Original research

Comparing the analgesic effect of hydroalcoholic extract of green tea and sodium salicylate in the formalin test in rats

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Abstract: ***Background:*** Green tea is believed to have some health promoting benefits. Among them anti-inflammatory properties are proposed to cause an analgesic effect. Few studies also have given it a central role to inhibit pain. The aim of this experiment was to compare the anti-nociception effects of green tea extract and sodium salicylate on neurogenic and inflammatory pain induced by formalin injection.

Methods: The experiment was done on 31 male Wistar rats. They were randomly divided into four groups: normal saline treated (control), sodium salicylate treated and the other two groups were treated with different dosages of green tea extracts (200 and 300 mg/kg) intraperitoneally. All subjects underwent the standard formalin test 30 minutes after the treatments and were followed for 60 minutes.

Results: In the early and the intermediate phases of the study, treatment with 200 mg/kg (p=0.01 for early phase; p = 0.02 for intermediate phase) and 300 mg/kg (p = 0.02 for intermediate phases) of green tea extract demonstrated lower levels of pain in comparison with sodium salicylate but not with the control group (p > 0.05). In the late phase of the test, the control group had significantly higher nociception scores than the other groups (P = 0.018 for green tea 200 mg/kg, P = 0.038 green tea 300 mg/kg, and p = 0.016 for sodium salicylate). The green tea treated groups and those treated with sodium salicylate showed similar nociception scores in the late phase of formalin test (p > 0.05).

Conclusion: Green tea extract administration seems to be effective against painful feelings at least in an inflammatory condition. This effect of green tea is comparable with sodium Salicylate. This finding is such a confirmatory data according to other investigations and might be clues to utilize proven safer analgesics.

Keyword: Green Tea; Analgesic Effects; Formalin Test; Sodium Salicylate

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1. Introduction

NNowadays considerable attentions are paid to the health promoting benefits of green tea. Prevention of cancer and cardiovascular diseases, anti-inflammatory effect, anti-arthritic, anti-bacterial, anti-angiogenic, anti-oxidative, antiviral, neuroprotective, and cholesterol-lowering effects are the most important beneficial effects of green tea consumption (1-3).

Pain and its procrustean burden on health systems is one of the most frequent and disabling symptoms that occurs in most medical conditions and push patients and physicians to seek for different analgesics (4-7). Undesirable side effect of current analgesics, despite their approved efficacy in some condition, has led researchers to continue their efforts to find an effective and safe analgesic drug. Among the mentioned benefits of green tea, some may be therapeutic (8).

Arzi et al. demonstrated that green tea extract (200 mg/kg) produces dose-related antinociception in chemical pain model and is proposed that one of its possible mechanisms involves opioid pathways (9). A further Indian study done in 2012 supposed anti-inflammatory and analgesic properties for green tea and recommend it as a health drinks (10). Green tea mouthwash is also considered as an appropriate and safe choice to control post-operative pain of molar surgery (11). Leong et al

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showed that in an osteoarthritis animal model, green tea epigallocatechin-gallate (EGCG) significantly slowed osteoarthritis symptom's progression and exerts a palliative effect. Raposo et al. evaluated the efficacy of EGCG on alleviation of hyperalgesia in streptozotocin diabetic. The results suggest therapeutic potential of EGCG in the treatment of diabetic hyperalgesia through the attenuation of oxidative stress (12). Choi et al suggest that intrathecal EGCG could produce an anti-allodynic effect against spinal nerve ligation-induced neuropathic pain. This effect is mediated by blockade of neural nitric oxide synthase protein expression and inhibition of the pronociceptive effects of nitric oxide (13).

Alongside the numerous studies on the properties of green tea and mechanisms of its action, the review of literature revealed a lack of enough attention to its antinociceptive properties. No study compares the analgesic effect of green with the common antinociceptive drug. Therefore, the current study was performed to address if there are comparable anti-nociceptive effects between green tea extract and common proven drug such as sodium salicylate. Sodium salicylate is a non-steroidal anti-inflammatory drug which along it's anti-inflammatory and antipyretic effects frequently used as a pain relieving drug.

2. Method

2.1. Study design

This experimental study was conducted at the Physiology Department of Iran University of Medical Sciences, Tehran, Iran in accordance with the rules established for experimental and animal researches by the university. Ethical approval was provided by the local Ethics and Research Committee. The researchers adhered to the principles of using laboratory animals as suggested in the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) over the course of the study.

2.2. Animals and Procedures

A total of 31 male Wistar rats (Pasteur's institute, Tehran, Iran) weighting 200-250 grams were randomized into four groups: group one (n=7) received 0.5 ml normal-saline, group two (n=8) receive 300 mg/kg sodium salicylate (Sigma-Aldrich, Germany) dissolved in normal saline, group three (n=8) received 200 mg/kg green tea extract and group four (n=8) received 300 mg/kg green tea extract as trials. All injections (except for that of formalin test) were intraperitoneally in a volume of 0.5 ml applied by one ml syringe 30 minutes before formalin injection. Rats supplied with normal special rat diet and were housed in an air-conditioned place with temperature of 22-24 degrees of Celsius and humidity of about 30-40% and a 12 h light/dark cycle.

2.3. Preparation of green tea extract

Iranian green tea was purchased from the market (Refah Co. Tehran, Iran). For preparation of hydroalcoholic extract, green tea was powdered and 300 g of the powder was macerated by 1500 ml of ethanol 70% (v/v) for 72 hours. The extract was then shaken, filtered and the solvent was removed in a vacuum evaporator to obtain semi-solid extract and then it was placed in an oven in 60°C for 72 hours (9).

2.4. Formalin test

A standardized classical formalin test (14) was applied for all four experimental groups 30 minutes after injections. The formalin test was carried out in a 30 × 30 × 30 cm clear plastic chamber with a mirror placed under the mesh floor to allow an unobstructed view of the hind paws. Fifty µl of formalin (2.5 %) was injected under the skin of plantar surface of the right hind paw. The rats were scored immediately after injection. Observations were continued for the next 60 minutes to record data for each subject and an independent pain score was signed up for each minute. Altogether, the mean score of the first five minutes was considered as the nociception score for the early phase, the mean score of minute five to minute 15 was considered as the nociception score for the intermediate phase and finally the mean score of the latest 45 minutes was labelled as the nociception score of the late phase.

2.5. Statistical analysis

Quantitative variables were described by means and

Table 1: Pain score (mean ± standard deviation) of studied animals in different phases of formalin test

Groups	Early phase	Intermediate phase	Late phase
Control	1.88 ± 0.32	1.00 ± 0.66	2.00 ± 0.01
Sodium salicylate	2.11 ± 0.15	1.03 ± 0.38	1.55 ± 0.31
Green tea 200 mg/kg	1.47 ± 0.26	0.22 ± 0.12	1.33 ± 0.54
Green tea 300 mg/kg	1.40 ± 0.68	0.23 ± 0.21	1.15 ± 0.57
P value	0.012	0.002	0.01

standard deviations (SD). According to the normal distributions of data, analysis of variances and post-hoc assessments were applied. Since there were paucities of homogeneity of variances among data, Brown-Forsythe and Games-Howell tests were used. All processes were performed by SPSS v.16 software (Chicago, IL, USA). A *p* value less than 0.05 considered to be statistically significant.

3. Result

All animals ended the procedure but the primary assessments showed some outlier recordings. Investigators decided to drop the outliers because of their impacts on the tests' assumptions. They were one of the nociception scores of the intermediate phase in 200 mg/kg of green tea treated group and one of the nociception scores of the late phase in control group.

The studied animals demonstrated different patterns of behavior against the formalin test. The mean values and standard deviation of perceived pain of studied rats have been presented in table 1. In the early phase of the test, animals treated with 200 mg/kg of green tea had lower nociception scores compered to sodium salicylate treated rats ($p = 0.01$) (Figure 1). In the intermediate phase both groups which received green tea extract showed statistically significant ($p = 0.02$) differences compared with animals injected with sodium salicylate (Figure 2). No significant difference observed between animals injected with normal saline with other animals ($p > 0.05$).

The analysis also revealed that green tea treated animals ($p = 0.018$ for dose of 200 mg/kg; $p = 0.038$ for dose of 300 mg/kg) and the subjects treated with sodium salicylate ($p = 0.016$) had a statistically significant difference with control rats injected with normal saline in the late phase of formalin test (Figure 3).

4. Discussion

The results revealed that the administration of green tea extract, as well as sodium salicylate, have decreased the level of pain perception among rats in the late phase of formalin test which is an indication of the inflammatory pain. In the early and the intermediate phases, the animals which received green tea injection had experienced lower pain than those treated with sodium salicylate, but not significantly lower than controls. Also the pain score of animals injected with sodium salicylate was comparable to the pain score of animals injected with normal saline which indicates that injection of sodium salicylate does not produce any pain reliving effect in the early and intermediate phase of formalin test. In the intermediate phase of formalin test green tea was more effective than sodium salicylate in reducing pain

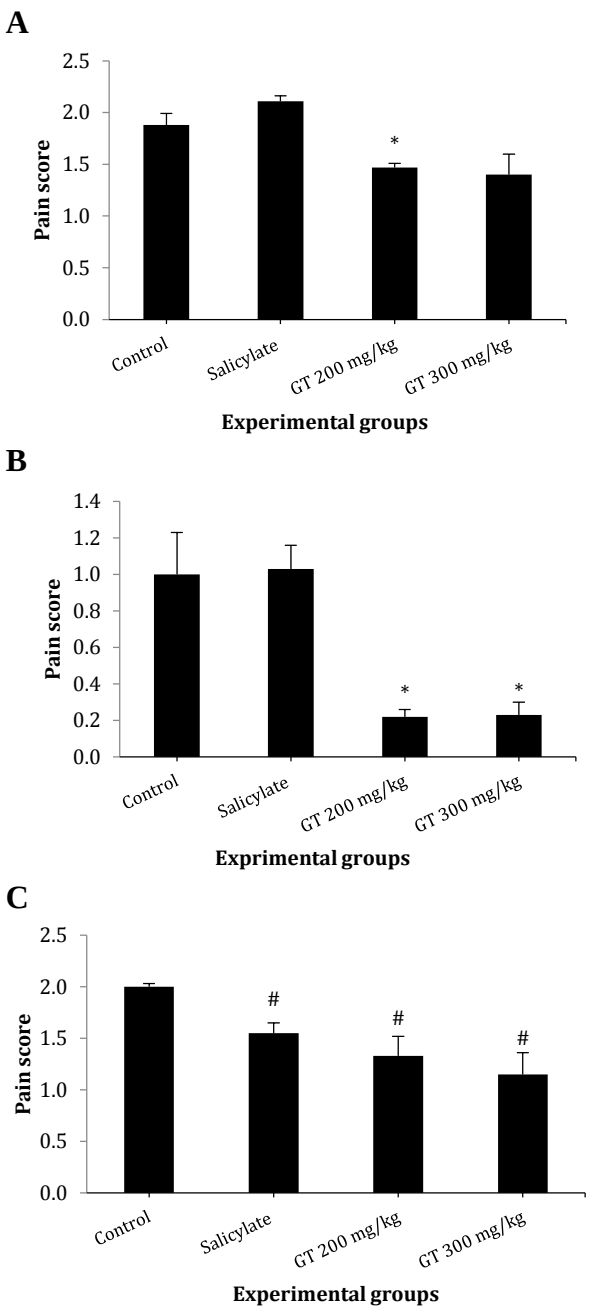

Figure 1: Effect of green tea (GT; n=8) extract, sodium salicylate (300mg/kg; n=8) and normal saline (control; n=7) injection on the early (A), intermediate (B), and late (C) phases of formalin test. *, $p < 0.05$ vs. sodium salicylate group; #, $p < 0.05$ vs control group.

which may represent some facts of interest.

The late phase of formalin test is thought to develop painful feelings because of the local inflammation (inflammatory pain). Thus a wide range of anti-inflammatory agents, including steroidal and non-steroidal drugs, have been shown to be effective against this inflammatory painful feeling (15), while centrally acting analgesics inhibit both early and late phases (16).

Green tea have anti-nociception effects mostly due to its anti-inflammatory properties (17, 18). These have been

mainly contributed to its major polyphenols such as EGCG (17). Consistent with the findings of some other studies (9, 11, 18-20), present experiment confirms the anti-nociception effect of green tea extract which is even more effective than non-steroidal anti-inflammatory drugs (NSAIDs). Hence, considering the doubtless importance and frequency of clinical use of analgesics, it would be a point of attention that many different side effects of common NSAIDs (21) may be avoided by providing the clinical utilization of green tea extract.

Trevisan G et al. have demonstrated that the Gallic acid, a polyphenolic compound commonly found in green tea acts as an analgesic in either an inflammatory pain model or a neuropathic pain model. It antagonizes the transient receptor potential ankyrin 1 (TRPA1) that has been identified as a relevant target for the development of novel analgesics (22). Arzi et al. also have concluded that the Iranian green tea extract might suppress the rats' pain in both early and late phases of formalin test and have suggested a central role for it beside its anti-inflammatory actions (9). Against Arzis' experiment, we have not seen any significant differences between animals injected with saline and animals received different doses of green tea in the early phase of formalin test. We don't have any explanation for these differences in the results. As mentioned, we have also found some improvements in pain reliving effect in the early (neurogenic phase of formalin test) and the intermediate phases of the test which was not statistically significant. The rats that had been treated with green tea showed a less mean nociception score than those treated with NSAID. This may be in accordance with central effects of tea or the antagonist action of green tea ingredients which act more effective than NSAIDs.

5. Limitation

The conflicting issue about the present study is the non-significant difference between green tea extract treated animals and controls in the early and intermediate phase of formalin test despite the significant lower pains in NSAID-receiving subjects. Limitations in sample size, dropping the outliers, and observational errors might explain such a conflict.

6. Conclusion

In conclusion, green tea extracts sound to play an important role in pain reduction, especially in comparison to wide-range-using NSAIDs. So according to the side effects of NSAIDs the authors recommend more specified clinical studies to provide more accurate data about probable replacement of green tea with NSAIDs.

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8. Conflict of interest

None.

9. Funding Source

None.

10. Author contribution

All authors passed four criteria for authorship contribution based on recommendations of the International Committee of Medical Journal Editor.

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