

Anti-Arthritic and Insecticidal Property of Crude Aqueous *Camellia sinensis* (Green Tea) Infusion and Decoction: Study by Two *In vitro* Methods

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Abstract: In order to search for an effective anti-arthritic candidate for pharmaceutical from natural source, an intensive attempt was made by screening crude aqueous *Camellia sinensis* (Green tea) infusion and decoction by two different methods, protein denaturation and membrane stability method. Both extract preparations showed anti-arthritic potential; however sharply significant results were more clear in decoction extract when compared with diclofenac sodium-a standard reference drug. Five different concentrations of test as well as standard drug i.e 50µg/ml, 100 µg/ml, 200µg/ml, 400µg/ml and 800µg/ml were used in the study. Action was observed in dose dependent manner. In protein denaturation method, decoction at 800µg/ml gave 96.5% protection and standard provided 99.2% protection on the contrary; infusion at 800 µg/ml gave 81.1% protection and standard provided 99.2% protection. Similarly, in membrane stabilization test, decoction at 800 µg/ml gave 78.9% protection and standard provided 81.1% protection on the contrary; infusion at 800 µg/ml gave 64.8% protection and standard provided 81.1% protection. Moreover, as far as insecticidal activity is concerned, both aqueous infusion and decoction showed mortality against all four insects *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Trogoderma granarium* tested, however; decoction showed much promising mortality results when compared with standard drug Permethrin (0.5% w/w). Both the activities clearly reflected that green tea possessed both insecticidal as well as antiarthritic activity.

Key words: Antiarthritic • Green tea • Insecticidal • Diclofenac sodium • Permethrin

INTRODUCTION

Rheumatoid arthritis is an autoimmune disease [1] in which there is joint inflammation, synovial proliferation and destruction of articular cartilage [2]. It is characterized

by pain, swelling and stiffness [3]. RA prevalence depends upon age. According to medical science there are more than 100 different kinds of arthritides. Among them, the three most common namely; are gout, osteoarthritis (OA) and rheumatoid arthritis (RA) [4].

Rheumatoid arthritis (RA) is two to three times more common in women than in men [5]. Insecticides of botanical origin are naturally occurring insecticides [6] that are obtained from plants [7]. Insects are getting resistant day by day from conventionally employed insecticidal agent [8] so there is a dire need to explore plant based such agents [9].

Camellia sinensis L. commonly called tea is largely used since ancient times and this plant of scientific interest for its numerous therapeutic properties now-a-days, related mainly to the contact in polyphenolic compounds [10]. Only young leaves are mostly used for drinks and extracts, such products are very expensive. However potential use of plant extracts from other parts of plants is being investigated. Depending on tea manufacturing methods, tea is divided mainly into: green and the black one [11]. Green tea is generally safe, non-toxic and having no side effects after use. However, over consumption may cause in treatment infection, human disease because to associated to lower side effects [12]. Therefore, the current study was undertaken to evaluate in vitro anti-arthritis as well as insecticidal activity of *Camellia sinensis* (Green tea) water based decoction and infusion.

MATERIALS AND METHODS

Purchase of Green Tea: The Green tea is easily available in the local market everywhere in Pakistan. After survey, a good quality green tea was purchased from the retail market in Karachi-Pakistan was brought to the laboratory in the Department of Microbiology, Federal Urdu University of Arts, Science and Technology (FUUAST)-Karachi-Pakistan for making extracts and exploiting their potential as anti-arthritis agent.

Preparation of Infusion: The aqueous infusion was prepared in 5 % concentration i.e. by taking 5g of *Camellia sinensis* (Green tea) in 100 ml distilled water and left for about 48 hours with occasional shaking at room temperature and later filtered by strainer to procure clear infusion and then also pass through 0.22µm filter and store the infusion into small eppendorf vials in freezer for further work [13].

Preparation of Decoction: The aqueous decoction was prepared by boiling in 5 % concentration i.e. 5g *Camellia sinensis* (Green tea) in 100 ml distilled water in a flask for 15-20 minutes. The flask was removed from heat and

allowed to cool. The content of flask was filtered to obtain clear decoction and then also pass through 0.22µm filter and store the infusion into small eppendorf vials in freezer till use [13].

Assessment of In-vitro-Arthritic Activity

Inhibition of Albumin Denaturation: The method of [14] was followed. The 5 ml of reaction mixture was comprised of 0.2 ml of eggs albumin (from hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentration of extracts so that final concentrations become 50, 100, 200, 400, 800 microgram/ml. Similar volume of double distilled water served as control. Then the mixture was incubated at 37°C in BOD incubator for about 15 mins and then heated at 70°C for 5 mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) at the final concentration of (50,100,200,400,800 µgram/ml) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ of inhibition} = 100 \times [V_t/V_c - 1]$$

where as;

V_t = Absorbance of test sample,

V_c = Absorbance of control.

Membrane Stabilization Property: For this test research study of [23] was followed:

Preparation of Red Blood Cells (RBCs) Suspension:

Fresh whole human blood (10ml) was collected and transferred to the heparin zed centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat Induced Hemolysis: The 2ml reaction mixture is consisted of 1 ml of test extract and 1 ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm

for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicate. Percent membrane stabilization activity was calculated by the formula mentioned below:

$$\% \text{ of inhibition} = 100 \times [V_t / V_c^{-1}]$$

where as;

V_t = Absorbance of test sample,

V_c = Absorbance of control.

Insecticidal Activity: The four insect species *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Trogoderma granarium* were graciously provided by a zoologist exclusively for this study. Different dilutions of the green tea both aqueous infusion and decoction extracts were prepared from the stock solution. Each extract was then used for insecticidal activity against four insects. The filter papers were cut according to the size of petri plate (9 cm or 90 mm) and were placed in the plates. The test sample was poured over the filter paper with the help of micropipette. The plates were left for 24 hrs at 25°C to evaporate the solvent properly. The number of the living insects was counted [6]. Standard drug Permethrin (0.5% w/w) was used as positive control and blank paper as negative control. The mortality percentage was calculated with the help of following formula:

$$\text{Mortality (\%)} = 100 - \frac{\text{Number of insects alive in test}}{\text{Number of insects alive in control}} \times 100$$

RESULTS AND DISCUSSION:

Literature are now full of scientific documentation today regarding medicinal plants and they have potential to cure various human diseases [15]. Thus, this superb feature further encourage to manufacture a pharmaceutical product procured from medicinal plants as they are safe and dependable as compared to synthetic drugs that are not only costly but also have adverse effects [16]. Similarly, for the sake of clinical management of arthritis, anti-inflammatory Drugs (NSAID's), disease modifying anti-rheumatoid drugs (DMARD's) and corticosteroids are meant to alleviate the patient's pain as well as joint inflammation, decline the loss of function and diminish the joint damage progression. However; it has been observed that these drugs are rarely perfectly effective rather some of them also exhibit serious side effects on the human body [17]. Naturally isolated anti-arthritis agents function

by suppressing the different types of inflammatory mediators involved in inflammation process [18]. In this study, two different methods were adopted to evaluate the antiarthritic property of *Camellia sinensis* (Green tea) infusion and decoction extracts with this belief that the treatment of extracts release various bioactive substances that could play a role in generating a particular pharmacological activity. Similarly, protein denaturation method and membrane stabilization test was done for this purpose. Protein denaturation is well documented method for this analysis and membrane stabilization also reflects the effect of extracts on cellular membrane like red blood cell. Five different concentrations of test as well as standard drug i.e. 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml and then 800 µg/ml were tested. Both methods indicated the anti-arthritis property in both decoction and infusion green tea extracts. However; the results were very promising in case of decoction extract than infusion. Dose dependent activity was also clearly noted. All results were compared with diclofenac sodium (standard drug). In protein denaturation method, decoction at 800 µg/ml gave 96.5% protection and standard provided 99.2% protection on the contrary; infusion at 800 µg/ml gave 81.1% protection and standard provided 99.2% protection. Similarly, in membrane stabilization test, decoction at 800 µg/ml gave 78.9% protection and standard provided 81.1% protection on the contrary; infusion at 800 µg/ml gave 64.8% protection and standard provided 81.1% protection. The findings clearly indicate that decoction preparation of extracts were better than infusion one and quite close to standard drug. As suggested by one of the studies that perhaps the mechanism behind the denaturation could be certain drastic changes in the hydrogen, electrostatic, hydrophobic and disulphide bonding [19]. In our study, both aqueous infusion and decoction extracts of green tea showed mortality against all four insects *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica* and *Trogoderma granarium* tested, however; decoction showed much promising mortality results in terms of percentages as mentioned in Table 5. According to one of the estimations, nearly one thousand species of insects have been associated with store products globally and among them a vast majority belong to Coleoptera (60%) and Lepidoptera (8 - 9%) [20]. The green tea contains 30 to 42% polyphenols on the dry weight basis and a cup of green tea contains about 300 to 400 mg of polyphenols. The public awareness of the health giving properties of tea has increased in the recent past. The majority of beneficial effects of tea have been attributed to primary

Table 1: *In vitro* anti-arthritis activity of crude *Camellia sinensis* (Green tea) aqueous decoction extract by Protein denaturation method:

Test Sample	Conc.(µg/ml)	% Protection
<i>Camellia sinensis</i> (Green tea) aqueous decoction extract	50	36.5%
	100	46.8%
	200	68.8%
	400	80.9%
	800	96.5%
Effect of diclofenac sodium	50	48.2%
	100	55.7%
	200	70.2%
	400	88.5%
	800	99.2%

Table 2: *In vitro* anti-arthritis activity of crude *Camellia sinensis* (Green tea) aqueous infusion extract by Protein denaturation method:

Test Sample	Conc.(µg/ml)	% Protection
<i>Camellia sinensis</i> (Green tea) aqueous infusion extract	50	25.7%
	100	42.3%
	200	62.7%
	400	79.8%
	800	81.1%
Effect of diclofenac sodium	50	48.2%
	100	55.7%
	200	70.2%
	400	88.5%
	800	99.2%

Table 3: *In vitro* anti-arthritis activity of crude *Camellia sinensis* (Green tea) aqueous decoction extract by Membrane Stabilization method:

Test Sample	Conc. (µg/ml)	% Protection
<i>Camellia sinensis</i> (Green tea) aqueous decoction	50	20.2%
	100	39.9%
	200	54.3%
	400	62.2%
	800	78.9%
Effect of diclofenac sodium	50	22.2%
	100	42.7%
	200	59.2%
	400	64.5%
	800	80.2%

Table 4: *In vitro* anti-arthritis activity of crude *Camellia sinensis* (Green tea) aqueous infusion extract by Membrane Stabilization method:

Test Sample	Conc. (µg/ml)	% Protection
<i>Camellia sinensis</i> (Green tea) aqueous infusion extract	50	12.5%
	100	28.9%
	200	41.1%
	400	53.5%
	800	64.8%
Effect of diclofenac sodium	50	22.2%
	100	42.7%
	200	59.2%
	400	64.5%
	800	80.2%

Table 5: Insecticidal activity of *Camellia sinensis* (Green tea) aqueous infusion and decoction extracts:

Test Sample	Insects tested	% Mortality
<i>Camellia sinensis</i> (Green tea) aqueous decoction extract	<i>Tribolium castaneum</i>	30%
	<i>Sitophilus oryzae</i>	80%
	<i>Rhyzoperth dominica</i>	50%
	<i>Trogoderma granarium</i>	30%
<i>Camellia sinensis</i> (Green tea) aqueous infusion extract	<i>Tribolium castaneum</i>	50%
	<i>Sitophilus oryzae</i>	100%
	<i>Rhyzoperth dominica</i>	80%
	<i>Trogoderma granarium</i>	20%

polyphenolic constituents of green tea. Strong antioxidant potential of these polyphenols is thought to mediate most of the beneficial effects of tea [21]. Tea has been shown to have a wide range of beneficial physiological and pharmacological effects. Among these are slowing the catabolism of catecholamines, strengthening capillaries (vitamin P effect), exerting an anti-inflammatory effect by enhancing the effectiveness of ascorbic acid [22, 23] acting as an antioxidant, inhibiting angiotensin-converting enzyme, having a hypo cholesterol emicaction as well as inhibiting the growth of implanted malignant cells [24]. Some other researches also pointed out that certain phytoconstitueints present in diverse and rich composition of plant extracts are responsible for anti-arthritis activity includes alkaloids, glycosides, tannins, phenolics, anthocyanins, sterols, triterpenoids *etc* [25].

CONCLUSIONS

From the above results of the study, it can be concluded although both decoction and infusion extract of *Camellia sinensis* (Green tea) possessed insecticidal as well as antiarthritis property. However; one should try to further figure out decoction extract more as having much better activity in quest of active candidate or chemical molecule that is mainly responsible for this activity via detailed experimentation.

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