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EFFECT OF TEMPERATURE ON SOME PHARMACOLOGICAL PROPERTIES OF ETHANOLIC EXTRACT OF *ZINGIBER OFFICINALE*

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ABSTRACT

Zingiber Officinale (Ginger) is one of the most common plants used for food and for medicinal purposes. There is scanty data on the possible effect of temperature on the efficacy and/ or toxicity of the ethanolic extract of ginger. The present study was to evaluate the effect of temperature on the anti-inflammatory, analgesic, antibacterial and antioxidant properties of ginger. The extracts did not show significant differences ($P > 0.05$) in both their anti-inflammatory and analgesic activity at the temperatures of 26°C, 50°C and 100°C. However, at 100°C, ginger had no antibacterial activity against *Escherichia coli* compared with the inhibitory activity at 26°C and 50°C. Ginger extracted at 26°C demonstrated significant ($P < 0.05$) antioxidant effect while ginger extracted at 100°C had no antioxidant activity compared to control ($P > 0.05$). This study has demonstrated that temperature affects the antibacterial and antioxidant properties of *Zingiber Officinale* but not the anti-inflammatory activity. This temperature dependent decrease in the antioxidant and antibacterial activities of ginger may be due to the degradation of the thermally labile gingerols present in *Zingiber officinale* to shogaols.

KEYWORDS: *Zingiber officinale*, anti-inflammatory, analgesic, antioxidant, Temperature.

INTRODUCTION

Zingiber officinale, commonly known as ginger, is an indigenous plant found in the tropics of Asia, India and southern China. The rhizomes have a powerful smell and are widely used as spice and drug. Ginger has been reported to be effective in treatment of inflammation, pain, bacterial infections and conditions resulting from oxidative stress although the exact

mechanisms of action is poorly understood. Its free radical scavenging property and antioxidant properties have been demonstrated.[1] Previous studies have demonstrated the antibacterial property of ginger against both gram negative and gram positive bacteria as well as anti-inflammatory property.[2-5]

Increase in temperature during boiling or heating of some medicinal plants has been shown to have both negative and positive effects on their medicinal properties depending on the plant and the duration of exposure to an elevated temperature. It has been found that boiling of onions for more than twenty minutes causes onions to lose anti-platelet aggregatory property and instead promote platelet pro-aggregation.[6] In addition, boiling for thirty minutes has been demonstrated to cause a significant loss of some of nutrients in beniseed whereas the antioxidant property of its aqueous extract was potentiated.[7] Likewise, boiling has been demonstrated to improve the radical scavenging activity of pumpkin when compared to raw pumpkin.[8] However, information on the effect of temperature on pharmacological properties of *Zingiber officinale* is limited and has been shown clearly. The aim of present study therefore is to evaluate the effect of temperature on the anti-inflammatory, analgesic, antibacterial and antioxidant properties of *Zingiber officinale*.

MATERIALS AND METHODS

Collection and identification of the *Zingiber officinale* Rhizome

Ginger rhizome was obtained from Farin Gada market, Jos, Plateau State, Nigeria. Stem, leaves and rhizome were identified and authenticated by plant taxonomist, Mr. Simon Iliya, at the Department of Plant Science, University of Jos, Nigeria. The voucher number of the plant specimen was UJH00334. The rhizomes were washed and air dried. The dried ginger was then ground using pestle and mortar to obtain a fine powder. The powder was kept in airtight bottles until it was required.

Extraction of *Zingiber officinale*

Samples of 204 g of ginger were weighed in three different glass containers and mixed with 70% ethanol. The samples were extracted at temperatures of 26°C, 50°C and 100°C respectively for three days in a water bath. The extracts were then filtered, air dried, collected and packaged in plastic containers. They were kept in a refrigerator until required.

Ethical Clearance

Ethical clearance to use the experimental animals (albino rats) was obtained from the Ethical

Committee of the Department of Pharmacology and Toxicology, University of Jos, Plateau.

Experimental Animals

Experiments were performed on adult albino rats (100-200 g) obtained from the animal house, University of Jos. They were kept in rat cages and maintained with food and water *ad libitum* until the time of use.

Acute Toxicity Test

Acute toxicity test was conducted on albino rats to determine median lethal dose (LD_{50}). Nine healthy albino rats were used in phase I of the study. The animals were subdivided into three groups each containing three rats and 10, 100 and 1000 mg/ kg of the ginger extracts were administered to each of the groups. The albino rats were then observed for behavioral changes and mortality over a period of 24 hours. The phase II of the experiment involved three albino rats and each one was given either 1600, 2900 or 5000 mg/ kg dose of the ginger extracts.[9] The LD_{50} was calculated using the formula:

$LD_{50} = \sqrt{(D_o \times D_{100})}$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that gave mortality

The LD_{50} was determined for all the extracts obtained at different temperatures.

Evaluation of Effect of Temperature on the Anti-inflammatory Property of *Zingiber officinale* Extracts in Albino rats

Fresh egg white albumin was used to induce edema on the rat hind paws.[10] Extracts of ginger were tested separately depending on the temperature at which they were extracted (26°C, 50°C and 100°C). Twenty five adult albino rats of either sex weighing (100-200 g) were used to test the anti-inflammatory activity of each extract and the rats were divided into 5 groups. Different doses of the ginger extracts (175 mg/ kg, 250 mg/ kg and 500 mg/ kg) in normal saline were prepared.

The doses of the ginger extracts (175 mg/ kg, 250 mg/ kg and 500 mg/ kg) in normal saline were administered intraperitoneally to rats in groups I, II and III. Groups IV and V rats received normal saline (5 mL/ kg) as a control and diclofenac sodium (10 mg/ kg) as a reference drug. After 30 minutes, hind paw edema was induced on the rats by the injection of 0.1mL of egg white albumin into the sub-plantar surface of the right hind paw of the rats.

The diameter of the thickness of the hind paws were measured using digital vernier caliper every 30 minutes after egg white albumin injection.[11] The readings were taken up to the 180th minute.

Evaluation of Effect of Temperature on the Analgesic Property of the *Zingiber officinale* Extracts in Albino Rats

Hot plate model was used to evaluate central analgesic activity of various extracts of ginger. Male and female albino rats weighing (100-200 g) were put into three treatment groups (I, II and III) according to the extraction condition (26°C, 50°C and 100°C). Group IV was used as control and were given normal saline (5 mL/ kg) orally and group V as a reference group which were injected Pentazocine (30 mg/ kg) intraperitoneally. The three treatment groups: I, II and III received the three ginger extracts separately through oral administration at doses of 150 mg/ kg, 300 mg/ kg and 600 mg/ kg. The hot plate was maintained at 55 ± 0.5°C and the animals were placed on it. The time taken for the rats to either lick the paws or jump were recorded at 0, 30, 60 and 90 minutes after the administration of the treatments.[12]

Evaluation of Effect of Temperature on Antimicrobial Property of the *Zingiber officinale* Extracts

Four strains of bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae and Staphylococcus aureus* were used to conduct this study. The bacteria were put in a nutrient agar and incubated at 37°C overnight to be reenergized before using them for the antimicrobial sensitivity test.

Antimicrobial assay using agar well method

Mueller Hinton agar was used as a medium for bacterial growth. The inoculum was prepared by mixing 20 mL of Muller Hinton agar (for each plate) with 10^7 CFU of test bacterial microorganism cultures. The inoculum was spread evenly on the surface of each plate. The wells were made by punching the agar inoculum using a sterile cork-borer (5 mm diameter). The ginger extracts were dispensed into the wells at different concentrations of 100 mg/ mL, 200 mg/ mL and 400 mg/ mL. The agar plates were then left at room temperature for 30 minutes. The plates were then incubated at 37°C for 24 hours before zones of inhibition were observed.[13]

Evaluation of Effect of Temperature on Antioxidant Property of the *Zingiber officinale* Extracts

The antioxidant property test of extracts of ginger was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Various concentrations of the ginger extracts were prepared using 95% methanol as a solvent. The concentrations prepared were; 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95 and 0.98 µg/ mL. DPPH solution (4 mL of 50 µM) in methanol was mixed with 2 mL of each concentration of the prepared extracts. This was done in triplicate. The mixtures were vortexed for about 10 seconds to make a homogeneous mixture. The test tubes containing the test mixtures were left in a dark room for 30 minutes. Ultraviolet-visible Spectrophotometer was used to measure absorbance at 515 nm. Lower absorbance indicated higher antioxidant effect. Blank solution was prepared by adding 2 mL of 95% methanol in 4 mL of 50 µM 2, 2- diphenyl-1- picrylhydrazyl solution (in methanol), which served as a control. The reference known antioxidant used for the experiment was ascorbic acid in the concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195 µg/ mL.[14-15]
The percentage inhibition was calculated:
% inhibition = (Abs control – Abs sample) / Abs control x 100 %

STATISTICAL ANALYSIS

Data were expressed as Mean ± SEM and analyzed using two way analysis of variance (ANOVA) with Tukey- Kramer Multiple Comparison tests. Microsoft Excel 2013 and GraphPad Instat version 3.10, 32 bit for windows were the softwares used for data analyses. Differences between groups were considered significant for $P < 0.05$.

RESULTS

Acute toxicity test

There was no mortality in the animals treated in phase I of the study. However, mortality was observed in some animals during phase II. The values of the LD_{50} were determined to be 2154. 07 mg/ kg for ginger extracted at 26°C and 1264. 91mg/ kg for ginger extracted at 50°C. The ginger extracted at 100°C did not give any mortality therefore the LD_{50} was greater than 5000 mg/kg.

Effect of Temperature on the Anti-inflammatory Property of *Zingiber officinale*

All the extracts showed significant ($P < 0.05$) anti-inflammatory activity compared to the control but no significant differences in the anti-inflammatory effect ($P > 0.05$) was observed between the extracts of different temperatures (Table 1). The 500 mg/ kg and 250 mg/ kg

doses demonstrated significant ($P< 0.05$) anti-inflammatory effect compared to the lowest dose administered (175 mg/ kg) for the ginger extracted at 26°C and 50°C.

Table 1: Anti-inflammatory Effect of Ginger Extracts in Albino Rats.

Extracts / drug	dose (mg/kg)	Egg white 0 min	induced 30min	Edema 60min	(diameter 90min	in mm) 120min	150min	180min
Normal saline		5.19±0.13	5.83±0.11	6.44±0.2	6.92±0.23	6.33±0.17	6.34±0.19	6.18±0.21
Ginger at 26 °C	175	4.81±0.07*	7.13±0.15*	7.44±0.15*	6.66±0.15	6.39±0.09	6.18±0.15	6.1±0.19
	250	4.43± 0.06*	6.04±0.46	6.33±0.47	5.71±0.47*	5.29±0.5*	4.93±0.48*	4.72±0.39*
	500	4.34±0.28*	5.87±0.51	5.89±0.33	5.73±0.28*	5.38±0.35*	4.73±0.26*	4.67±0.23*
Ginger at 50 °C	175	5.55±0.21*	5.75±0.29	6.34±0.34	7.52±0.52	6.52±0.29	6.23±0.29	5.87±0.36
	250	5.21±0.9	5.44±0.18*	5.88±0.38	6.95±0.44	5.94±0.32	5.82±0.28*	5.37±0.21*
	500	4.78±0.14*	5.6 ±0.20	6.02 ±0.3	6.33 ±0.20*	5.62± 0.15*	5.29 ±0.14*	5.03 ±0.24*
Ginger at 100 °C	175	4.95±0.17	5.62±0.14	6.14±0.13	5.91±0.33*	6.14±0.15	5.80±0.16*	5.74±0.13*
	250	5.13±0.28	5.91±0.23	6.55±0.24	6.08±0.23*	6.13±0.18	5.89±0.13*	5.70±0.12*
	500	4.80±0.22	6.39±0.31	6.72±0.36	6.21±0.27*	5.86±0.25	5.55±0.20*	5.11±0.28*
Diclofenac	10	5.18±0.07	5.40± 0.2*	5.66±0.28*	5.63± 0.21*	5.48±0.24*	5.40±0.22*	5.29±0.22*

Data presented as Mean ± SEM. *Statistical difference ($P< 0.05$) between treated and control groups, n=5.

Effect of Temperature on the Analgesic Property of *Zingiber officinale*

There was no significant ($P> 0.05$) analgesic activity demonstrated by the extracts when compared to control as shown in Table 2. However pentazocine (30 mg/ kg) showed significant ($P< 0.05$) analgesic effect compared with the control and the ginger extracts.

Table 2: Analgesic Effect of Ginger Extracts in Albino Rats.

		Time to lick the paw/ to jump in seconds			
Drug/extracts	**Dose (mg/ kg)**	**0 min**	**30 min**	**60 min**	**90 min**
Normal saline		8.76±1.06	15.04 ± 2.82	8.90±1.75	9.04±1.02
Ginger at 26 °C	150	6.78±1.47	7.12 ± 0.69	11.82±2.05	11.14±1.52
	300	9.36±1.08	8.28 ± 1.73	9.86 ±1.23	8.80±1.75
	600	8.10±1.34	8.44 ± 1.24	10.80±3.09	4.80±0.75
Ginger 50 °C	150	8.44±1.68	11.72 ± 1.92	16.20±6.10	9.80±3.63
	300	9.98±2.18	10.76 ± 4.89	11.54±2.49	12.62±2.25
	600	10.62±1.34	10.52±1.20	12.94±2.80	12.10±2.46
Ginger at 100 °C	150	13.42±3.67	15.36± 4.08	10.12±1.90	7.76±1.30
	300	10.00± 2.13	12.00±2.44	11.82±1.24	12.32±1.65
	600	8.84±1.95	10.88±1.75	12.06±1.62	6.92±1.02
Pentazocine	30	9.70±0.69*	24.10±7.24*	18.08±3.30*	17.08±1.51*

Data presented as Mean ± SEM, n=5.

Effect of Temperature on the Antibacterial Property of *Zingiber officinale*

The extracts showed significant ($P< 0.05$) antibacterial effect against all the test micro-organisms compared to the control except for the ginger extracted at 100°C which did not

show antibacterial activity against *Escherichia coli*. Gentamycin demonstrated significant antibacterial affect ($P< 0.001$) against all the microorganisms compared to the ginger extracts (Table 3).

Table 3: Antibacterial Effect of Ginger Extracts.

		Diameter of zones of inhibition in mm			
Extracts/drug	**Dose**	***P. auroginosa***	***S. pneumoniae***	***S. aureus***	***E. coli***
Distilled water		0	0	0	0
Ginger at 26 °C	100 mg/ mL	13±1.00	11±0.58	13±1.00	10±0.00
	200 mg/ mL	15±1.16	11±0.58	14±0.00	11±0.58
	400 mg/ mL	17±2.00	17±1.00	18±2.08	13±1.00
Ginger at 50 °C	100 mg/ mL	22±0.58	11±1.00	11±0.58	11±0.00
	200 mg/ mL	14±1.53	12±1.16	12±1.53	12±1.16
	400 mg/ mL	22±1.53	13±1.53	17±1.53	13±0.58
Ginger at 100 °C	100 mg/ mL	11±1.00	11±0.58	13±1.53	0
	200 mg/ mL	12±1.16	11±1.00	14±1.53	0
	400mg/ mL	14±1.16	12±1.16	15±1.55	0
Gentamycin	40 µg/ mL	25±1.53	20±1.16	19±1.53	19±1.53

Data presented as Mean ± SEM, n=3.

Effect of Temperature on the Antioxidant Property of *Zingiber officinale*

Ginger extracted at 26 °C showed significant ($P< 0.05$) antioxidant effect compared to the other extracts. The ginger extracted at 100 °C did not show significant ($P> 0.05$) free radical scavenging activity on 2, 2- diphenyl-1- picrylhydrazyl compared to control (Table 4). The ascorbic acid demonstrated significant ($P< 0.05$) antioxidant effect compared to all the extracts except for the highest dose of ginger, 500 µg /mL, which produced a greater inhibition on the 2, 2- diphenyl-1- picrylhydrazyl than the highest dose of ascorbic acid, 100 µg/ mL (Table 5).

Table 4: *In Vitro* Antioxidant Effect of Ginger extracts.

	% inhibition of DPPH by ginger extracts		
Concentration in µg/mL	**Ginger at 26 °C**	**Ginger 50 °C**	**Ginger at 100 °C**
500	87.7	79.4	-6.1
250	85.9	78.4	-9
125	83.4	65.7	-19.5
62.5	62.7	51.4	-10.3
31.25	53.1	41.5	-5.6
15.62	47.2	36	-44.4
7.8125	38.4	32.8	-55.2
3.91	32.2	30	-65.7
1.95	30.4	27.7	-67.8
0.98	29.1	25.2	-89.3

n = 10

Table 5: *In vitro* Free Radical Scavenging Effect of Ascorbic Acid on 2, 2- diphenyl-picrylhydrazyl.

Conc. µg/Ml	% inhibition
100	83.3
50	81.7
25	81.3
12.5	78.1
6.25	75.1
3.12	73.2
1.56	71.5
0.78	65.6
0.39	56.8
0.19	53.3

n=10

DISCUSSION

Effect of Temperature on the Anti-Inflammatory Property of *Zingiber officinale*

Medicinal plants have been used to relieve inflammatory conditions over a long period of time. Unlike modern conventional drugs that target specific biosynthetic pathways in the host, herbal medicines contain many active compounds that work in synergism.[16] *Zingiber officinale* has been shown to possess good anti-inflammatory action against acute inflammatory reactions in animal models.[10] The anti-inflammatory property of *Zingiber officinale* has been attributed to the presence of gingerols and shogaols in the rhizome. It has been reported that shogaols and gingerols act by inhibiting the synthesis of prostaglandins and leukotrienes by suppressing the action of prostaglandin synthetase or 5- lipoxygenase enzymes.[17]

In this study, the effect of extraction at various temperatures on the anti-inflammatory property of ginger was evaluated by inducing swelling, using phlogistic agent (egg yolk albumin), on the hind paws of albino rats. Edema produced on the hind paws of rats is thought to be due to the release of pro-inflammatory mediators from the site of injury. These pro-inflammatory mediators include serotonin, histamine and bradykinin.[18] The anti-inflammatory action exhibited by ginger has been suggested to be due to its inhibitory activity on the release of these inflammatory mediators.[10] It has also been reported that 6-shagaol possess anti-inflammatory property through the inhibition of cyclooxygenase enzyme in the arachidonic acid pathway thereby reducing production of prostaglandins.[19]

Endogeneous and exogeneous free oxygen radicals have also been implicated in the inflammatory processes and development of inflammatory disorders.[20] *Zingiber officinale* is also known to contain flavonoids which are known for their anti-inflammatory property. Catechin and quercetin are flavonoids in ginger which have been reported to possess free radical scavenging properties.[21] Therefore radical scavenging ability of the flavonoids and other polyphenolic compounds to reactive oxygen species promote the anti-inflammatory property of ginger.

From the study, the extracts demonstrated anti-inflammatory effect against egg yolk albumin induced inflammation. The results showed that there was no temperature dependent effect on the anti- inflammatory property of ethanolic extract of ginger ($P > 0.05$). This implies that heat up to 100°C does not have significant detrimental effect on the anti-inflammatory property of ethanolic extract of ginger. Though it has been shown that heat above 70°C readily degrades gingerols to shogaols (Purnomo *et al*., 2010), the present study demonstrated that the decrease in the gingerol as a result of degradation to shogaol does not affect its anti-inflammatory property up to 100°C. The anti-inflammatory property of *Zingiber officinale* at 100°C could be attributed to the presence of shogaols. However, it was found that heat potentiates the anti-inflammatory activity of *Zingiber officinale*, the present study has not found any significant difference in the anti-inflammatory property of ginger extracted at 26°C, 50°C and 100°C.[22]

Effect of Temperature on Antibacterial Property of *Zingiber officinale*

The antimicrobial activity of *Zingiber officinale* was demonstrated in the present study to be temperature dependent particularly against *Escherichia coli* with complete loss of activity at 100°C. It has been reported that this activity is due to the presence of sequesterpenes especially Zingiberine.[23] Sesquiterpenes are volatile compounds which decrease in activity with increase in temperature.[24] This volatilization process may contribute to the loss of antibacterial property of *Zingiber officinale* extracted at 100°C. Moreover, it has been demonstrated that chemical and physical changes in phytochemicals occur when medicinal plants are exposed to heat.[25] This could have contributed to the loss of antibacterial activity of ginger extracted at 100°C.

Effect of Temperature on the Antioxidant Property of *Zingiber officinale*

Zingiber officinale is rich in phytochemicals that possess antioxidant property.[26-27] The major phytochemicals in *Zingiber officinale* are polyphenolic compounds which include

gingerols, shogaols, Zingerone and paradol.[17] Gingerols, shogaols and paradol have been reported to possess antioxidant property.[28-29] However, the high antioxidant activity in *Zingiber officinale* is due to the active principal, gingerol.[30] Other polyphenolic compounds in *Zingiber officinale* which have demonstrated antioxidant property are flavonoids some of which are rutin, quercetin, catechin, kaempferol, epicatechin and naringenin.[21]

The present study has demonstrated that the antioxidant property of *Zingiber officinale* is dependent on the temperature used during extraction. The extract obtained at 26°C demonstrated statistically significant ($P< 0.05$) antioxidant property compared to the extract obtained at 50°C and at 100°C. However, the extract obtained at 100°C had no antioxidant activity. This observation is in agreement with past studies that have reported that gingerols are thermally labile and degrade to shagaols readily at temperatures above 70°C.[31-32] This degradation to shogaols is due to the presence of a beta- hydroxyl keto group which undergo degradation at high temperatures. It is possible that the degradation of gingerols could have been responsible for the decreased antioxidant activity of *Zingiber officinale* extracted at 50°C and the complete loss of antioxidant property at 100°C.

CONCLUSION

The study has demonstrated that temperature affects the antibacterial and antioxidant properties of *Zingiber officinale.* Though there are conflicting reports about the analgesic activity of *Zingiber officinale*, this study did not demonstrate any analgesic activity at all the temperatures investigated. In addition, the anti-inflammatory property of *Zingiber officinale* was shown not to be temperature dependent. It is of interest to note that the temperature dependence on some pharmacological actions of *Zingiber officinale* may be due to the thermally labile properties of gingerols. Temperature of extraction should always be considered in studies on *Zingiber officinale.*

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