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Biological Activity of Methanolic Extract of *Waltheria indica* Roots

[1]Raga Eltayeb Osman, [2]Elamin Ibrahim Elnima and [3]Mohammed Elfatih Ahmed Omer

Waltheria indica used by traditional healer in Sudan for treatment of many diseases. In this study antibacterial activity, toxicological and pharmacological screen of methanolic extracts of *Weltheria indica* roots was reported. One hundred eighty clinical isolates were obtained from diabetic wound infection, 30 *Escherichia coli*, 15 *Klebsiella pneumoniae*, 45 *Proteus* sp. 15 *Pseudomonas aeruginosa*, 60 *Staphylococcus aureus* and 15 *Staphylococcus epidermidis*. Cup-plate method used for antibacterial activity and the extract had an inhibitory effect against (91.7%) of the isolates. In the toxicity studies the results indicate that the LD_{50} of the extracts was more than 2000 mg kg^{-1}. The extract had no effect on smooth muscle and skeletal muscle. The result of this study indicated that the methanolic extract of *Waltheria indica* roots with high antibacterial activity. In the toxicity study the result indicate that LD_{50} of the extract was more than 2000 mg kg^{-1} and the pharmacology study indicated at least the safety of the extract regarding the studied tissues.

Key words: Antibacterial activity, pharmacological, toxicological, *Waltheria indica*, roots

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Raga Eltayeb Osman
Medicinal and Aromatic Plants Research Institute,
National Centre for Research,
P.O. Box 2404,
Khartoum, Sudan

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[1]National Center for Research, Department of Microbiology and Parasitology, Medicinal and Aromatic Plants Research Institute, Sudan
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Khartoum, Sudan
[3]Faculty of Pharmacy, University of Elnilin, Sudan

INTRODUCTION

During the past decade, traditional systems of medicine have become a topic of global importance. In many developing countries people relies heavily on traditional practitioners and medicinal plants to meet primary health care needs (WHO, 1999).

Eisenberg *et al*. (1993) and Hostettmann *et al*. (2000) reported traditionally, plants are used for treatment of diseases in different parts of the world and they have significant part in primary health care delivery (Holetz *et al*., 2002). They are considering important sources of pharmaceutical products (Olajuyigbe *et al*., 2011).

The substances that have an effect on microorganisms only not host cell are appropriate for developing new antimicrobial drug (Garba *et al*., 2012).

Waltheria indica widespread plant belongs to the family Sterculiacae used in Sudan for treatment of ulcer, diarrhea, diabetic wound infection, tooth and stomach pain, rheumatism and as a tonic.

MATERIALS AND METHODS

Collection of plant: The plant used in this study was collected from Tandalti (Northern Kordofan), Sudan by Herbalists in collaboration with the Institute of Traditional medicine. They were authenticated by the researcher Haider Abdelgadir and Wail Elsadig Abdalla, Medicinal and Aromatic plants Research Institute (MAPRI). Voucher specimens were deposited at the herbarium of the institute.

Preparation of crude extracts: The coarsely powdered plant material (50 g) was exhaustively extracted for 20 h with chloroform in Soxhlet apparatus. The chloroform extract was filtered and evaporated under reduced pressure using Rota-vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The methanolic extract was filtered and evaporated under reduced pressure again using Rota-vap. The residue was weighed and the yield percentage was determined. The methanol residue (2 g) was dissolved in methanol 20 mL (100 mg mL^{-1}) and kept in refrigerator until used.

Tested organisms: The clinical isolates were obtained from diabetic wound infection. The purified isolates were identified by microscopical examination, cultural characters and biochemical tests and then stored in a refrigerator until they were used.

Preparation of bacterial suspensions: One milliliter aliquots of a 24 h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 h. The bacterial growth was harvested and washed off with 100 mL sterile normal saline, to produce a suspension containing about 10^8-10^9 CFU mL^{-1}. The suspension was stored in the refrigerator at 4°C till used (Al-Magboul, 1992).

Testing for antibacterial activity: The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial activity of the prepared extract.

Animal: Thirty five 8-week-old male Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum.

Oral acute toxicity test: The methanolic extract of *Waltheria indica* was evaluated for oral acute toxicity and LD_{50} in rats (Walum, 1998).

The rats were allotted at random to four groups, each of 5 rats. Group 1 served as control. Groups 2, 3 and 4 received 500, 1000 and 2000 mg kg^{-1} of methanolic extract of *Waltheria indica*, respectively. After two weeks all the rats in different groups anaesthetized with diethyl ether and killed. Body weight and body weight gain for each group were recorded. Blood samples were collected at slaughter. At necropsy, all rats were examined to identify gross lesions and specimens of the liver, kidneys, heart, spleen and intestines were fixed in 10% neutral buffered formalin and processed for histopathology.

Serobiochemical methods: Blood samples were collected at slaughter in dry test tubes and serum was separated and stored at -20°C until analyzed for the activities of aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and alkaline phosphotase (ALP) and for the concentrations of total protein, albumin, globulin, cholesterol and urea.

Haematological methods: The parameters measured were Hb, PCV, RBC, WBC, MCV, MCH and MCHC, using an Automated Haematology Analayzer.

Pathological methods: Necropsy was conducted to identify gross lesions and specimen of the liver, kidneys, heart, spleen and intestines were immediately fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μm and stained routinely with haematoxylin and eosin (H and E) and examined.

Statistical analysis: The data was analyzed statistically through the program of statistical analysis (SPSS) significance of differences between means was compared using one-way ANOVA test (Snedecor and Cochran, 1989).

Pharmacology methods: This set of experiments was carried out to evaluate the pharmacological effects of the methanolic extracts of *Waltheria indica* roots, Rabbit jejunum: Isolated rat uterus preparation and Isolated frog rectus abdominous muscle (Kitchen, 1984).

RESULTS

Antibacterial activity: The methanolic extract of *Waltheria indica* roots had inhibitory effect against 91.7% of isolates at concentration of 100 mg mL^{-1}. It showed high activity against (70%) of the *E. coli* isolates (60%) of the *K. pneumoniae* and *P. aruginosa* isolates (57.8%) of the *Proteus* spp. and (55%) of the *S. aureus* isolates (Table 1).

Evaluation of *Waltheria indica* toxicity in Wistar rats

Pathological changes: The microscopic changes shown in rats in group that received 500 mg kg^{-1} were congestion; hemorrhage and hepatic cell necrosis, kidney exhibited necrosis of epithelial cell of renal tubules. Cardiac muscle showed degeneration hemorrhage and congestion. Intestine revealed necrosis of epithelial cell. Hemorrhage, congestion, depletion of lymphocytes and deposition of haemosiderin pigment. The rats in groups received 1000 and 2000 mg kg^{-1} the kidney showed congestion and hemorrhage and necrosis of renal tubules. There was severe liver cells necrosis, congestion and vacuolation.

Table 1: Activity of *Waltheria indica* roots against clinical isolates

	MeOH		
Organism	Active	Moderate	Resistant
E. coli	70	30.0	0.0
K. pneumonia	60	26.7	13.3
Protus spp.	57.8	35.5	6.7
P. aeruginosa	40	46.7	13.3
S. aurues	55	35.0	10.0
S. epidermidis	40	46.7	13.3

Total No. of clinical isolates: 180, No. of *Escherichia coli* clinical isolates: 30, *Klebsiella pneumoniae* clinical isolates: 15, *Proteus* spp. clinical isolates: 45, *Pseudomonas aeruginosa* clinical isolates: 15, *Staphylococcus aureus* clinical isolates: 60, *Staphylococcus epidermidis* clinical isolates: 15 Interpretation of sensitivity results: (MIZD)>18 mm = sensitive (MIZD) 14-18 mm = Intermediate, (MIZD)<14 mm = Resistant, Concentration of extracts 100 mg mL^{-1}

Serobiochemical changes: The activities of serum AST did not change in all groups. The activities of serum of ALT and ALP did not change in groups that received 500 and 2000 mg kg^{-1} but ALT was higher ($p<0.05$) in group that received 1000 mg kg^{-1} than control and in the same group ALP was lower ($p<0.05$) than the control. The concentration of total protein did not change but the concentration of albumin was higher ($p<0.05$) and globulin was lower ($p<0.05$) in all groups. The concentration of cholesterol was higher ($p<0.05$) in all groups than the control. The concentration of urea was higher ($p<0.05$) only in group that received 2000 mg kg^{-1} (Table 2).

Haematological changes: The values of Hb, RBC, MCV, MCH and MCHC did not change but those of WBC were lower ($p<0.05$) in all groups than control. The values of PCV were lower ($p<0.05$) in group that received 1000 and 2000 mg kg^{-1} than the control (Table 3).

Effect of methanolic extract of *Waltheria indica* roots on different isolated tissue preparation:

Rabbit jejunum preparation: Increasing concentration of the extract (0.2-0.8 mg mL^{-1}) produced a dose-dependant contraction on the smooth muscle. The stimulant effect of the extract on the jejunum was effectively blocked by the muscarinic antagonist Atropine (10 μg mL^{-1}) (Fig. 1).

Non-contracting rat uterus preparation: The extract in a dose up to 1.6 mg mL^{-1} had neither agonistic nor antagonistic effects on the uterine muscle (Fig. 2).

Table 2: Hematological changes in rats receiving methanolic extract of *Waltheria indica*

	Treatment groups			
Parameters	Control	500 (mg mL^{-1})	1000 (mg mL^{-1})	2000 (mg mL^{-1})
Hb (mg dL^{-1})	11.78±0.166	10.76±0.700^{NS}	11.24±0.37^{NS}	10.96±0.75^{NS}
RBC (×10^6 mm)	7.890±0.390	7.89±0.469^{NS}	7.52±0.29^{NS}	6.83±0.32^{NS}
WBC (×10^3 mm)	6.480±0.590	3.44±0.690**	2.80±0.52**	2.43±0.41**
PCV	49.48±0.900	44.26±1.110*	46.58±1.28^{NS}	41.32±1.73*
MCV (m^3)	60.20±0.580	60.40±1.600^{NS}	62.20±0.86^{NS}	60.40±1.6^{NS}
MCH (pg)	16.50±2.090	15.00±0.670^{NS}	14.96±0.19^{NS}	15.32±0.58^{NS}
MCHC (%)	24.20±0.370	24.84±0.540^{NS}	24.12±0.22^{NS}	24.68±0.49^{NS}

Values are Mean±SE, NS: Not significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$

Table 3: Serobiochemical changes in rats receiving methanolic extract of *Waltheria indica*

	Treatment groups			
Parameters	Control	500 (mg mL^{-1})	1000 (mg mL^{-1})	2000 (mg mL^{-1})
AST	11.67±0.825	15.3±2.153^{NS}	8.98±1.39^{NS}	16.63±2.740^{NS}
ALT	8.310±1.630	14.31±0.90^{NS}	19.25±4.24**	13.13±0.390^{NS}
ALP	60.32±6.630	50.37±6.75^{NS}	33.29±2.98*	40.11±9.410^{NS}
Total protein (g dL^{-1})	8.050±0.240	8.05±0.36^{NS}	7.82±0.33^{NS}	7.84±0.180^{NS}
Albumin (g dL^{-1})	4.350±0.180	6.29±0.22**	5.92±0.08*	5.87±0.450*
Globulin (g dL^{-1})	3.730±0.070	1.72±0.31***	1.70±0.20***	1.96±0.310*
Cholesterol (mg dL^{-1})	43.07±5.660	140.97±5.85***	155.74±9.68***	144.60±90.34***
Urea (mg dL^{-1})	36.78±3.470	49.67±3.30^{NS}	60.86±8.19^{NS}	67.59±10.08*

Values are Mean±SE, NS: Not significant, *p<0.05, **p<0.01, ***p<0.001

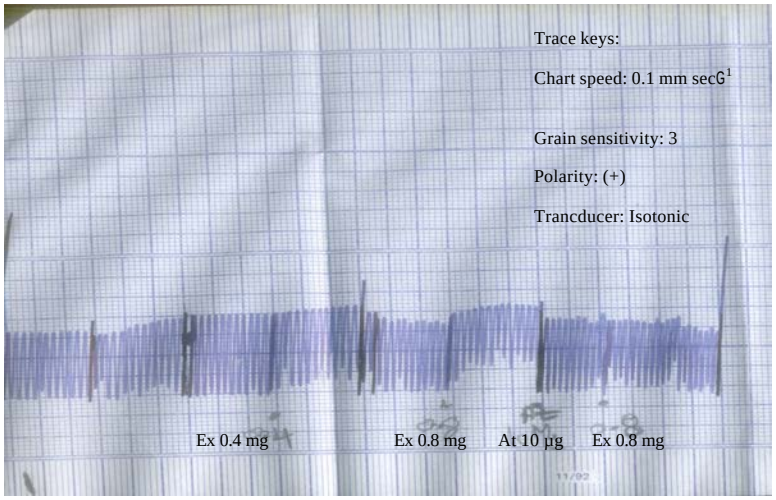

Fig. 1: Effect of methanolic extract of *Waltheria indica* root in doses (0.4, 0.8 mg mL^{-1}) on isolated Rabbit jejunum, the extract showed contraction inhibit by Atropine in dose of (10 µg mL^{-1})

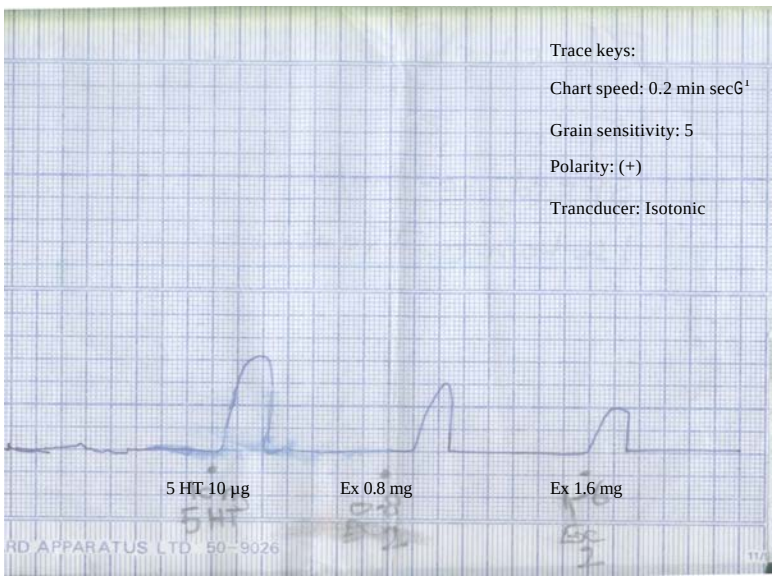

Fig. 2: Effect of methanolic extract of *Waltheria indica* root in doses (0.8 and 1.6 mg mL^{-1}) on isolated Rat uterus, the extract showed no effect on the contraction exerted by 5 HT (10 µg mL^{-1})

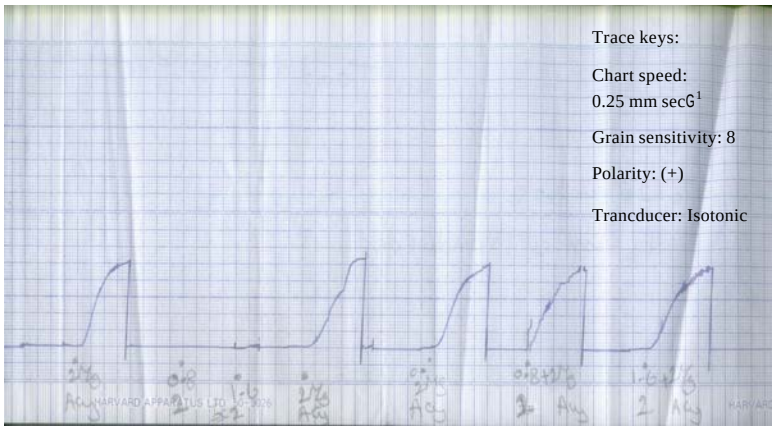

Fig. 3: Effect of methanolic extract of *Waltheria indica* root in doses (0.8 and 1.6 mg mL^{-1}) on isolated Frog-rectus abdominous muscle, the extract showed no effect on contracting of acetylcholine in a dose of 2 μg mL^{-1}

Frog rectus abdominous muscle preparation: The extract in doses 0.8 and 1.6 mg mL^{-1} has no effect on isolated Frog rectus abdominous muscle. Also it did not affect the contracting activity of Acetyl Choline in a dose of 2 μg mL^{-1} (Fig. 3).

DISCUSSION

The methanolic extract of *Waltheria indica* roots had inhibitory effect against 91.7% of isolates at concentration of 100 mg mL^{-1}. Elegami *et al*. (2001) found that leaf and fruit extract of *Waltheria indica* exhibit antibacterial activity against *Bacillus subtillis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Kone *et al*. (2007) assessed twenty crude extracts from 17 species out of 11 families for their antibacterial activity against *Streptococcus pneumoniae* (*Pneumococcus*). Only 7 extracts showed a promising *in vitro* bactericidal activity against *Peumococcus*, The most active extracts included *Waltheria indica* (Sterculiaceae). Garba *et al*. (2012) studied the antioxidant and antimicrobial activity of some Nigerian medicinal plants and the result showed that ethanolic extract of *Waltheria indica* and *Mucuna piurin* gave the best antimicrobial activity against *Salmonella typhi*, *Bacillus subtillis* and *Escherishia coli*.

At dose of 1000 mg kg^{-1} of *Waltheria indica* root extract the serum ALT was higher and ALP was lower than the control. The concentration of albumin was higher and globulin was lower in all groups. The concentration of cholesterol was high in all groups but the concentration of urea was high only in group that received 2000 mg kg^{-1}. The values of WBC were lower in all groups while the value of PCV were low in group that received 1000 and 2000 mg kg^{-1}.

In the histopathological studies the two extracts showed hepatocellular necrosis, haemorrhage and congestion Kidney exhibited necrosis of renal tubules and haemorrhage. Muscle degeneration hemorrhage and congestion in heart. Intestine showing necrosis of epithelial cells. Depletion of lymphocytes hemorrhage and haemosiderin pigment deposit was also observed in spleen. Mohammed *et al*. (2007) studied the analgesic effect of the aqueous extracts of the leaves, stem and root of *Waltheria indica*. The result showed that the root had the most analgesic activity and showed maximum activity at 20 mg kg^{-1} b.wt. The extract had intraperitoneal (i.p) LD_{50} of 363, 14 and 69 mg of leave, stem and root, respectively.

Hamidu *et al*. (2008) found that aqueous ethanolic extract of *Waltheria indica* showed bioactivity in acetic-acid induced stretches in animal model and the acute toxicity study showed low toxicity (LD_{50}, 875 mg kg^{-1}) in mice when orally administered.

CONCLUSION

The methanolic extract of *Waltheria indica* apart of the mild cholinergic effect revealed by their effects on rabbit intestine were without effect on the uterine smooth muscle and the skeletal muscle studied. These finding indicated at least the safety of the extract regarding the studied tissues.

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