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Study of Analgesic Effect of Aqueous and Methanolic Extracts of Dry Powder of Zingiber Officinale in Wistar Rats.

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ABSTRACT

Background

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are commonly used for pain relief but often lead to adverse effects including gastrointestinal, renal and hepatic complications. Ginger widely used in traditional medicine, exhibits potential analgesic, anti-inflammatory and hepatoprotective properties. However, its efficacy in modern analgesic applications is not well-documented. Objective

To investigate the analgesic effects of aqueous and methanolic extracts of dry powder of Zingiber officinale (Z. officinale) in an experimental rat model. To compare analgesic activity of both extracts with that of standard drug.

Materials and methods

The study was conducted after animal ethics committee approval. Animals were divided into six groups. Control group received 0.2ml NS IP while test groups received aspirin (150mg/kg) , Aqueous extract of dry powder of Z. officinale (100 and 200mg/kg) , Methanolic extract of dry powder of Z. officinale (100 and 200 mg/kg). Rat tail flick method was used to investigate analgesic activity of Ginger.

Results

Both aqueous and methanolic extracts showed significant analgesic activity when compared with control.

Conclusion

Aqueous and methanolic extracts of dry powder of Z. officinale extracts show promising analgesic effects in rats. Ginger extracts may offer a safer alternative or adjuvant to NSAIDs, particularly for chronic pain management.

INTRODUCTION

Drugs like Non-Steroidal Anti Inflammatory drugs have shown to be non specific analgesics drugs and can be used for acute or chronic pain [1]. But they are having adverse effects like peptic ulcer, nephrotoxicity, hepatic damage. Whereas Ginger is digestive and hepato protective.

Ginger, Botanical name Zingiber officinale (Z.Officinale) (Family: Zingiberacae) is a plant distributed worldwide [2]. It has been used as a spice, flavouring agent in food, it is also used by traditional Indian and Chinese medicine for more than 25 centuries. It is grown widely in India. It is an underground root or rhizome, which is used in traditional medicine for antiemetic effect, improving blood circulation, stimulating digestion etc. [3]. Various animal studies, pilot studies in human, clinical trials suggest the analgesic [4,5], anti inflammatory [6 ,7], hepatoprotective [8], hypouricaemic [9], antidiabetic [10], anticancer [8] effects of either crude extract or pure gingerol. Use of Ginger extract for acute and chronic analgesic purpose is not established in modern medicine and not documented in textbooks. Only in traditional medicine of different countries many uses of crude Ginger extract are mentioned. They are not yet proven. Different experimental studies have shown its analgesic effects in animals but few studies report no activity. If analgesic effect of Ginger extract is proved it will be step forward towards formation of new safe drug which will be useful for patients suffering from pain and inflammation.

MATERIALS AND METHODS

ANIMALS

Adult wistar rats of either sex weighing 150-200 grams, aged 12-16 weeks were procured from the Central Animal House, Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad. Total 36 animals were used for experiments in six different groups having six animals in each groups.

DRUGS

Dry powder of Z. officinale was obtained from local market. Aqueous and methanolic extracts of powder were prepared by using Soxhlet Apparatus . All dried extracts were dissolved in 0.9 % normal saline for injection intraperitoneally. All the extracts and standard drug were used in experiment as mentioned in Table 1.

Doses were selected as per previous research of ginger extract [11]. Acute toxicity study was done. No mortality was seen upto 2000mg/kg methanolic extract of Z. officinale. All experiments were conducted after approval of institutional animal ethics committee of Krishna Institute of Medical Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad. Experiments were conducted as per CPCSEA guidelines.

EVALUATION OF ANALGESIC ACTIVITY

Radiant heat analgesiometer was used for evaluating analgesic activity . The animals were placed in a restrainer which had an aperture for the tail. A timer was started simultaneously, and the time taken by the animal to withdraw (flick) its tail was taken as the endpoint of the test. A cut off time of 20 second was followed to avoid tissue damage. Loss of tail flick response by rat was taken as end point and positive response. Reaction time in seconds was used as the unit for measurement of pain and an increase in reaction time was indicative of analgesia. Rats received the respective drug (saline/Aspirin/extract) and were tested for tail flick response zero minute , 30 minutes , 60 minutes , 120 minutes. After injection [12].

TABLE 1 : EXPERIMENTAL GROUPS AND DOSES

GROUP	Name of Drug /Extract	Short form used	Doses
Group I	0.9% Normal Saline	NS	0.2ml i.p.
Group II	Aspirin (Standard Control)	ASP	150mg/kg i.p
Group III	Aqueous extract of dry powder of Z. officinale	ADZ	100mg/kg i.p
Group IV	Aqueous extract of dry powder of Z. officinale	ADZ	200mg/kg i.p
Group V	Methanolic extract of dry powder of Z.	MDZ	100mg/kg i.p
GROUP	Name of Drug /Extract	Short form used	Doses
	officinale		
Group VI	Methanolic extract of dry powder of Z. officinale	MDZ	200mg/kg i.p

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for post-hoc analysis. $P < 0.05$ considered statistically significant, P: Probability Factor. All the statistical methods were carried out through the SPSS software.

OBSERVATIONS AND RESULTS

Effect of ASP 150 , ADZ 100 , ADZ 200 , MDZ 100 , MDZ 200 , treatments on Rat tail flick response compared with control

Time after treatment	**Time of Tail flick**						**ANOVA**
	Control	**ASP 150**	**ADZ 100**	**ADZ 200**	**MDZ 100**	**MDZ 200**	**P Value**
0 min	8.33±0.816	8.83±0.408	9.1±0.408	8.83±0.752	9±0.632	9.16±0.983	0.37
30 min	10.33±2.066	19.33±0.816	14.16±1.722	14.16±1.722	12.83±1.169	15.16±0.752	<0.0001
60 min	11±1.549	19.66±0.516	16±1.673	15.16±0.752	14±0.632	15.5±1.049	<0.0001
120 min	8±1.722	16.33±2.658	9.83±1.722	10.83±1.941	12.66±0.516	14.66±0.516	<0.0001

DISCUSSION

In the present study, analgesic effects of different doses of methanolic and aqueous extracts dry powder of plant Z. officinale were tested in experimental model of pain. Extracts were prepared with help of Soxhlet apparatus. For evaluation of analgesic activity, we selected Rat Tail Flick method. Total number of rats to be used for this study was decided after reviewing CPCSEA guidelines and previous studies, to have minimum number of animals for particular animal experiment, to get statistically significant data.

Pain is protective mechanism. Tissue damage can lead to pain [2]. It is unpleasant sensory and emotional experience which has tissue damage association. Currently used analgesic agents like NSAIDS, corticosteroids, despite of having high efficacy, lead to different adverse events generating major problems during their clinical use such as peptic ulcer, nephrotoxicity, hepatic damage etc [6]. Ginger, Botanical name Z. officinale is a plant distributed worldwide [7]. It is an underground root or rhizome, which is used in traditional medicine for antiemetic effect, improving blood circulation, stimulating digestion etc [8].

Various animal studies, pilot studies in human, clinical trials suggest the analgesic [9,10], anti-inflammatory [11,13], hepatoprotective [14], hypouricemic [15], antidiabetic [16], anticancer [14] effects of either crude extract or pure gingerol.There are minimum 115 constituents identified from fresh and dried Ginger. Among them Gingerols are the major ones. They are abundant in fresh Ginger and less in dry Ginger whereas Shogaols are abundant in dry than in fresh Ginger. They are referred to major Gingerol dehydration products [17]. There are different possible mechanisms for analgesic effect of Ginger. Ginger has capacity to inhibit prostaglandin and leukotriene biosynthesis [18]. It is suggested that inhibition of Arachidonate 5 - lipoxygenase also help in anti-inflammatory activity of Ginger [19]. Inhibition of cyclooxygenase 2 (COX 2)[20] and proinflammatory cytokines by Gingerol [21] also claimed to be responsible for anti-inflammatory activity of Ginger. Same mechanisms might be responsible for analgesic effect of ginger. Considering these results, it was proposed to evaluate analgesic activity of extracts of ginger.

AQUEOUS EXTRACT OF DRY POWDER (ADZ 100 AND ADZ 200)

We found that aqueous extract of dry powder 100 mg/kg and 200 mg/kg (ADZ 100 and ADZ 200) has shown statistically significant difference in time taken for rat tail flick response ($p < 0.05$) when compared to control at 30 minutes and 60 minutes whereas no statistically significant difference in time taken for rat tail flick response was seen when compared with control at 120 minutes ($p > 0.05$). When compared with aspirin, both of them have shown statistically significant difference in time taken for rat tail flick response ($p < 0.05$) at all time intervals.

ADZ 100 and 200 have shown analgesic activity when compared with the control group whereas their analgesic activity was not comparable with aspirin at 30 minutes and 60 minutes interval. We can also suggest that the weak analgesic activity of ADZ 100 and ADZ 200 is short lasting i.e. only for 1 hour.

METHANOLIC EXTRACT OF DRY POWDER (MDZ 100 AND MDZ 200)

We found that methanolic extract of dry powder at dose of 100 mg/kg and 200 mg/kg (MDZ 100 and MDZ 200) showed statistically significant difference in time taken for rat tail flick response ($p < 0.05$) when compared to control at 30 minutes, 60 minutes and 120 minutes. When compared with aspirin, both of them have shown statistically significant difference in time taken for rat tail flick response ($p < 0.05$) at all time intervals. So, we can say that MDZ 100 and MDZ 200 have shown analgesic activity when compared with control whereas their analgesic activity is less than that of aspirin. When the dose of methanolic extract of dry powder was increased from 100 mg/kg to 200 mg/kg, we observed increase in the time taken for rat tail flick response. So, we can say that MDZ is showing dose dependent analgesic activity.

Reza Sepahvand et al. [22] have done one study to evaluate the analgesic activity of dry rhizome of ginger in which they have selected radiant heat induced tail-flick test in rats. They have selected three different doses of ginger extracts of 200 mg/kg, 400 mg/kg and 600 mg/kg. These drugs were administered intraperitoneally. They observed decrease in nociception in the extract treated group at all 3 doses. Their results have shown that the duration of action of the extracts was increasing with the subsequent increase in dose i.e., 200 mg/kg has shown analgesic effect up to 60 minutes; 400 mg/kg has shown analgesic effect up to 90 minutes and 600 mg/kg of extracts has shown analgesic activity up to 120 minutes. They have also observed that co- administration of ginger extracts with morphine have produced significant analgesic effect when compared with control and morphine alone. Our study has shown analgesic activity as compared to control but it is not comparable to aspirin. We also have not done co-administration of extracts with our standard drug aspirin. We might get potentiation of analgesic effect of aspirin by ginger extract. Thus, we plan that in future analgesic effects of coadministration of ginger extract with aspirin or diclofenac will be done.

Another study done by Raji Y et al. [23] have evaluated analgesic activity of the rhizome extract of Z. officinale. They have selected acetic acid induced writhing in mice. They have selected two doses of 50 mg/kg & 100 mg/kg of ginger extract which was administered intraperitoneally. Their results have shown decrease in number of acetic acid induced writhing at both the doses of 50 mg/kg & 100 mg/kg when compared to the control group. They have

observed that extract at high dose of 100 mg/kg have shown comparable analgesic activity with the aspirin group (standard drug).

One more study was done by John. A. O. Ojewole et.al. [24] to evaluate the analgesic activity of ethanolic extract of dried rhizome of Z. officinale. They have selected different doses of 50, 100, 200, 400 & 800 mg/kg. They administered these extracts intraperitoneally. They have selected two different models for evaluating analgesic activity i.e hot-plate test method & acetic acid test methods in mice. They observed that Z. officinale dried rhizome ethanol extract have shown significant analgesic effect in both the models when compared with the control and standard drug group. Their results have shown that these extracts have shown dose-dependent analgesic activity, which we also observe in case of MDZ.

Thus, in conclusion, our results show that extracts of Z. officinale have analgesic property. Previously we also have concluded anti inflammatory activity of the same [25,26]. Considering problems of gastric ulceration and hepatotoxicity associated with higher doses of aspirin and other NSAIDs, it will be justifiable to combine ginger extract with aspirin (NSAIDs) for chronic treatments of senile arthritis, low back ache, rheumatoid arthritis etc. Ginger due to its analgesic activity might potentiate aspirin action, therefore we may be able to reduce dose of aspirin. Ginger due to its digestant and hepatoprotective properties might reduce chances of aspirin induced adverse effects. Therefore, we propose that further clinical studies should be done to prove benefit of coadministration of ginger extract and NSAIDs in chronic pain conditions.

LIMITATIONS

It is very primitive in the method and parameters as well which are used to evaluate the analgesic activity. These activities were carried out in a single species i.e. wistar rats. In our setup, we were unable to quantify active principle present in the extracts . The efficacy of the extracts as an analgesic agent can be further evaluated using other related models in different species and also in human studies.

CONCLUSION

From the findings of present experimental study, we conclude that Extracts of dry powder of Z. officinale have analgesic properties. 100 mg/kg and 200 mg/kg doses of aqueous and methanolic extracts of dry powder of plant Z. officinale have significant analgesic activity at all time intervals except ADZ at 120 minute interval as compared to control in Rat tail flick model but it is not comparable to aspirin at all time interval. AFZ and MDZ have shown dose dependent analgesic activity. Present study predicts that use of ginger may have beneficial analgesic effect in various pain conditions. As this study proves analgesic effect of ginger extracts, it will be a step forward towards formation of new safe drug which will be useful for patients suffering from pain considering the adverse effects of conventional analgesic drugs such as NSAIDs and Corticosteroids. Considering digestant and hepatoprotective activities of ginger extracts, it may be a good adjuvant to aspirin or other NSAIDs therapies for chronic inflammatory pain conditions like arthritis. Although further studies need to be done in various other analgesic models along with human studies to strengthen the results and prove their efficacy on long term administration as potential analgesic agent in routine clinical practice.

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