

Full Length Research Paper

Evaluating the antipyretic activities of aqueous and ethanol extracts of leaves of *Artemisia Annua* in mice

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Phytomedicines obtained from herbal sources are in great demand as they are able to cure many infectious diseases. *Artemisia annua* is an herbaceous plant that belongs to Composite family. As traditional medicine, it is used for cancer, ulcer, rheumatic pain and other ailments including fever in children. The objective of the present study was to evaluate the antipyretic activities of the aqueous and ethanol extracts of leaves of *A. annua* in mice. Rectal temperatures were recorded before and after inducing pyrexia as well as after administration of the respective extracts every half an hour for three hours. Parallel experiments were conducted with the standard antipyretic (aspirin) and the negative control (distilled water). Both extracts showed significant antipyretic activity at the specified dose levels except for 100 mg/kg aqueous extract. The antipyretic activities for both extracts were found to be dose dependent. No significant potency difference was observed for aqueous and ethanol extracts though the effects of aqueous extract were not statistically significant to the end of the experiment.

Key words: Antipyretic activity, *Artemisia annua*, extraction.

INTRODUCTION

Pathophysiology of fever

The febrile response is a complex physiologic reaction to disease involving a cytokine-mediated rise in body temperature, generation of acute-phase reactants and activation of endocrinology and immunologic systems. Understanding the basic mechanisms underlying this phenomenon helps to formulate rational approaches to treatment (Schafer et al., 2000).

Phytomedicines

Phytomedicines obtained from herbal sources are in great demand as they are able to cure many infectious

diseases. These plant based drugs provide outstanding contribution to modern therapeutics. They have proved efficacy and safety for primary health care. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, and immune disorders. The integration of phytomedicines into the health system should be developed in such a way to bring harmony between the traditional and modern system of health care with minimum threat to each other (Pandey et al., 2011).

Artemisia annua

A. annua L. is the plants from temperate regions, but can be developed in the tropics through breeding. In Indonesia

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demand for artemisinin is very large and all are imported, therefore, the development of cultivation of *A. annua* L. in Indonesia is a pretty big opportunity (Cao et al., 2010). *A. annua*, belongs to the family Composites, and is a fragrant shrub that grows widely in the Arabian area. Phytochemical analysis shows that it is a rich source of flavonoids including apigenin, cirsimaritin, and various novel compounds (Abdalla and Abu-Zagra, 1987; Schned and Silver, 1981). Approximately, 42% of the total artemisinin compounds are found in upper leave. The highest artemisinin compounds are found in plant aged 12 to 13 weeks (Breder et al., 1988). *A. annua* is used in the treatment of gastrointestinal disorders, enhanced eyesight, cardiovascular health, capillary strength, and structure of connective tissue, appearance of skin, and immune systems as well as decreased risk of atherosclerosis, cancer, fever and arthritis (Huh and Lichtiger, 1987). *Artemisia* family extract showed significant higher antioxidant effect (Grgesina et al., 1995). There are also evidences that *Artemisia* family can improve diabetes mellitus in addition to their antimicrobial and anti-fungal effects but the antipyretic activity is not well investigated and documented. The purpose of this study was, therefore, to evaluate the antipyretic activities of aqueous and ethanol extracts of dried leaves of *A. annua* in mice

MATERIALS AND METHODS

Collection of the plant material

After getting a support letter, to Ankober Woreda development association, the leaves of *A. annua* were collected from Ankober (170 km to the north from Addis Ababa) in October 2013. The plant was authenticated by an expert taxonomist and a specimen representing this collection was deposited in the Addis Ababa University Herbarium, Addis Ababa, for further reference (AA1500).

Chemicals and drugs

Chemicals and Drugs used were: distilled water, ethanol (70%), powder of acetyl salicylic acid (Barer Schering pharma AG, Germany) and yeast extract powder (Lot. Number0000076357).

Experimental animals

The experiment was performed in house bred albino mice (both sexes weighing 25-35 g) which were obtained from Department of Pharmacology, School Of Medicine, Addis Ababa University. They were kept in cages in animal house with a 12-h-natural light: 12-h-dark cycle. They fed on pellets and drank clean water *ad libitum*. Mice were allowed to adapt to the experimental room 1 h hour before experiments.

Preparation of the extract

800 g of the herbal material was air dried and coarsely powdered. 400 g of the powdered material was macerated with distilled water for 14 days with occasional shaking. The remaining 400 g was

macerated with ethanol. Both the aqueous and ethanol extracts were filtered. The aqueous extract was placed in deep freeze to solidity. The solidified aqueous extract was placed in lyophilizer machine and a gummy residue with a calculated yield of 2.5% was obtained. The ethanol extract was placed in oven for three days. After the ethanol was removed, it was solidified in freeze and kept in lyophilizer machine and powder with a yield of 3.7% was obtained. The gummy residue and the powder extracts were properly stored and finally reconstituted in distilled water to get the desired concentration for administration in to mice.

Acute toxicity study

The aqueous and ethanol extracts of *A. annua* dried leaves were studied for acute oral toxicity as per revised OECD guidelines No.423 (Organization for Economic Cooperation and Development Guidelines No. 423 (200); Revised draft guideline for testing of chemicals, Paris).

Antipyretic activity study

Antipyretic activities of both aqueous and ethanol extracts were evaluated by yeast extract induced pyrexia model in mice as described by (Naveed et al., 2012). Mice were fasted over night with water *ad libitum* before the experiments. The initial rectal temperature was measured by using digital thermometer. Then pyrexia was induced in all mice by injecting 30%w/v yeast extract powder suspension subcutaneously (10 ml/kg) for 16 h after the injection, the rectal temperature of each mouse was measured for the second time. Only mice that showed an increase in temperature of at least 0.5°C were used for the experiment. Animals were divided in to 8 groups (each containing 6 animals). Group one served as control (received equal volume of distilled water); Group two received the standard drug (aspirin 100 mg/kg); Group three received aqueous extract (100 mg/kg); Group four received aqueous extract (200 mg/kg) and the last group for aqueous extract received 300 mg/kg. The remaining three groups (6, 7 and 8) received 100, 200 and 300 mg/kg ethanol extracts respectively. Finally, the temperature for each mouse was measured at 0.5, 1, 1.5, 2, 2.5 and 3 hours after extracts administration.

Determination of LD₅₀ from the acute toxicity study

The LD₅₀ for both aqueous and ethanol extracts of leaves from *A. annua* was determined as per revised OECD guide line no 423 (limit test). A total of 20 mice (both sexes) were used. The highest dose levels (500 and 2000 mg/kg) were reasonably selected for administration in to mice. On the first day of the experiment, mice fasted overnight were given aqueous and ethanol extracts of leaves of *A. Annua* in a dose of 500 mg/kg (five mice for each extract). Then, mice were observed for 24 h for any lethality. On the next day both aqueous and ethanol extracts were administered orally in to the remaining ten mice (five mice for each extract at a dose of 2000 mg/kg). Then mice were observed for 24 hours.

Statistical analysis

All the values are expressed as mean± standard error of the mean and analyzed for ANOVA and post hoc dunnet's t-test (SPSS version 20).

RESULTS AND DISCUSSION

The results are presented in Tables 1 and 2 (change in

Table 1. Effects of oral aqueous extract of dried leaves *Artemisia annua* against yeast induced pyrexia in mice (mean± standard error of the mean) (n=6).

Group	Dose (mg/kg)	T0	TY	0.5HT	1HT	1.5HT	2HT	2.5HT	3HT
Control (Distilled water)	...	36.45± 0.11	37.18± 0.29	37.20±0.12	37.23±0.11	37.23±0.12	37.18±0.09	37.18±0.09	37.01±0.04
Standard (aspirin)	100	36.56± 0.09	37.32± 0.25	36.23± 0.10a	36.20±0.27 a	36.30± 0.14(b)	36.20± 0.28 a	35.26± 0.37b	35.50± 0.22a
Aqueous	100	36.43± 0.12	37.35± 0.28	36.71± 0.13n.s	36.81± 0.15n.s	36.91± 0.11n.s	36.78± 0.07n.s	36.66± 0.14n.s	36.61± 0.14n.s
Aqueous	200	36.58± 0.13	37.28± 0.30	36.33± 0.15a	36.13± 0.26a	36.53± 0.10b	36.05± 0.23b	35.71± 0.26n.s	35.81± 0.44n.s
Aqueous	300	36.48± 0.15	37.18± 0.29	36.12± 0.24b	36.15± 0.27a	36.41± 0.15b	36.25± 0.27a	35.60± 0.51a	35.80± 0.28n.s

0.5HT, rectal temperature after 0.5 hour of treatment; 1HT, rectal temperature after 1 hour of treatment; 1.5HT, rectal temperature after 1.5 hour of treatment; 2HT, rectal temperature after 2 hour of treatment; 3HT, rectal temperature after 3 hour of treatment; n.s,not statistically significant; a,significant (p<0.05) when compared with the corresponding value of control; b, extremely significant (P<0.005) when compared with the corresponding value of control;Equal volume (1 ml).

Table 2. Effects of oral ethanol extract of dried leaves *Artemisia annua* against yeast induced pyrexia in mice (mean± standard error of the mean) (n=6).

Group	Dose (mg/kg)	T0	TY	0.5HT	1HT	1.5HT	2HT	2.5HT	3HT
Control (distilled water)	-	36.45±0.11	37.18±0.29	37.20±0.12	37.23± 0.11	37.23± 0.12	37.18± 0.09	37.18± 0.09	37.01± 0.04
Standard (aspirin)	100	36.56±0.09	37.32±0.25	36.23±0.10 ^a	36.20± 0.27 ^a	36.30± 0.14 ^b	36.20± 0.28 ^a	35.26± 0.37 ^b	35.50± 0.22 ^a
Ethanol	100	36.56±0.14	37.43±0.26	36.36±0.18 ^a	36.08± 0.25 ^a	36.45± 0.19 ^a	36.65± 0.06n.s	36.05± 0.34n.s	36.58± 0.14n.s
Ethanol	200	36.45±0.12	37.32±0.34	35.98±0.14 ^b	36.10± 0.17 ^a	36.28± 0.11 ^b	36.16± 0.12 ^a	35.68± 0.40 ^a	35.65± 0.39 ^a
Ethanol	300	36.73±0.27	37.42±0.47	35.75±0.24 ^b	35.70± 0.17 ^b	36.01± 0.12 ^b	36.15± 0.22 ^a	35.48± 0.24 ^a	35.50± 0.13 ^a

0.5HT, rectal temperature after 0.5 hour of treatment; 1HT, rectal temperature after 1 hour of treatment; 1.5HT, rectal temperature after 1.5 hour of treatment; 2HT, rectal temperature after 2 hour of treatment; 3HT, rectal temperature after 3 hour of treatment; n.s,not statistically significant; a,significant (p<0.05) when compared with the corresponding value of control; b, extremely significant (P<0.005) when compared with the corresponding value of control;Equal volume (1 ml).

body temperature; time in hour). The ethanol extract of leaves of *A. Annua* showed a decrease against yeast induced fever at all doses employed (Table 2).

Aqueous extract (200 and 300 mg/kg) also showed a reduction in yeast induced pyrexia whereas the 100 mg/kg aqueous extract was not statistically significant for the whole period of the experiment (Table 1). This significant difference in antipyretic activities between aqueous and ethanol extracts at 100 mg/kg dose level might be due to the difference in the chemical nature of active constituents between the two extracts. Since water is polar solvent, it is expected to

isolate polar components only. Unlike water, ethanol has predominant hydrophilic and some lipophilic properties that is ethanol is capable of extracting both polar and non-polar components from *A. annua* dried leaves. These non-polar constituents of ethanol extract (100 mg/kg) might be responsible for lowering rectal temperature in mice (Titus and Kalu, 2008).

The antipyretic activities for both extracts were found to be dose dependent. The antipyretic activities for ethanol extract (at 200 and 300 mg/kg dose levels) were comparable with the antipyretic activity of aspirin (100 mg/kg) but the lowest dose level of ethanol extract was observed

to be less potent than aspirin. The aqueous extract at 200 and 300 mg/kg dose levels showed similar degree of antipyretic activity during the initial period of measurement whereas at the end of the experiment, aspirin was found to be more potent than aqueous extract at all doses employed. This time dependent antipyretic activity observed for aqueous extract might indicate that the active constituents in aqueous extract have short duration of action than aspirin.

Time dependent antipyretic activity was also observed for ethanol extract (decreased with time). This result also provides some clue regarding onset of action for both aqueous and

ethanol extracts. Both extracts at specified dose levels (except 100 mg/kg aqueous extract) exhibited antipyretic activity immediately after administration in to mice indicating that both extracts possess rapid onset of action.

Significant potency difference was observed between aqueous and ethanol extracts. The ethanol extract at all doses administered was found to be more potent than the aqueous extract with respect to the corresponding dose levels.

Regarding the duration of action secondary metabolites which are found in ethanol extract might have longer duration of action than those in aqueous extract because significant antipyretic activity was observed for ethanol extract (at high dose levels) long time after administration.

Both aqueous and ethanol extracts possess a significant antipyretic activity which is comparable to the standard antipyretic drug aspirin. Previously, around 100 active ingredients have been identified in this plant (Ghasemi et al., 2013). Among those constituents, the flavonoids were reported to be responsible for most of pharmacological effects (Shakeri et al., 2012). Like the previous reports, the antipyretic activities of this plant might be due to the presence of flavonoids and the probable mechanism for lowering temperature in yeast induced pyrexia may be by decreasing the synthesis of prostaglandins and other mediators secondary to inhibiting the enzymes responsible for prostaglandin production.

The acute oral toxicity study of both aqueous and ethanol extracts of dried leaves of *A. annua* was carried out as per OECD guide line number 423 (Organization for Economic Cooperation and Development Guidelines No. 423 (200); Revised draft guideline for testing of chemicals, Paris). At the end of the study, both extracts were found to be safe in mice when given large dose up to 2000 mg/kg by oral route. The LD₅₀ for both extracts was also determined from the acute toxicity study based on OECD guide line.

Based on the guide line, mice were initially given 500 mg/kg dose then observed for 24 h for lethality. Since both extracts were safe at 500 mg/kg, the remaining mice were provided with 2000 mg/kg aqueous and ethanol extracts and observed for 24 h. At the end of the observation, no mouse died even at 2000 mg/kg indicating that the LD₅₀ for both aqueous and ethanol extracts was greater than 2000 mg/kg.

In the present pharmacological evaluation, the aqueous and ethanol extracts of dried leaves of *A. annua* were extensively investigated for antipyretic activities against yeast extract induced pyrexia model in mice. The statistically processed result supports that both extracts possess antipyretic activities. The statistically processed result showed that both extracts of leaves from *A. annua* possess antipyretic activity against yeast extract induced pyrexia in mice. This positive result initiates the need to conduct further studies on the same plant regarding the mechanism of action at molecular level, the particular

active ingredient responsible for antipyretic activity and determination of LD₅₀ (from the current acute toxicity study, the LD₅₀ is greater than 2000 mg/kg). Spectroscopic characterization is proposed to study the constituents of both aqua and ethanol extracts of *A. annua*.

Ethical approval

Ethical approval was obtained from the ethics review board of Debreberhan University, Debreberhan, Ethiopia. The care and handling of animals was as per the internationally accepted ethical guidelines (9).

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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