Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (2): 123-135, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.123.135

Antibacterial Activity and Toxicological Evaluation of Semi Purified Hexane Extract of *Urtica dioica* Leaves

[1]R. Singh, [2]S.A. Dar and [2]P. Sharma

[1]Department of Biomedical Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India
[2]Department of Zoology, Bundelkhand University, Jhansi, Uttar Pradesh, India

Corresponding Author: Poonam Sharma, Department of Zoology, Institute of Basic Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India Tel: 91-09415587302 Fax: +91-0510-2320761

ABSTRACT

Herbs are largely unexplored source of drug repository. Medicinal plants have great potential for providing novel drug leads with novel mechanism of action. The present study describes the antibacterial activity, phytochemical profile and toxicological evaluation of *Urtica dioica*. *U. dioica* leaves were subjected to solvent extraction by hexane, ethyl acetate, chloroform, methanol and water. All the extracts were tested for antibacterial activity against five clinical isolates of Gram positive and Gram negative bacteria by disc diffusion method. Hexane extract showed good antibacterial activity against all the five bacterial strains, hence it was further purified using silica column chromatography and Minimum Inhibitory Concentration (MIC) of the semi purified fraction-B (F_B) of hexane extract was determined by serial tube dilution method. F_B showed MIC value of 31.25, 250, 7.81, 31.25 and 125 μg mL^{-1} against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri*, respectively. F_B was subjected to GC-MS analysis in search of potent antibacterial compound(s). Neophytadiene (26.97%) butyl tetradecyl ester (9.53%), Dibutyl phthalate (7.45%), Bis (2-ethyl hexyl) maleate (8.80%) and 1,2-benzenedicarboxylic acid (9.89%) were the major constituents which may be responsible for the antibacterial activity of F_B of *U. dioica*. Sub-acute oral toxicity of F_B was carried out in wistar rats at doses of 100, 200, 400 and 800 mg kg^{-1} Body Weight (b.wt.) to assess index of safety. F_B showed MIC value as low as 7.81 μg mL^{-1} against *Salmonella typhi*, exhibiting that a promising antibacterial agent is present in this fraction. Hematological and biochemical parameters showed that the F_B was safe at tested concentrations. Hence further purification of F_B is required to obtained potential antibacterial compound.

Key words: *Utrica dioica*, antibacterial activity, serum, *in vivo* toxicity, lipid profile, phytochemical analysis

INTRODUCTION

Microbial infections are the major cause of morbidity and mortality particularly in immune-compromised patients (Black *et al*., 1982). Although a numbers of antibiotics are available for treatment and management of microbial infections, development of resistance against present day antibiotics is a major health concern (Al-Bari *et al*., 2007). Therefore, there is a perpetual and urgent need to discover new antimicrobial compounds with high safety index (Erturk *et al*., 2006). Historically, medicinal plants have been a source of inspiration for novel drug compounds. Plant derived medicines have made large contributions to human health and well being. Phytochemicals

may become the base for the development of medicines by providing a pharmacophore which could be used for the development of new drug with novel mechanism of action. Many laboratories across the globe are involved in systematic screening of plant species for discovering new bioactive compounds but still a very meager portion of this tremendous potential drug-repertoire has been scientifically screened (Menghani *et al*., 2011). Hence, there is a need for scientific evidence based validation of bioactive phytochemicals (Davies, 1994; Adeniyi and Ayepola, 2008; Karim *et al*., 2011). Isolation, purification and characterization of lead antimicrobial phytochemicals is an exciting area of search, especially developing drugs against multi drug resistance microbes (Rahman *et al*., 2000). A number of medicinal plants have been screened for antimicrobial activity in recent times (Premanath *et al*., 2011; Oboh, 2010) and efforts have been done to identify their active constituents (Tijjani *et al*., 2009). Safety of herbal extracts and their purified constituents is another major concern during natural product based drug discovery research (Hassan *et al*., 2011). The plant extracts possessing bioactivity are essentially evaluated for toxicity (Chavda *et al*., 2010). The extracts are usually tested for short or long term toxicity in animal models (Abdelgadir *et al*., 2010; Diallo *et al*., 2010). Non toxic extracts possessing good bioactivity may provide potential antimicrobial leads.

Urtica dioica or stinging nettle or common nettle, is a herbaceous perennial flowering plant, native to Europe, Asia, Northern Africa and North America and is the best-known member of the nettle genus *Urtica* (Emmelin and Feldberg, 1949). *Urtica dioica* is used in Indian traditional medicine for genitourinary ailments, kidney disorders, allergies, diabetes, internal bleeding and anemia, GI tract ailments, musculoskeletal aches, osteoarthritis and alopecia (Matsingou *et al*., 2001). However, only a few of these pharmacological activities have been experimentally proved (Lourdes *et al*., 2008). Recently anti-proliferative (Durak *et al*., 2004), anti hyperglycemic (Bnouham *et al*., 2003), antioxidant (Abdullin *et al*., 2002), antiarthritic (Riehemann *et al*., 1999), antidandruff, galactogogue and haemostatic (Hadizadeh *et al*., 2009) activity of *U. dioica* are also reported. The main phytoconstituents of *U. dioica* include lectins, plastocyanins, glycoproteins, carotenoids, fatty acids, sterols, flavonoids, polysaccharides, terpenes and lignans (Sajfrtova *et al*., 2005).

In present study, *in vitro* bioassay guided purification of antibacterial compound(s) from leaves of *U. dioica* was carried out. The active sub fraction was subjected to *in vivo* toxicological evaluation to assess its safety index and GC-MS analysis to identify potential antibacterial lead compound(s).

MATERIALS AND METHODS

Collection and extraction of plant material: *U. dioica* leaves were collected from the Anantnag district of Kashmir valley, India in the month of October, 2009 and authenticated by the Centre of Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir and a voucher specimen (28100 KASH) was deposited. The leaves were air dried, chopped in small pieces and grinded to obtain coarse powder. Powder (1 kg) was extracted successively with hexane, chloroform, ethyl acetate and methanol in soxhlet apparatus for 24 h. The extracts were filtered over Whatman No. 1 paper and the organic solvent extracts were concentrated under vacuum using rotary evaporator (Singh *et al*., 2002). The residue left after methanol extraction was soaked in distilled water with continuous stirring for 24 h. Filtered water extract was centrifuged at 5000 rpm for 10 min at room temperature to remove any suspended material and lyophilized. All the extracts were stored at 4°C until used for the antibacterial assay.

Antibacterial susceptibility test: All five solvent extracts were investigated for the antibacterial activity by disc diffusion method against clinical isolate of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia* and *Shigella flexneri* (Bauer *et al*., 1996) obtained from Govt. Medical College, Jhansi, UP, India. Sterile whatman filter paper discs with 6 mm diameter were impregnated with 2000 μg of each extract (dissolved in 8 μL DMSO) as per standard procedures (Chandrasekaran and Venkatesalu, 2004). Gentamycin (30 μg $disc^{-1}$) was used as a positive control and a paper disc impregnated with DMSO (8 μL) was used as negative control. The bacterial cultures were grown in nutrient broth liquid medium at 35°C and kept under refrigeration (4°C) until use. The nutrient agar plates were prepared for disc diffusion assay and each bacterial strain at a concentration of 1.5×10^6 cells mL^{-1} (adjusted to the 0.5 McFarland turbidity standards) was used (NCCLS, 2005). All experiments were performed in triplicate and results were recorded by measuring the zones of growth inhibition around the discs. The test samples with inhibition zones equal to or greater than 7 mm, were considered active.

Bioassay guided isolation of active compound(s): A 60 cm long glass column with a diameter of 10 cm was filled with 1.5 kg silica gel, mess size 60-120. Hexane extract (HE, 100 g) was dissolved in a minimal amount of mobile phase and mixed with 200 g silica gel. The column was eluted with gradient mobile phase of hexane-ethyl acetate, 20 fractions of 50 mL each were collected, analyzed by silica gel TLC (Merck) and pooled to give 5 sub fractions F_A, F_B, F_C, F_D and F_E. The subfractions were tested for antibacterial activity by disc diffusion assay.

Minimum Inhibitory Concentration (MIC): Serial tube dilution technique was used to determine MIC (Rahman *et al*., 2000). F_B was dissolved in DMSO to prepare a stock solution. Ten serial dilutions of stock, ranging from concentration of 1000 μg to 0.97 μg mL^{-1} were prepared in the test tubes. The tubes were inoculated with 100 μL of bacterial strain inoculums at a concentration of 10^6 cell mL^{-1}. Standard antibiotic Gentamycin was included in the assay for comparison. Nutrient broth with the inoculums only was used as growth control. All experiments were carried out in triplicates. The tubes were incubated aerobically at 37°C for 12-18 h and 50 μL of 0.2 mg mL 2-(4-iodophenyl)3-(4-nitrophenyl)-5 phenyltetrazolium chloride (INT) solution was added. The tubes were observed for color change (Anders *et al*., 2002). The concentration of F_B at which a decrease in red color was apparent compared to the next dilution was taken as MIC value. Bacterial growth was indicated by the red color of INT reduced to formazan by bacteria.

GC-MS analysis: GC-MS analysis of F_B was carried out by using Perkin Elmer-Clarus 500 GC-MS with PE-5 (equivalent to DB-5) capillary column (length-30 m) and helium as carrier gas (flow rate-3.3 mL min^{-1}). Samples were analyzed with the column held initially at 100°C for 1 min after injection, then increased to 170°C with 10°C/min programme rate and up to 215°C with 5°C/min programme rate. Final temperature was increased to 270°C with 10°C/min heating ramp for 10.5 min. The injections were performed in split mode (30:1) at 250°C. Detector and injector temperatures were 260 and 250°C, respectively. Pressure was established as 50 psi. Run time was 35 min. Temperature and nominal initial flow for Flame Ionization Detector (FID) were set as 250°C and 3.1 mL min^{-1}, correspondingly. The constituent compounds were determined by comparing their retention times and mass weights with those of authentic samples obtained by GC as well as the mass spectra from the Wiley and Nist database searches by GC-MS.

Toxicity studies: The F_B was subjected to *in vivo* sub-acute toxicity studies. Thirty male wistar rats weighing about 150-200 g, obtained from Central Drug Research Institute (CDRI), Lucknow were used in the experiment. Animals were kept for acclimatization at ambient temperature of 25°C and 45-55% relative humidity, 12 h each of dark and light cycles and were fed pelleted diet and water *ad-libitum*. Animal experimental protocols were in accordance with the recommendations of the institutional animal ethical committee. Animals were divided into five groups of six rats in each. First group served as a control group and the remaining four groups (A, B, C and D) received the doses of 100, 200, 400 and 800 mg kg^{-1} body weight of F_B, respectively, for 14 days by gavaging (Mosaddik and Haque, 1999). The behavior and body weight of the animals was observed daily. Animals were sacrificed on 15th day and blood was collected from the jugular vein with the help of sterilized hypodermic syringe. Half of the blood was transferred into Ethylene Diamine Tetra Acetic acid (EDTA) vials for hematological studies. Serum for various biochemical investigations was isolated from other half. Hematological and biochemical parameters were determined using standard procedures and reagents supplied by diagnostic kit (Siemens Medical Solution Diagnostics Ltd, India).

Statistical analysis: All experiments were performed in triplicate. Statistical analysis was carried out using GRAPHPAD Prism 5. Results were expressed as Mean±SD. The statistical analysis involving two groups was performed by means of paired t-test and Analysis of Variance (ANOVA) followed by Tukey's test. $p<0.01$ was considered significant and $p<0.001$ was considered highly significant.

RESULTS AND DISCUSSION

Herbal drugs are in great demand currently due to their varied pharmacological properties and source of novel bioactive compounds. Natural plant product based antibiotic drug discovery attained paramount importance as newly discovered drugs are likely to be effective against multi drug resistant microbes. There is only one report of antimicrobial activity from *Urtica dioica* plant (Uzun *et al*., 2002) and no antimicrobial the compounds are isolated so far, hence this study was planned to isolate antibacterial molecule(s) from this plant.

Antibacterial susceptibility test: The HE exhibited good antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Shigella flexneri*. The antibacterial activity produced by HE was comparable to that of the standard antibiotic Gentamycin (Table 1). The crude HE showed good antibacterial activity against all the tested bacteria as compared to other extract. The results suggest that antibacterial principle lies in non polar solvent extract as compared to polar extracts. There are a number of reports of hexane extracts of plant possessing antibacterial activity (Elzaawely *et al*., 2005). These results indicate that the extracting solvent has a definite effect on bioactive principles. Usually, the extract having large inhibition zone diameter with low minimum inhibitory concentration can be recognized as more potent drug than that of small inhibition zone diameter and high minimum inhibitory concentration (Semwal *et al*., 2009).

Bioassay guided isolation of active compound(s): HE was fractionated on silica column and 20 fractions of 50 mL each were collected. The fractions were analyzed by silica TLC and similar fractions were pooled to give 5 sub fractions F_A, F_B, F_C, F_D and F_E. All subfractions were tested for antibacterial activity by disc diffusion assay. F_B showed potent antibacterial activity against gram positive and negative bacteria as compared to other fractions in disc diffusion assay (Table 2). The

Table 1: Antibacterial activity of different solvent extract of leaves of *Urtica dioica*

	DIZ (mm)						
Microorganisms	H	C	E	M	A	Ct	Sd
Pseudomonas aeruginosa	++	-	-	-	-	-	+++
Staphylococcus aureus	++	+	-	-	-	-	+++
Salmonella typhi	++	+	-	-	-	-	+++
Klebsiella pneumoniae	++	+	-	-	-	-	+++
Shigella flexneri	++	+	-	-	-	-	+++

H: Hexane extract, C: Chloroform extract, M: Methanol extract, E: Ethyl-acetate extract, A: Aqueous extract, Ct: Control (Dimethyl Sulphoxide, DMSO), Sd: Standard (Gentamycin). The results were represented as inactive (-, inhibition zone diameter ≤7 mm), moderate activity (+, inhibition zone diameter ≤10 mm), good activity (++, inhibition zone diameter ≤15 mm) and very good activity (+++, inhibition zone diameter>15 mm)

Table 2: Antibacterial activity of different column eluted fractions of Hexane Extract (HE) of *Urtica dioica*

	DIZ (mm)						
Microorganisms	F_A	F_B	F_C	F_D	F_E	Ct	Sd
Pseudomonas aeruginosa	-	++	+	+	+	-	+++
Staphylococcus aureus	-	+++	+	+	+	-	+++
Salmonella typhi	-	+++	+	+	+	-	+++
Klebsiella pneumoniae	-	+++	+	+	+	-	+++
Shigella flexneri	-	+++	+	+	+	-	+++

F_A: Combined fractions 1-4, F_B: Combined fractions 5-7, F_C: Combined fractions 8-10, F_D: Combined fractions 11-14C, F_E: Combined fractions 15-19, Sd: Standard; Gentamycin and Ct: Control; DMSO. The results were represented as inactive (-, inhibition zone diameter ≤7 mm), moderate activity (+, inhibition zone diameter ≤10 mm), good activity (++, inhibition zone diameter ≤15 mm) and very good activity (+++, inhibition zone diameter >15 mm)

Table 3: The Minimum Inhibitory Concentration (MIC) of the fraction F_B of Hexane Extract (HE)

Test organisms	MIC range (μg mL^{-1})	Gentamycin (μg mL^{-1})
Pseudomonas-aeruginosa	250.00	<7.81
Staphylococcus-aureus	31.25	<7.81
Salmonella-typhi	7.81	<7.81
Klebsiella- pneumoniae	31.25	<7.81
Shigella-flexneri	125.00	<7.81

activity of F_B was comparable with standard antibiotic. A semipurified fraction consisting of around 20 compounds, possessing antibacterial activity comparable to standard antibiotic is very promising.

Minimum Inhibitory Concentration (MIC): The MIC values of F_B were 250, 7.81, 31.25, 125 and 31.25 μg mL^{-1} against *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia*, *Shigella flexneri* and *Staphylococcus aureus*, respectively (Table 3). The MIC as low as 7.81 μg mL^{-1} of a semi purified fraction against gram negative bacteria is suggestive of good antibacterial potential of the compounds of F_B. Hence F_B may yield potential molecules in the treatment of infections caused by pathogenic bacteria which have developed resistance against the known antibiotics (Singleton, 1999).

GC-MS analysis: GC-MS analysis of F_B (20 compounds) is shown in Fig. 1. The GC-MS Retention Time (RT), molecular formula, molecular weight and percentage peak of the individual compounds were presented in the (Table 4). F_B showed five major compounds i.e., 2,6,10-Trimethyl,14-ethylene-14-pentadecne (Neophytadiene) (26.97%) butyl tetradecyl ester (Phthalic acid) (9.53%), dibutyl ester (Dibutyl phthalate) (7.45%), Bis(2-ethyl hexyl) maleate (8.80%) and 1,2-benzenedicarboxylic acid (9.89%) which may be responsible for antibacterial activity. Antimicrobial activity of plant essential oil containing neophytadine (Palic *et al.*, 2002) and other aromatic carboxylic acids and ester is reported (Roy *et al.*, 2010). Neophytadiene is also reported to possess antibacterial activity as well as helping in treatment of headache, rheumatism and some skin disease (Suresh *et al.*, 2010). Fatty acids are also known to have antibacterial and antifungal activities (Russel, 1991). Fatty acid esters namely phthalic acid, dibutyl ester, bis(2-ethyl hexyl) maleate and 1,2-benzenedicarboxylic acid were reported to possess anti inflammatory (Li *et al.*, 2004) and antibacterial activity (Modupe *et al.*, 2010). Essential oils rich in terpenes have been shown to possess good antibacterial activity (Taylor *et al.*, 1996). The appreciable presence of the terpenes (neophytadiene 26.97% and olean-1-ene 1.97%) in the F_B of HE, could explain its antibacterial activity against the tested bacterial strains. Since, *Urtica dioica* is reported to possess a wide range of medicinal value, these compounds may be analyzed for other biological activities reported from this plant.

Toxicity studies: Increase in popularity and scarcity of scientific studies on safety of herbal remedies have raised concern regarding toxicity and adverse effect (Saad *et al.*, 2006). Body weight is one of the sensitive indicators of toxicity if it is monitored frequently and correctly during the study (Wilson *et al.*, 2001). Average body weights of all rats before and after treatment with F_B are presented in Fig. 2. After 14 days, control group gained an average weight of 2.46% and the experimental groups A, B, C and D gained an average weight of 3.76, 2.71, 2.81 and 3.11%,

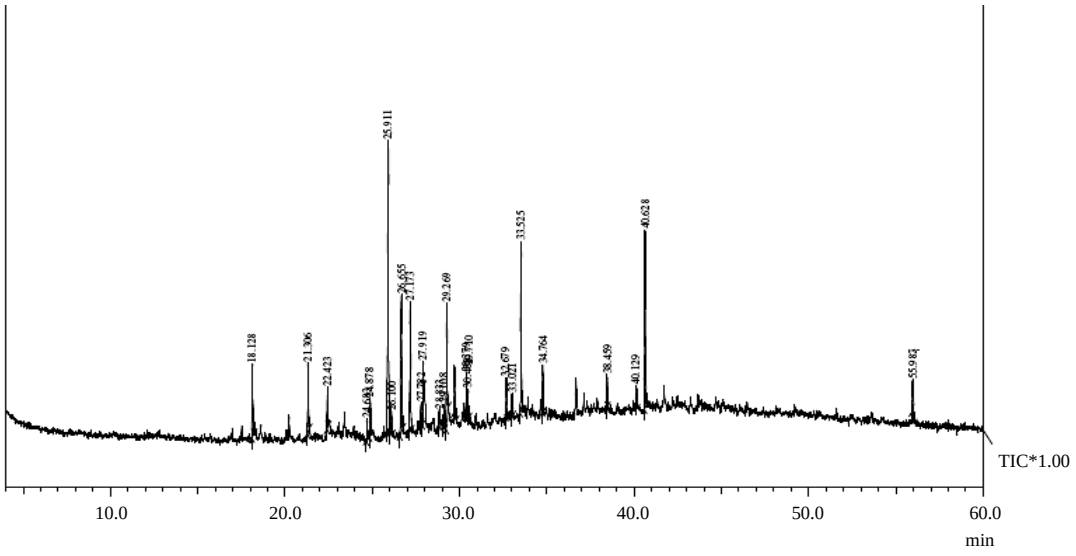

Fig. 1: Chromatogram of F_B of hexane extract *Urtica dioica*

Table 4: GC-MS analytical report for fraction F_B of Hexane Extract (HE)

RT	Name of the compound	Molecular formula	MW	Peak area (%)
18.12	2,4-di-t-butylphenol	$C_{14}H_{22}O$	206	4.56
21.30	Phosphoric acid tributyl ester	$C_{12}H_{27}O_4P$	266	3.56
22.42	8-Methylheptadecane	$C_{18}H_{38}$	254	1.62
24.68	1-Heptadecene	$C_{17}H_{34}$	238	1.62
24.87	Eicosane	$C_{20}H_{42}$	282	2.86
25.91	2,4,10-trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278	26.97
26.10	3,7,11,15-tetramethyl-2-hexadecyl ester	$C_{20}H_{40}$	280	2.06
26.65	Butyl tetradecyl ester	$C_{26}H_{42}O_4$	418	9.53
27.78	2,6,10,15-tetramethylheptadecane	$C_{21}H_{44}$	296	0.90
27.91	Olean-18-ene	$C_{30}H_{50}$	410	1.97
28.83	3,5-di-tert-butyl-ortho-benzoquinone	$C_{14}H_{20}O_2$	220	1.08
29.10	2,6,10,14-tetramethylpentadecane	$C_{19}H_{40}$	268	1.06
29.26	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	7.45
29.71	Unknown			3.04
30.37	Heneicosane	$C_{21}H_{44}$	296	2.00
30.48	Unknown			1.20
32.67	Hexacosane	$C_{26}H_{54}$	366	1.93
33.02	Unknown			1.04
33.52	Bis(2-ethyl hexyl) maleate	$C_{20}H_{36}O_4$	340	8.80
34.76	Nonacosane	$C_{29}H_{60}$	408	2.30
38.45	Pentacosane	$C_{25}H_{52}$	352	1.36
40.62	1,2-benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	9.89
55.98	2-tert-Butyl-4,6-bis (3,5-di-tert-butyl-4-hydroxybenzyl)phenol	$C_{40}H_{58}O_3$	586	3.19

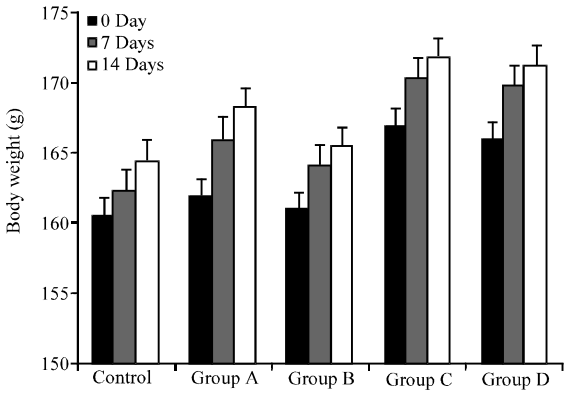

Fig. 2: Body weight changes before and after the treatment of Fraction B of Hexane extract of *Urtica dioica*. Each bar represents Mean+SEM of 6 rats

respectively. No significant treatment related variation was observed in body weight and behavior of wistar rats during 14 days exposure to F_B of HE indicating safety of the semi purified plant extract.

The hematopoietic system is very sensitive to toxic compounds (Mukinda and Syce, 2007) and it ranks with liver and kidneys in pre clinical and clinical safety evaluation (Bloom, 1993). Analysis of blood parameter is relevant in risk evaluation as the changes in hematological system have a higher predictive value for human toxicity, when the data translated from animal studies (Rhiouani *et al*., 2008). The hematological profiles of the experimental and control group wistar rats were determined at 15th day after treatment with F_B in this study. No significant variation in the values of Red Blood Cell Count (RBC), White Blood Cell count (WBC) (Fig. 3), neutrophils, monocytes, basophils and eosinophils (Fig. 4). Significant decrease in level of lymphocytes was observed (Fig. 4) in group D. Hemoglobin percentage (Hb), Mean Corpuscular Hemoglobin Concentration (MCHC), bilirubin and total protein showed no significant variation in any of the treatment group as compared to control except significant decrease in Packed Cell Volume (PCV)

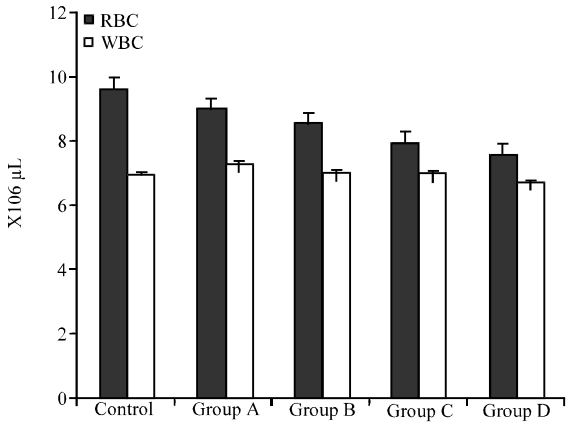

Fig. 3: Effect of fraction B of Hexane extract of *Urtica dioica* on Red Blood Corpuscles (RBC) and White Blood Cells (WBC) count in wistar rats. Each bar represents Mean+SEM of 6 rats

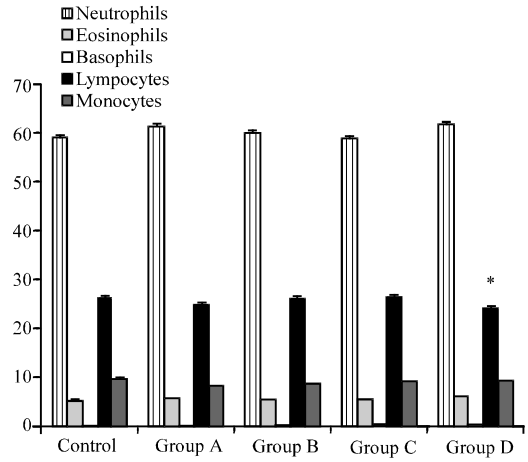

Fig. 4: Effect of fraction B of Hexane extract of *Urtica dioica* on Differential Leucocytes Counts (DLC) in wistar rats. Each bar represents Mean+SEM of 6 rats. *Significantly different from control group at $p<0.05$

in group A. Significant increase in Mean Corpuscular Volume (MCV) in group C and D, while significant increase in Mean Corpuscular Hemoglobin (MCH) was observed in group B, C and D (Table 5).

Detoxification and clearance of toxic substances from the body are primarily a function of liver and kidneys and they are often the first to be affected by toxic herbs (Elvin-Lewis, 2001). Damage to these organs often results in elevation in clinical chemistry parameters. Biochemical parameters from serum were determined after treatment with F_B and compared with control group. No variation in liver function markers like Aspartate amino Transferase (AST), Alanine amino Transferase (ALT) (Fig. 5). Alkaline Phosphates (ALP) was significantly decreased in treated animals in group B, C and D as compared to control (Fig. 6), however this decrease was with in the normal reference range of ALP values. Cholesterol (CHO), High Density Lipoprotein-cholesterol (HDL-C), Triglycerides (TG), Low Density Lipoprotein-cholesterol (LDL) and very low density lipoprotein-cholesterol (VLDL-C) (Fig. 7) were found under the normal reference values in treated animals. CHO, HDL-C, LDL-C and VLDL-C, TG can give useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Toyin *et al.*, 2008). High blood cholesterol concentrations are an important risk factor for cardiovascular disease (Abolaji *et al.*, 2007). Therefore, the reduced levels of serum CHO at 100, 200, 400 and 800 mg kg^{-1} body weight of the F_B may be clinically beneficial and indicates

Table 5: Effect of 14 days oral administration of F_B of hexane extract (HE) on hematological and biochemical parameters

Parameters	Control	Group-A (100 mg kg^{-1})	Group-B (200 mg kg^{-1})	Group-C (400 mg kg^{-1})	Group-D (800 mg kg^{-1})
Hb	14.91±0.2	14.41±0.1	14.86±0.1	15.20±0.3	15.20±0.3
PCV	42.48±0.3	39.06±0.4*	40.83±0.3	43.15±1.0	41.40±1.3
MCV	44.29±0.8	43.41±0.7	47.74±0.9	54.25±1.4**	54.46±1.63**
MCH	15.39±0.5	16.02±0.2	17.37±0.3*	19.10±0.4**	20.03±0.5**
MCHC	36.09±0.5	36.92±0.5	36.42±0.5	35.28±0.9	36.99±1.8
Total protein (g dL^{-1})	6.84±0.1	6.43±0.1	6.73±0.2	6.40±0.1	6.27±0.2
Bilirubin (mg dL^{-1})	0.34±0.0	0.48±0.0	0.37±0.0	0.36±0.0	0.32±0.0

*p<0.05, **p<0.01

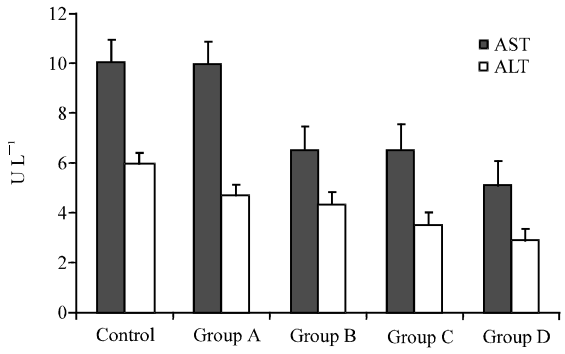

Fig. 5: Effect of fraction B of Hexane extract of *Urtica dioica* on Aspartate amino Transferase (AST) and Alanine amino Transferase (ALT) level. Each bar represents Mean+SEM of 6 rats

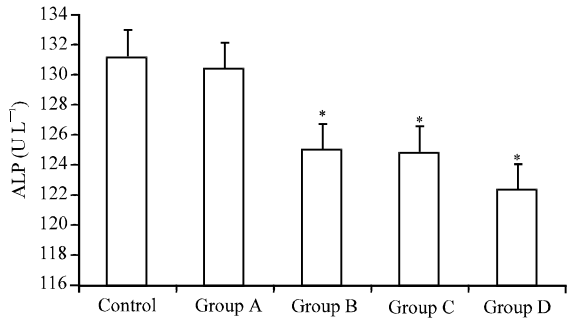

Fig. 6: Effect of fraction B of Hexane extract of *Urtica dioica* on Alkaline Phosphatase (ALP) level in wistar rats. Each bar represents Mean+SEM of 6 rats. *Significantly different from control group at $p<0.05$

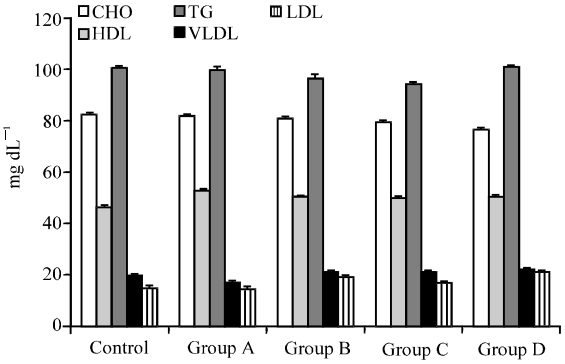

Fig. 7: Effect of fraction B of Hexane extract of *Urtica dioica* on Cholesterol (CHO), High Density Lipoproteins (HDL), Triglycerides (TG), Very Low Density Lipoproteins (VLDL) and Low Density Lipoproteins (LDL) level in wistar rats. Each bar represents Mean+SEM of 6 rats

hypolipidemic activity. Cholesterol lowering activity of *U. dioica* is reported in hypercholesterolemic rats (Nassiri-Asl *et al*., 2009). The TG, HDL, LDL and VLDL cholesterol did not change significantly ($p>0.05$) indicating that the mechanism involving lipid alteration may not have been disturbed by the administration of the F_B at these doses.

CONCLUSIONS

Hexane extract of *Urtica dioica* showed good antimicrobial activity against all the tested pathogenic clinical strains of bacteria. The extract showed better activity against gram negative bacteria as compared to gram positive. Owing development of multidrug resistant strains treating gram negative bacterial infection is more difficult, it will be interesting to purify the active subfractions for potential lead antibacterial compounds. GC-MS analysis of the subfraction F_B indicated that terpenes, fatty acid esters and phenols are the active antimicrobial principles present in F_B. F_B was non toxic in wistar rats up to the tested concentration of 800 mg kg^{-1} bw indicating that it is safe for *in vivo* consumption.

ACKNOWLEDGMENTS

The authors are thankful to Bundelkhand University, Jhansi, India, for providing research facilities for undertaking this work.

REFERENCES

Abdelgadir, E.H., R.H. Ahmed, S.I.Y. Adam and A.M. Husein, 2010. Evaluation of toxicological activity (Acute and sub-chronic toxicities) of the aqueous extract of *Lawsonia inermis* seeds on wistar rats. J. Pharmacol. Toxicol., 5: 324-333.

Abdullin, I.F., E.N. Turova, G.K. Gaisina and G.K. Budnikov, 2002. Use of electrogen erated bromine for estimating the total antioxidant capacity of plant raw materials and plant-based medicinal preparations. J. Anal. Chem., 57: 557-560.

Abolaji, A.O., A.H. Adebayo and O.S. Odesanmi, 2007. Effect of ethanolic extract of *Parinari polyandra* (Rosaceae) on serum lipid profile and some electrolytes in pregnant rabbits. Res. J. Med. Plants, 1: 121-127.

Adeniyi, B.A. and O.O. Ayepola, 2008. The phytochemical screening and antimicrobial activity of leaf extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* (Myrtaceae). Res. J. Med. Plant, 2: 34-38.

Al-Bari, M.A.A., A. Khan, M.R. Islam, M. Kudrat-E-Zahan, M.M.S. Rahman, M.A. Ul-Islam and M.A. Mosaddik, 2007. Isolation and *in vitro* antimicrobial activities of ethyl acetate extract from *Streptomyces bangladeshiensis*. Res. J. Microbiol., 2: 272-277.

Anders, R.J., K. Bendixen and U. Karlson, 2002. Detection of microbial growth on polycyclic aromatic hydrocarbons in microtiter plates by using the respiration indicator WST-1. Applied Environ. Microbiol., 6: 2683-2689.

Bauer, A.W., E. Kirby, E.M. Sherris and M. Turk, 1996. Antibiotic by standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Black, R.E., K.D. Brown, S. Becker and M. Yunus, 1982. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh. Am. J. Epidemiol., 115: 305-314.

Bloom, J.C., 1993. Principles of hematology: Laboratory assessment and interpretation of data. Toxicol. Pathol., 21: 130-134.

Bnouham, M., F.Z. Merhfour, A. Ziyat, H. Mekhfi, M. Aziz and A. Legssyer, 2003. Antihyperglycemic activity of the aqueous extract of *Urtica dioica*. Fitoterpia, 74: 677-681.

Chandrasekaran, M. and V. Venkatesalu, 2004. Antibacterial and antiyeast activity of *Syzygium jabolanum* seeds. J. Ethnopharmacol., 91: 105-108.

Chavda, R., K.R. Vadalia and R. Gokani, 2010. Hepatoprotective and antioxidant activity of root bark of *Calotropis procera* R. Br (Asclepediaceae). Int. J. Pharmacol., 61: 937-943.

Davies, J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science, 264: 375-382.

Diallo, A., K. Eklu-Gadegbeku, A. Agbonon, K. Aklikokou, E.E. Creppy and M. Gbeassor, 2010. Acute and sub-chronic (28-Day) oral toxicity studies of hydroalcoholic extract of *Lannea kerstingii* Engl. and K. Krause (Anacardiaceae) stem bark. J. Pharmacol. Toxicol., 5: 243-349.

Durak, I., H. Biri, E. Devrim, S. Sozen and A. Avci, 2004. Aqueous extract of *Urtica dioica* makes significant inhibition on adenosine deaminase activity in prostate tissue from patients with prostate cancer. Cancer Biol. Ther., 3: 855-857.

Elvin-Lewis, M., 2001. Should we concerned about herbal remedies. J. Ethnopharmacol., 75: 141-164.

Elzaawely, A.A., T.D. Xuan and S. Tawata, 2005. Antioxidant and antibacterial activities of *Rumex japonicus* HOUTT. aerial parts. Biol. Pharm. Bull., 28: 2225-2230.

Emmelin, N. and W. Feldberg, 1949. Distribution of acetylcholine and histamine in nettle plants. New Phytol., 48: 143-148.

Erturk, O., H. Kati, N. Yayli and Z. Demirbag, 2006. Antimicrobial properties of *Silene multifida* (Adams) Rohrb. plant extract. Turk. J. Biol., 30: 17-21.

Hadizadeh, I., B. Peivastegan and M. Kolahi, 2009. Antifungal activity of nettle (*Urtica dioica* L.), colocynth (*Citrullus colocynthis* L. schrad), oleander (*Nerium oleander* L.) and konar (*Ziziphus spina-christi* L.) extracts on plants pathogenic fungi. Pak. J. Biol. Sci., 12: 58-63.

Hassan, S.W., M.G. Abubakar, R.A. Umar, A.S. Yakubu, H.M. Maishanu and G. Ayeni, 2011. Pharmacological and toxicological properties of leaf extracts of *Kingelia africana* (bignoniaceae). J. Pharmacol. Toxicol., 6: 124-132.

Karim, A., M. Nouman, S. Munir and S. Sattar, 2011. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. Int. J. Pharmacol., 7: 419-439.

Li, R.W., D.N. Leach, P. Myers, G.J. Leach, G.D. Lin, D.J. Brushett and P.G. Waterman, 2004. Anti-inflammatory activity, cytotoxicity and active compounds of *Tinospora smilacina* Benth. Phytother. Res., 18: 78-83.

Lourdes, R.F., R.E. Jorge, B. Scott, H.R. Dea and T. Eliseo, 2008. Risks and benefits of commonly used herbal medicines in Mexico. Toxicol. Applied Pharmacol., 227: 125-135.

Matsingou, T.C., M. Kapsokefalou and A. Salifoglou, 2001. Aqueous infusions of Mediterranean herbs exhibit antioxidant activity towards iron promoted oxidation of phospholipids, linoleic acid and deoxyribose. Free Radical Res., 35: 593-605.

Menghani, E., A. Pareek, R.S. Negi and C.K. Ojha, 2011. Search for antimicrobial potential from certain Indian medicinal plants. Res. J. Med. Plants, 5: 295-301.

Modupe, O., O. Wesley, A. Morufu and A.O. Elizabeth, 2010. Analysis of essential oil from the stem of *Chasmanthera dependens*. J. Nat. Prod., 3: 47-53.

Mosaddik, M.A. and M.E. Haque, 1999. Toxicological evaluation of goniothalamin isolated from *Bryonopsis laciniosa* Linn in rats. Pharm. Pharmacol. Commun., 5: 411-413.

Mukinda, J.T. and J.A. Syce, 2007. Acute and chronic toxicity of aqueous extract of *Artemisia afra* in rodents. J. Ethnopharmacol., 112: 138-144.

NCCLS, 2005. National Committee for clinical laboratory standards for antimicrobial disk susceptibility tests: Approved standards. NCCLS, Wayne, Pennsylvania, USA.

Nassiri-Asl, M., F. Zamansoltani, E. Abbasi, M.M. Daneshi and A.A. Zangivand, 2009. Effects of *Urtica dioica* extract on lipid profile in hypercholesterolemic rats. J. Chin. Integr. Med., 7: 428-433.

Oboh, G., 2010. Antioxidant and antimicrobial properties of ethanolic extract of *Ocimum gratissimum* leaves. J. Pharmacol. Toxicol., 5: 396-402.

Palic, R., G. Stojanovic, S. Alagic, M. Nikolic and Z. Lepojevic, 2002. Chemical composition and antimicrobial activity of the essential oil and CO_2 extracts of the oriental tobacco, Prilep. Flavour Fragr. J., 17: 323-326.

Premanath, R., J. Sudisha, N. Lakshmi Devi and S.M. Aradhya, 2011. Antibacterial and antioxidant activities of Fenugreek (*Trigonella foenum graecum* L.) leaves. Res. J. Med. Plants, 5: 695-705.

Rahman, M.M., M.A. Mosaddik, M.I. Wahed and M.E. Haque, 2000. Antimicrobial activity and cytotoxicity of *Trapa bispinosa*. Fitoterapia, 71: 704-706.

Rhiouani, H., J. El-Hilaly, Z.H. Israili and B. Lyoussi, 2008. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. J. Ethnopharmacol., 118: 378-386.

Riehemann, K., B. Behnke and K. Schulze-Osthoff, 1999. Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-κB. FEBS Lett., 442: 89-94.

Roy, S., K. Rao, C. Bhuvaneswari, A. Giri and L.N. Mangamoori, 2010. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World J. Microbiol. Biotechnol., 26: 85-91.

Russel, A.D., 1991. Mechanisms of bacterial resistance of nonantibiotics: Food additives and food pharmaceutical preservatives. J. Applied Bacteriol., 71: 191-201.

Saad, B., H. Azaizeh, G. Abu-Hijleh and S. Said, 2006. Safety of traditional Arab herbal medicine. Evid. Based Comp. Alternative Med., 3: 433-439.

Sajfrtova, M., H. Sovova, L. Opletal and M. Bartlova, 2005. Near-critical extraction of β-sitosterol and scopoletin from stinging nettle roots. J. Supercrit. Fluids, 35: 111-116.

Semwal, D.K., U. Rawat, A. Bamola and R. Semwal, 2009. Antimicrobial activity of *Phoebe lanceolata* and *Stephania glabra*: Preliminary screening studies. J. Sci. Res., 1: 662-666.

Singh, R., R. Chandra, M. Bose and P.M. Luthra, 2002. Antibacterial activity of various rhizome extracts of *Curcuma longa* on pathogenic bacteria. Curr. Sci., 83: 737-740.

Singleton, P., 1999. Bacteria in Biology, Biotechnology and Medicine in Africa. John Wiley and Sons, Chichester.

Suresh, L., R.M. Veerabah and S.R. Gnanasingh, 2010. GC-MS analysis of ethanolic extract of *Zanthoxylum rhetsa* (Roxb.) dc spines. J. Herbal Med. Toxicol., 4: 191-192.

Taylor, R.S.L., F. Edel, N.P. Manandhar and G.H.N. Towers, 1996. Antimicrobial activity of Southern Nepalese medicinal plants. J. Ethnopharmacol., 50: 97-102.

Tijjani, M.B., I.A. Bello, A.B. Aliyu, T. Olurishe, S.M. Maidawa, J.D. Habila and E.O. Balogun, 2009. Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium* A. rich. (Cochlospermaceae). Res. J. Med. Plant, 3: 16-22.

Toyin, Y.M., A.M. Adewumi and O.A. Temidayo, 2008. Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia agrestis* stem. Res. J. Med. Plant, 2: 66-73.

Uzun, Y., A. Kele, A. Imali, E. Ogun and A. Kaya, 2002. Antimicrobial activity of *Urtica dioica* L. and *Rheum ribes* L. BioSci. Res. Bull., 18: 43-50.

Wilson, N.H., J.F. Hardistry and J.R. Hayes, 2001. Short Term, Sub-Chronic and Chronic Toxicology Studies. In: Principle and Method of Toxicology, Hayes, A.W. (Ed.). 4th Edn. Taylor and Francis, Philadelphia, pp: 917-957.