4*

T1=measurements after 30 minutes, * = $P< 0.05$.

In spite of the natriuretic and diuretic effects of U. dioica, there was no significant changes in the urinary K+ excretion rate this is unlike furosemide and thiazide diuretics in which they increase K+ excretion rate and their main side effect is hypokaleamia, which may precipitate arrhythmias, therefore potassium supplementation or concomitant treatment with potassium sparing agents should generally be used (28,29). This property makes this plant superior to other diuretics; therefore U. dioica may not need potassium supplementation. Thus the mechanism of diuretic effect of U. dioica is unlike furosemide (loop diuretic) which acts by inhibiting the activity of the Na+-K+-2Cl- symporter in the thick ascending limb of the loop of Henle and unlike thiazide diuretics that acts by inhibiting the Na+-Cl- symporter (30). Also the diuretic effect of U. dioica did not resemble K+ sparing diuretics which they cause hyperkalaemia (31).

The diuretic effect of U. dioica began shortly after intravenous injection and remained for 2 hours this indicate that this plant has an immediate effect and a long duration of action that does not resemble furosemide which has a rapid onset of action (intravenously 5 minutes) and short duration of action (32).

In the present study, no detectable changes seen in the GFR and percentage of Na+ reabsorption of the filtered load; this indicates that this diuretic effect is not due to increase in the GFR. The mechanism of diuretic property of the plant extract was not due to effect on the antidiuretic hormone because there was a significant increase in TSE (19).

This diuretic and natriuretic properties of U. dioica aqueous extract may be attributed to its antagonistic effect on Adenosine A1 receptor since adenosine plays a comparable role in the renal regulation of fluid homeostasis in normotensive rats (33).

The action of U. dioica as a diuretic drug, it seems that it looks like the diuretic effects of selective A1 antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and CVT-124 drugs which produce diuresis and natriuresis without significantly increasing potassium excretion (34-36).

Effect of U. dioica aqueous extract on the blood pressure and heart rate of rat

The result obtained from the experiment on the rat for detecting the effect of intraperitonial injection of U. dioica aqueous extract on the blood pressure and heart rate showed that there was significant decrease in blood pressure and heart rate. In folk medicine this plant is used to treat arterial hypertension, this is suggested by a survey which was undertaken in different areas of oriental Morocco, that select the main medicinal plants used for arterial hypertension and U. dioica was among 18 vegetal species which was used for hypertension (37). Tahri et al. (27) studied the effect of continuous intravenous perfusion of U. dioica aqueous extract on anaesthetized male wister rat, that resulted in a decrease in arterial blood pressure and it was reduced proportionally to the dose of the perfusion of the plant extract.

The hypotensive and bradycardial effects of U. dioica also agreed with a report explained the hypotensive responses of U. dioica root extract, through a vasorelaxing effect mediated by the release of endothelial nitric oxide and the opening of potassium channels, and through a negative inotropic action (23). The study performed on the isolated rat heart shows a dose dependent bradycardia and increase left ventricular pressure after perfusion of (1 and 2 g/l) of the aqueous extract of nettle (AEN), higher concentration (5 g/l) even led to cardiac arrest (25) this effect also confirms the bradycardia observed in vivo by Bromcamo et al. (38). The inotropic and chronotropic effects of AEN resemble those of carbachol a muscarinic receptor agonist. However, the effect of AEN persisted in the presence of atropine (a muscarinic reseptor antagonist) (26) showing that it was independent of cholinergic pathway.

Moreover, the AEN activates rather than inhibits α1-adrenergic receptors and prazocine neither mimicked nor modified the cardiac effects of AEN (11) as it was seen previously in this study, this plant has neither a vasodilator activity nor it blocked the vasoconstrictor effect of Alpha adrenergic receptor agonist (phenylephrine). This indicates that the hypotensive effect of the plant extract was not due to vasodilatation or inhibiting α receptor. The hypotensive result could be explained by the diuretic property of the plant extract (39), while further studies are needed to elucidate the mechanism of decreasing heart rate or the hypotensive and bradycardial effect might be due to presence of a Beta-1 antagonistic substances in the constituent of this plant.

Conclusion

The following conclusions are drawn from the results and their interpretation:

The aqueous extract of U. dioica produced a significant increase in urine flow and an increase in the urinary excretion of Na+ without significant increase in K+ excretion in rabbits. No changes occur in GFR and %Na+ reabsorption of filtered load. Intraperitoneal injection of the aqueous preparation produced a profound fall in blood pressure of rat which was accompanied by a decrease in heart rate. The hypotensive result could be explained by the diuretic property of the plant extract.

References

1. Brill S, Dean E. Identifying and Harvesting Edible and Medicinal Plants in Wild. 1st Edition, Harper Collins 1994.
2. Konrad L, Muller H, Lenz C. Laubinger H, Aumuller G, Lichius J. Antiproliferative effect on human prostate Cancer cells by a stinging nettle root *Urtica dioica* extract. Planta Med. 2000;66:44-47.
3. Ji, T, Liu C, Wang A, Yang J, Su Y, Yuan L, Feng X. Studies on the chemical constituents of *Urtica dioica* L. grown in Tibet Autonomous Region. Zhong Yao Cai Jur. 2007;30: 662-4.
4. Westfall E. Galactagogue herbs: a qualitative study and review. Canadian Journal of Midwifery Res Pract. 2003;22: 22-27.
5. Teucher T. Cytokine secretion in whole blood of healthy subjects following oral administration of *Urtica dioica* L. plant extract. Arzneimittelforschung. 1996;46:906-910.
6. Obertreis B, Giller K, Teucher T, Behnke B, Schmits H. Anti-inflammtory effect of *Urtica dioica* folia extract in comparison to caffeic malic acid. Arzneimittelforschung. 1996;46:52-56.
7. Schneider T. Stinging nettle root extract (Bazoton-uno) in long term treatment of benign prostatic syndrome (BPS). Results of a randomized, double-blind, placebo controlled multicenter study after 12 months. Urologe. 2004;43:302-6.
8. De Smet P. Herbal remedies. N Eng. J Med. 2002; 347:2046-2056.
9. Gagnier J, DeMelo J, Boon H, Rochon P, Bombardier C. Quality of reporting of randomized controlled trials of herbal medicine interventions. Am J Med. 2006;119:1-11

10. Randall C, Randall H, Dobbs F, Hutton C, Sanders H. Randomized controlled trial of nettle sting for treatment of base-of thumb pain. J R Soc Med. 2000;93:305-309.
11. Legssyer A, Ziyyat A, Mkhfi H, Bnouham M, Tahri A, Serhrouchni, M. Cardiovascular effects of *Urtica dioica* L. in isolated rat heart and aorta. phytother Res. 2002;16:503-507.
12. Bush TM, Rayburn KS, Holloway SW, Sanchez-Yamamoto DS, Allen BL, Lam T. Adverse interactions between herbal and dietary substances and prescription medication: a clinical survey. Altern ther Health Med. 2007;13:30-35.
13. Heinrich M. Ethnobotany and natural products, the search for new molecules new treatments of old diseases or a better understanding of indigenous cultures. Curr. Topics Med Chem. 2003; 3:141-154
14. Federici E, Multari G, Gallo FR, Palazzino G. Herbal drugs fromtraditional use to regulation. Ann 1st Super sanita. 2005; 41:49-54.
15. Barnes J, Anderson L A, Phillipson JD: Herbal Medicine, 3rd edition, Pharmaceutical Press 2007.
16. Dizaye K, Hamed B. Cardiovascular studies of White squill (Urginea Maritima) Extract. Zanco journal of medical science. Z J MS 2010;14:37-42
17. Kawadizaye FS, Aljeboury A, Al-jaf H. The Pharmacological studies of procyanidine isolated from craeaegus azarolus. Pakistan J Pharmaco. 2005;22:57-79
18. Laird, K, Swindle M, Fleckneel P. Rodent and Rabbit Medicine. 1st Edition, BPC Wheatons Ltd, Exter U.K 1996.
19. Al-Merani S, Dizaye K. Natriuretic and pressor responses to central hypernatremia persist during inhibition of central sympathetic outflow by Clonidine. Zanco J Med Sci. 2007;11:1-7.
20. Varley, H. Gowenlock, A. H.; and Bell, M.: Practical clinical biochemistry, Volume 1, General topics and commoner tests. Fifth edition, William Heinemann Medical books, LTD, London 1980.
21. Pitts R F. Physiology of the kidney and body fluids. Second edition. Year book Medical publisher, Chicago 1968.
22. Adamski R, Bieganska J. Studies on substances present in *Urtica dioica L.* leaves. Analysis for protein amino acids and nitrogen containing non-protein amino acids. Herba Polonica 1984;30:17-26.
23. Testai L, Chericoni S, Calderone V, Nencioni G, Nieri P, Morelli I, Martinotti E. Cardiovascular effects of *Urtica dioica* L. (Urticaceae) roots extract: in vitro and in vivo pharmacological studies. J. Ethnopharm. 2002; 81:105-109.
24. Chaurasia N, Wichtl M. Flavinoglykoside Aus *Urtica dioica.* Planta Med 1987; 53: 432-434.
25. Andriambeloson E, Magnier C, Hean-Archipoff G. Natural dietary polyphenolic compounds cause endothelium-dependant vasorelaxation in rat thoracic aorta. J Nutr. 1998;128:2324-2333.
26. Legssyer A, Ziyyat A, Mkhfi H, Bnouham M, Tahri A, Serhrouchni M. Cardiovascular effects of *Urtica dioica* L. in isolated rat heart and aorta. phytother Res. 2002;16: 503-507.
27. Tahri A, Yamani A, Legssyer A, Aziz H M, Bnouham M, Ziyyat A. Acute diuretic, natriuretic and hypotensive effects of continuous perfusion of aqueous extract of Urtica dioica in the rat. J Ethnopharma. 2000;73:95-100.
28. Davis M, Gibbs C. ABC of heart failure management: diuretics. ACE inhibitors, and nitrates. BMJ; 2000;320-428.
29. Eva L. Clinical review. Drug treatment in heart failure BMJ. 2000; 320: 1188-1192.
30. Hebert SC: Molecular mechanisms. Semin Nephrol. 1999;19:504-523.
31. Carter B L, Ernst M E, Cohen J D. Hydrochlorthiazide versus Chlorthalidone: Evedence supporting their Interchangability. Hypertention. 2004;43: 9-4.
32. Brater D. Clinical pharmacology of loop diuretics. Drugs. 1991;41:14-22.
33. Kost C, Herzer W, Jackson E. Diuretic and natriuretic response to A1 receptor blockade in normotensive and hypertensive rats: Role of Gi-proteins. J Am Soc Nephrol. 1998; 9: 310.
34. Balakrishnan V, Coles G, Williams J. A potential role for endogenous adenosine in control of human glomerular and tubular function. Amere J Renal Physiol,1993;265: 504-510.
35. Gellai M, George F, Robert R, Ruffolo J: CVT-124, a Novel Adenosine A1 Receptor Antagonist with Unique Diuretic Activity. J Pharmaco. 1998;286: 1191-1196.
36. Curtis K, William A, Herzer B, Rominski M, Edwin K. Diuretic Response to Adenosine A1 Receptor Blockade in Normotensive and Spontaneously Hypertensive Rats: Role of Pertussis Toxin-Sensitive G-proteins. Pharmaco experiment Therape. 2000; 292:752-760.
37. Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Marrocco. J Ethno pharmacol. 1997;58:45-54.
38. Bromcamo FJ, Revuelta M, Vivas JM, Serranilos M: Etude de leffet sur le centre cardiovasculalre de quelques preparations de l *Urtica dioica* L. Pl Med Phytother, 1983; 4: 222-229.
39. Brenner G. Pharmacology. 1st Edition, Harcourt Health Science 2001.