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Evaluation Of The Anti-Inflammatory Pharmacological Effects Of Artemisia Annua Extracts Artemisinin In Vitro And In Vivo

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Abstract: *Artemisia annua L. is acknowledged as a traditional medicinal plant bearing potent antimalarial, antileishmanial, antibacterial, antifungal and antioxidant potential. Therefore, this study was carried out to explore the anti-inflammatory impact of A. annua both in vitro and in vivo in rabbit models. We procured fresh A. annua plant material from local nurseries and Artemisinin was extracted from its leaves. The anti-inflammatory potential of the herb was found significant (p<0.05) in terms of IC-50 against the control. For in vivo trial, 50 rabbits were procured and 5-HT were injected parenterally into their paws to generate inflammation and were subsequently treated with Artemisinin, acetylsalicylic acid, and normal saline for the control group. Rabbits were treated using three different concentrations of Artemisinin 100mg/ml, 200mg/ml and 500mg/ml in Group A, B and C, respectively, while, Group D using Acetylsalicylic acid 80mg/Kg and Group E served as control. The rabbits of Group C demonstrated the greatest reduction in inflammation in the inflamed paws of model rabbits within the first four hours of treatment and significantly (p<0.05) reduced the swelling after two and four hours by 40 and 60 per cent, respectively. Artemisinin has a thus potent anti-inflammatory effect and can be employed as commercial anti-inflammatory medicine.*

Key Words: Anti-inflammatory, Cytokines, Inflammatory mediators, Interleukines, Potent drugs

Introduction

There has been a worldwide rise in the reassessment of traditional medicinal plants, with substantial studies being conducted on numerous plant species and their therapeutic potential. Traditional herbal medicinal treatments have been recognized as complementary and alternative medications (CAM) bearing fewer adverse side effects as compared to chemicals created synthetically (Kim et al., 2015).

Artemisia annua L. (sweet-wormwood plant) is a herbaceous plant belonging to the 500-species-strong Artemisia genus and the Asteraceae family (Compositae). Interest in the chemical structure

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and biological activity of several species of Artemisia has grown since the Sesquiterpene-Lactone Artemisinin, isolated from Artemisia annua and demonstrating the efficacy of its antimalarial action, received the 2015 Nobel Prize in Medicine (Ekiert, Świątkowska, Klin, Rzepiela, & Szopa, 2021). Among these, *A. annua's* phytochemical and pharmacological profile has made it the subject of modern, professional scientific investigation. This herb is crucial to conventional Asian medicine (mainly Hindu and Chinese). And it has been documented as a medicinal plant in Asia, Europe, the Americas, and Australia (Pellicer et al., 2018). In Asian medicine, it was used to cure jaundice and bacterial dysentery, as well as a fever associated with malaria and tuberculosis. It proved beneficial in treating wounds and haemorrhoids, as well as a variety of viral and bacterial infections and autoimmune disorders (Willcox, 2009). It is also included in the WHO-published International Pharmacopoeia. *Artemisiae annuae herba* and *Artemisiae annuae folium* are therapeutic ingredients in raw form (Organization, 2006).

Leaf extracts of *Artemisia annua* have antifungal, antibacterial, and antileishmanial activities, in addition to anticancer potential. Artemisinin, the primary bioactive component of A. annua, transformed the paradigm profoundly in research and therapy of malaria and saved millions of people from this ravaging infection. Professor Youyou was honoured with the Nobel Prize for her discovery of Artemisinin in Physiology or Medicine and rigorous testing of its effectiveness against malaria (Su & Miller, 2015). Evidently, the data indicated its potential to alleviate osteoarthritis-related pain, stiffness, and complications. Several studies have demonstrated *in vitro* efficiency of Artemisinin and *A. annua* against small-cell lung cancer (Allemailem, 2022). Artemisia species including *A. annua*, have been in traditional use to treat inflammation, pain and pyrexia (Habib & Waheed, 2013) owing to their anti-inflammatory, inhibition of angiotensin-converting enzyme, cytokinin and antitumor features (Chu, Wang, Chen, & Hou, 2014), anti-inflammatory effects of prostaglandin E2 (PGE2) and nitric oxide (NO), and the expression levels of cyclooxygenas (Yun et al., 2016).

Keeping in mind these pharmacological actions of *Artemisia annua* herb, the current study was designed and carried out to evaluate the anti-inflammatory effects of *A. annua* herb using both *in vitro* and *in vivo* research trials on rabbit models.

Materials And Methods

Artemisia annua herb

Fresh *Artemisia annua* plant material was procured from local nurseries in District Dera Ismail Khan (Figure 1), and identified at the Pharmacy Department, Gomal University, Dera Ismail Khan, bearing specimen number PHGU-3546, dated 03-03-2022. The plant samples' leaves were cleaned with tap water to eliminate dust and dirt (Figure 2). The obtained plant components were then dried in the shade at 25 C and crushed using a mortar and pestle in the Laboratory facility.

Figure 1

Artemisia annua plant

Figure 2

Fresh vs dried leaves of *A. annua* herb

Artemisinin Extract from the Artemisia Annua Plant

Artemisinin extract was acquired from the *Artemisia annua* plant using the Soxhlet method after cyclohexane defatting. Four hours were spent dissolving 100 grammes of dried, powdered leaves in 300ml of cyclohexane in a beaker while stirring continually. The defatted powder after overnight drying at 40 degrees Celsius, and after 10 minutes of centrifugation at 5000 rpm, supernatant comprising cyclohexanes, soluble lipids and soluble cyclohexane were removed. Then powder was put in thimble, and Soxhlet equipment was used to extract it automatically (SoxtecTM 8000). The extraction process was continued for several hours and distilled water served as a solvent in the collecting beaker; ultimately, the volatile solvent was let evaporated and left behind the gummy residues of *A. annua* containing Artemisinin (Allemailem, 2022). Then each day of the experiment, a fresh solution of Artemisinin (1mg/ml in dimethyl sulfoxide) was prepared from the stock solution (DMSO, Merck).

Evaluation of Artemisinin Anti-inflammatory Activity in Vitro

Using the Luminol-amplified chemiluminescence (CL) method, the efficiency of *A. annua* extracts (Artemisinin) was determined *in vitro* (Helfand, Werkmeister, & Roder, 1982). The experiment was performed on 96-welled white plates (Costar-USA). A cell suspension of whole blood diluted in Hanks Balanced Salt Solution (HBSS) (Sigma, USA) was incubated with three different doses of Artemisinin in HBSS, namely 1, 10, and 100g/ml. As control wells, only HBSS-containing medium and cells were employed. The luminometer thermostat chamber was incubated at 37°C for 15 minutes. Then luminol along with the serum-opsonized zymosan was applied (25µl) to each well, excluding those containing HBSS. Artemisinin extract concentrations were measured in RLUs (Relative Light Units), and the result was expressed as an IC50 (concentration inhibiting growth by 50%). Tests were conducted in triplicate to ensure precision (Bedouhène, Moulti-Mati, Hurtado-Nedelec, Dang, & El-Benna, 2017).

Evaluation of in vivo Anti-inflammatory Effects of Artemisinin

The present study was done on local breed rabbits weighing between 1.5-2 Kg body weight and possessing both types of sexes. The rabbits were caged in uniform conditions, temperature and humidity were (25 C) and (60-65%). For this purpose, 50 rabbits were procured and classified into five equal groups (n=10) and confined in separate cages. Before the start of the trial, all rabbits were acclimatized for 02 weeks, for their adaptation to the laboratory environment and to rule out infections.

In order to evaluate the anti-inflammatory potency of Artemisinin with the control group, inflammation was produced in the planter aspect of rabbit models (paw) through injecting histamine and 5-HT, parentrally, post 02 weeks acclimatization period. Histamine and 5-HT increased vascular permeability and induced inflammation through the production of prostaglandins. Introducing histamine subplantarly stimulated the release of plasma proteins and fluid into the extracellular spaces. It enhanced lymph flow and produce oedema (Patil et al., 2019), and then rabbits were treated with Artemisinin in conjunction with a conventional anti-inflammatory drug and a control group (table 1**).**

Table 1

Treatment protocol for the experimental rabbit models.

Treatment Group	No. of Rabbit Models	Treatment P/O
Group A	10	Artemisinin 100mg/ml/Kg
Group B	10	Artemisinin 200mg/ml/Kg

Treatment Group	No. of Rabbit Models	Treatment P/O
Group C	10	Artemisinin 500mg/ml/Kg
Group D	10	Acetyl salicylic acid 80mg/Kg
Group E	10	Normal saline 1ml/Kg

Statistical Analysis

The mean and standard error were displayed for all the data (SEM). ANOVA with Post Hoc Tukey's test was implied to examine the statistically significant differences. P-values of 0.05 or below were considered statistically significant and were shown as P< 0.05*. All the analysis was performed on SPSS Version-20, software.

Ethical Approval

All research protocols, methods, euthanasia, and ethical consent were duly approved by Gomal University Ethical Board of Conduct (GUEBC), Dera Ismail Khan, Pakistan. Moreover, all selected animals were handled following study ethics.

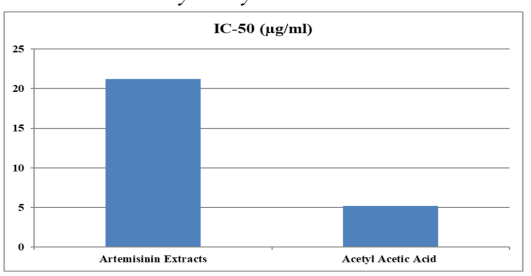
Results & Discussion

Evaluation of in vitro Artemisinin anti-inflammatory activity

Comparatively to the control group, the Artemisinin herb extracted with cyclohexane exhibited statistically significant (P< 0.05) anti-inflammatory activity using the CL technique in a 96-well plate. Comparing the half-maximal inhibitory concentration (IC-50) of Artemisinin extracts to that of the control groups revealed a statistically significant difference between the treatments. Mean+SD IC50 values for Artemisinin extracts and Acetylsalicylic acid (a standard anti-inflammatory drug), were determined to be 21.22 ± 3.98 µg/ml and 5.15 ± 1.65 µg/ml, respectively (Figure 3). A study that assessed *in vitro* effectiveness of *A. annua* extracts in comparison to diclofenac and reported an IC50 of 20g/ml for the *A. annua* extracts substantially corroborated our findings (Parameswari, Devika, & Vijayaraghavan, 2019). Similar observations were made in an executed study that reported significant anti-inflammatory effects of herbs *in vitro* (Salah-Abbès et al., 2008). Our results were also correlated with a study in which the acetone extracts of *A. annua* showed maximum anti-inflammatory potential posing inhibitory effects on COX-enzymes, nitric oxides, PGE_2 (prostaglandins) and cytokine production (Kim et al., 2015).

Figure 3

In vitro comparison of IC-50 of Artemisinin extracts and Acetylsalicylic acid



Evaluation of in Vivo Anti-inflammatory Effects of Artemisinin

Histamine and 5HT-induced paw oedema in rabbits were treated using three different doses of Artemisinin 100mg/ml, 200mg/ml and 500mg/ml in Group A, B and C, respectively, while, Group D was treated using Acetylsalicylic acid 80mg/Kg and Group E was the control group and treated with placebo (Normal saline 1ml/Kg body weight). The per cent decrease in edematous swelling was recorded 2 and 4 hours post-treatment (Figures 4 and 5). In comparison to the control group, Group C rabbits treated with Artemisinin at 500 mg/ml/kg showed the greatest reduction in inflammation in the inflamed paws of model rabbits within the first four hours of treatment and significantly (P< 0.05) reduced the swelling after hours two and four by 40 and 60 per cent, respectively (table 2). Artemisia annua (Artemisinin) extracts were found to have a potent anti-inflammatory impact in animal models by

inhibiting inflammatory mediators such as tumour necrosis factor and interleukin. Our findings corroborated these findings (Efferth & Oesch, 2021). It was also reported the same that Artemisinin bears significant anti-inflammatory potential and can be used potently against the induced as well as natural inflammatory conditions of the body. He also suggested the development of commercial anti-inflammatory drugs from Artemisinin (Shi, Li, Yang, & Hou, 2015). Another study also revealed that Artemisinin had the potency to reduce prostaglandin E_2, nitric oxide and interleukins and ultimately reduce the inflammatory conditions of the body (Jeong, Kim, & Min, 2018). Our results were in agreement with an *in vivo* trial conducted in mice, in which the anti-inflammatory effects of Artemisinin were investigated in mice and rats. The results of the study revealed that Artemisinin was directly connected to the decrease in macrophage-produced inflammatory mediators and the generation of inflammatory cytokines in inflamed tissuesm (Yun et al., 2016). Similar findings were reported in which Artemisnin was suggested as a potent herb against inflammatory conditions (Bode, Ehlting, & Häussinger, 2012).

Figure 4

Per cent decrease in the edematous swelling of rabbit paw after 02 hours.

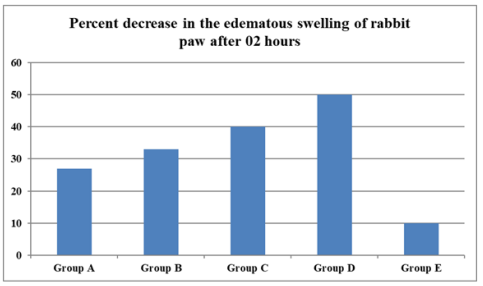


Figure 5

Decrease (%) in edematous swelling of rabbit paw after 04 hours.

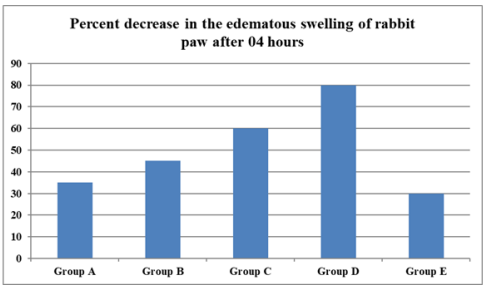


Table 2

Reduction in percentage inflammation in rabbits of test groups

Treatment Group	Treatment P/O	Per cent reduced paw swelling		P- value
		2 hours post-treatment	4 hours post-treatment	
Group A	Artemisinin 100mg/ml/Kg	27.2	35.2	
Group B	Artemisinin 200mg/ml/Kg	33.5	45.4	
Group C	Artemisinin 500mg/ml/Kg	40.7	60.6	0.000423
Group D	Acetyl salicylic acid 80mg/Kg	50.4	80.4	
Group E	Normal saline 1ml/Kg	10.9	30.3	

Statistics applied between the treatment groups
SS = 330
df = 4
MS = 82.5
F = 8.25
P = 0.000423; Statistically significant at (P<0.05)

Conclusion

Artemisinin includes potential anti-inflammatory components, can reduce inflammation in the body by reducing inflammatory mediators comprising TNF, ILs, Cytokines *etc* and can be employed as a commercial anti-inflammatory medicine in the future, according to both *in vivo* and *in vitro* tests.

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