THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN-2319-2119

RESEARCH ARTICLE

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POSSIBLE ANTI-INFLAMMATORY EFFECT OF AQUEOUS GREEN TEA EXTRACT AGAINST AN ACUTE INFLAMMATION INDUCED BY EGG WHITE IN RATS

ABSTRACT

Background

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs [Dolor (pain), Calor (heat), Rubor (redness), Tumor (swelling) and Functio laesa (loss of function].

Green tea (Camellia sinensis) belongs to the family theacaceae is the most widely consumed beverage in the world as dietary supplement that represents approximately 20% of world tea consumption. Many reports have demonstrated the usefulness of the extracts of green tea in many diseases and conditions like cancer prevention, hyperlipidemia, diabetes mellitus, obesity and in microbial diseases; but others were demonstrated a contrary results concerning their usefulness in diseases and conditions.

Objective:

The present study was carried out to assess the possible anti-inflammatory effect of aqueous green tea extract (AGTE) using egg white-induced edema in rats (acute model of inflammation).

Materials and methods:

The anti-inflammatory effect of AGTE was evaluated in acute inflammation model using 18 Sprague Dawley rats and divided into three groups including distilled water1 ml/kg orally, diclofenac 30 mg/kg IP and AGTE 1.5 % orally. Thirty minutes post treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin into the sub plantar surface of the right hind paw and mean increase in paw edema was measured 30 min, 60 min and 90 min after induction of inflammation using a digitalized vernier caliper.

Suppression of paw inflammation by either diclofenac or AGTE and the percentage of inhibition of paw edema were assessed.

Results:

The data obtained from this study reported that significant decrease ($P<0.05$) in paw edema were seen in rats treated with diclofenac (30mg/kg) IP prior to egg albumin (group II) after 30 min and 60 min, respectively compared to group I animals . A significant increase in paw edema ($P<0.05$) were observed in the group II animals compared to group I after 90 min from induction of acute inflammation by egg albumin. The percent inhibition of paw edema provoked by IP injection of diclofenac prior to egg albumin (group II) was 3.9%, 3.6% and after 30 and 60min, respectively; while there was no inhibition of paw thickness observed after 90 min (20.6%).

Concerning the effect of orally-administered AGTE (1.5g %) to rats prior to egg albumin (group III), the results of this study were demonstrated that the extract produced significant increase in paw edema after 30 min, 60 min and 90 min compared to either group I and group II animals.

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The percent of inhibition of paw edema provoked by AGTE given orally to animals prior to egg albumin were not observed but rather there were an increase in paw thickness (2.77%, 3.4% and 32.2%), after 30, 60 and 90 min, respectively.

Conclusion:
In conclusion, AGTE orally-administered to rats did not show anti-inflammatory activity at different times selected in this study.

KEY WORDS: Acute inflammation, egg white, aqueous green tea extract, rats

INTRODUCTION

Acute inflammation is usually of sudden onset and short duration following the injury of tissues. The damage may be purely physical, or it may involve the activation of an immune response [1]. Three main processes occur [2]:

- Increased blood flow due to dilation of blood vessels (arterioles) supplying the region
- Increased permeability of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces
- Migration of neutrophils (and perhaps a few macrophages) out of the venules and into interstitial spaces.

Green tea (Camellia sinensis) belongs to the family theacaceae, is the most widely consumed beverage in the world as dietary supplement that represents approximately 20% of world tea consumption. Many reports have demonstrated the usefulness of green tea and its major constituents on human health where it has been consumed in many countries for a very long time and today interest is growing because scientific reports indicate that tea could bring benefits for health and may help prevent chronic diseases due to anti-oxidant properties of its poly phenol constituents [3-6]; while other reports demonstrated the contrary, where, the tea catechins, including (-)-epigallocatechin-3-gallate (EGCG), are unstable under cell culture conditions and undergo oxidative polymerization with co-generation of H_2O_2 [7].

Mandel et al in 2004 demonstrated that, EGCG possesses both antioxidant and pro-oxidant activities because of its unique ability to auto-oxidation and acts as a hydrogen donor [8].

The chemical composition of green tea varies with climate, season, horticultural practices and position of the leaf on the harvested shoot. The major components of interest are the polyphenols. The major polyphenols in green tea are flavonoids. The four major flavonoids in green tea are the Catechins Epicatechin (EC), Epigallocatechin (EGC), Epicatechin Gallate (ECG) and Epigallocatechin Gallate (EGCG). Epigallocatechin gallate is viewed as the most significant active component. The leaf bud and first leaves are richest in EGCG. The usual concentration of total polyphenols in dried green tea leaves is about 8 to 12%. Other compound of interest in dried green tea leaves include terpenes, gallic acid, quercetin, kaempferol, myricetin, caffeic acid and chlorogenic acid [9].

This study was designed to assess whether or not AGTE possess an anti-inflammatory effect by attenuating egg white-induced edema in rats (acute model of inflammation).

Materials and Methods:
The aqueous extract of green tea was made by soaking for 10 minutes 1.5gm of green tea leaves in 100 ml of distilled water whose temperature is 90 ^{o}C.

Eighteen Sprague Dawley rats weighing 170-220gm of both sexes were obtained from the Animal House of the College of Pharmacy/Baghdad University. They were maintained on normal conditions of temperature, humidity and light/dark cycle.

The animals were fed standard rodent pellet diet and have free access to water except when starvation needed during the investigation.

They were allocated into three groups:

Group I- Six rats were received distilled water1 ml/kg using oral needle.
Group II- Six rats were treated with intraperitoneal injection of 30 mg/kg diclofenac.

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Group III- six rats were received 1.5g % AGTE orally by feeding bottle.

Thirty minutes post treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin into the sub plantar surface of the right hind paw. The volume of edema produced was measured in millimeters before and 30 min, 60 min and 90 min after induction of inflammation using a digitalized vernier caliper [10].

The ability of the either diclofenac or AGTE to suppress paw inflammation was expressed as a percentage of inhibition of paw edema according to the following equation [11]:

Percentage of inhibition (%) = 100X [(1- (x/y)] Where,
X= mean increase in paw volume, thickness or weight of treated rats of
Either (group II or III).
Y= mean increase in paw volume, thickness or weight of group I rats.

STATISTICAL ANALYSIS:
Results were expressed as the mean ± S.E.M. Analysis of data was carried out using one-way analysis of variance (ANOVA) followed by Student's t-test. Differences in mean were considered to be significant when $p< 0.05$.

RESULTS:

The data obtained from this study revealed that significant ($P<0.05$) decrease in paw edema was seen in rats treated with IP injection of diclofenac (30mg/kg) (group II), prior to egg albumin after either30 min or 60 min, respectively compared to control (group I) animals; while significant increase in paw edema ($P<0.05$) were observed in the group of animals treated with IP injection of diclofenac (30mg/kg) (group II) prior to egg albumin compared to group I after 90 min from induction of acute inflammation by egg albumin as shown in table 1 and figure 1.

The percent of inhibition in paw edema provoked by IP injection of diclofenac prior to egg albumin were 3.9% and 3.6% after 30 and 60 min, respectively; while diclofenac injection produced no inhibition in paw edema 90 min after induction of acute inflammation by egg albumin but rather an increase in paw thickness (20.6%). Table 1.

Concerning the effect of orally-administered AGTE (1.5 %) to rats prior to egg albumin (group III), the results of this study were demonstrated that, the extract produced significant increase ($P<0.05$) in paw edema after 30 min (2.77%), 60 min (3.4%) and 90 min (32.2%) compared to group I and group II of animals. Table 1 and figure 1.

Treatment Group	Mean increase in paw thickness (mm)			% of inhibition		
	30 min	60 min	90 min	30 min	60 min	90 min
Group I N=6	5.77±0.06 a	5.54±0.05 a	5.06±0.07 a	-	-	-
Group II N=6	5.54±0.05 b	5.34±0.06 b	6.37±0.05 c	3.9%	3.6%	20.6% **
Group III N=6	5.93±0.05 d	5.73±0.06 d	6.69±0.06 e	2.77% **	3.4% **	32.2% **

Table 1- Effect of prior treatment with aqueous green tea extract (group III) against egg albumin-induced acute inflammation compared to group I and group II animals.

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- Data were expressed as mean ± SEM.
- Values with non identical subscripts (a, b, c and d) among different groups are considered significantly different (P<0.05).
- Group I= Distilled water (1ml/kg) 30 min prior to (0.1ml) egg albumin.
- Group II= Diclofenac (30mg/kg) 30 min prior to (0.1ml) egg albumin.
- Group III=AGTE (1.5 %) 30 min Prior to (0.1ml) egg albumin.
- * *, increase in percent of paw edema.
- N= number of animals.

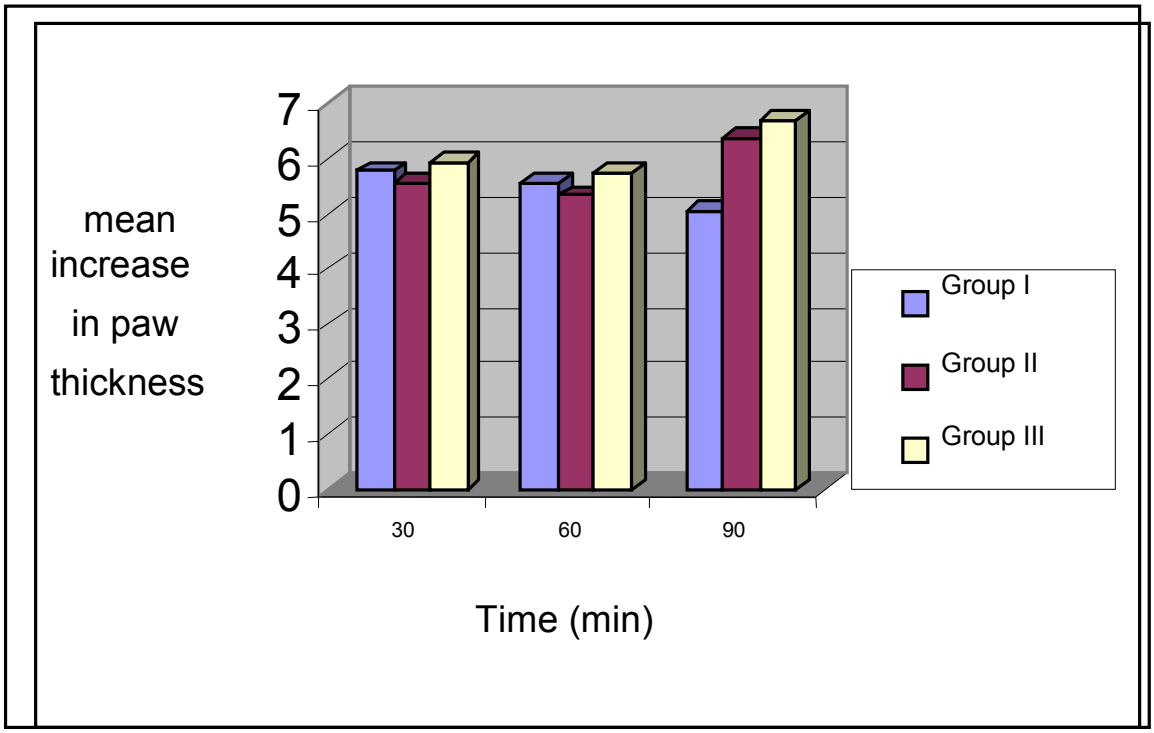

Figure 1- Mean increase in paw thickness of rats treated with either diclofenac (group II) or AGTE (group III) prior to egg white-induced acute inflammation.

DISCUSSION:

The inflammatory response is a physiological characteristic of vascularized tissues [12]. Exudation, which is a consequence of increase vascular permeability, is considered as a major feature of acute inflammation [13]. Egg albumin-induced paw edema in rats is an in vivo model of inflammation used to screen agents for anti-inflammatory effect [14]. The characteristic swelling of the paw is due to edema formation. Inhibition of increased vascular permeability and hence the attendant edema modulate the extent and magnitude of the inflammatory reaction. The paw edema that was induced by injection of egg albumin is peaked after 30min and then progressively declined with time.

Many chemical mediators like histamine, serotonin (5-HT), kinins and prostanoids mediate an acute inflammation induced by phlogistic agents including egg albumin [15]. Inflammation occurs through three distinct phases: an initial phase mediated by histamine and 5-HT

(up to 2 hours); an intermediate phase involving the activity of bradykinin and a third (late) phase with prostanoid synthesis by cyclooxigenase (COX) enzyme [16].

The anti-inflammatory activity of AGTE was evaluated by egg albumin-induced paw edema utilizing vernier caliper method and the results were shown in table 1 and figure 1.

The concentration (1.5 %) of AGTE was evaluated for the aim of finding the possible anti-inflammatory of AGTE; the results indicated a non-significant anti-inflammatory activity of this extract in the intended concentration utilized compared to standard anti-inflammatory agent (diclofenac) used in this respect. Although the intended concentration of AGTE (1.5 %) was selected based on the study performed by Hesham A El-Beshbishy [17] in 2005, where the extract has the capacity to scavenge free radical and can protect against oxidative stress induced by tamoxifen intoxication due to its antioxidant properties.

It has been demonstrated that the green tea anti-inflammatory effects may be possibly mediated through their anti-oxidant properties [18] ; while, Chan et al in 1995, observed that green tea also inhibited production in peritoneal exudates (macrophage cells) [19]. Similarly, lin and lin in 1997 [20] , showed that green tea inhibited lipopolysaccharide –stimulated nitric oxide production and inducible nitric oxide synthesis gene expression in peritoneal macrophages by decreasing nuclear kappa-B (NF-kB) factor.

Furthermore, it has been demonstrated that, green tea extracts may be helpful in treating chronic inflammatory states [21]; and the mechanism of tea's anti-inflammatory effects involves the regulation of gene transcription where, tea affects a number of important molecular targets including TNF-α [22], interleukin 2 (IL-2) [23], signal transducer and activator of transcription-1α (STAT-1α) [24], and affects DNA and RNA directly [25, 26].

Conflicting reports obtained from results of other investigators who demonstrated that EGCG, the major catechin of green tea is cytotoxic and higher consumption of green tea can exert acute cytotoxicity in liver cells [27].

Furthermore, intra-peritoneal injection of EGCG (200 and 400 mg/kg), resulted in the formation of EGCG-O-quinone, which then reacts with the sulfhydryl group on cysteine and likely other cysteine-containing molecules such as glutathione [28].

In conclusion, AGTE orally-administered to rats did not show anti-inflammatory activity at different times (30min, 60 min and 90 min) selected in this study to counteract the acute inflammation induced by egg white albumin. This may be due to inability of the AGTE concentration (1.5%) utilized in this study for attenuating acute inflammation induced by egg albumin . Thus, further studies are needed to evaluate its anti-inflammatory effects utilizing various concentrations of green tea aqueous extract.

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