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Original Article

ANTI-ATHRITIC EFFECTS OF LIME, MAIZE HUSK EXTRACT AND ITS CO ADMINISTRATION ON WISTAR RATS

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Abstract: This study investigated the anti-arthritic effects of lime juice (LJ) and lime juice with maize husk extract (LJMHE) in Wistar rats. Arthritis was produced by formaldehyde model after the commencement of oral administration of LJ, LJMHE, vitamin C (ViC), saline and indomethacin. Daily changes in paw sizes were measured for 10 days. Serum urea (BUN), ferritin (SF), serum creatinine (SC) and C-reactive protein (CRP) were also measured.

Results: The results showed that LJ, LJMHE and indomethacin produced consecutive reductions ($p<0.05$) in paw sizes from the 5th - 10th day. LJ and LJMHE performed better than Indomethacin while ViC did not produced any significant reduction in paw size. Likewise, LJ, LJMHE and ViC increased ($p<0.05$) BUN and reduced the serum concentration of CRP. There were no significant changes in the SF in groups treated with LJ (2.5mg/Kg) LJMHE and Indomethacin compared with control group (13.80 ± 0.60) whereas SF was increased in the groups treated with LJ (5mg/Kg) and ViC (21.12 ± 0.78 and 22.38 ± 0.10 ng/ml respectively).

Conclusion: The study established the anti-arthritic effect of Lime Juice and Lime Juice with Maize Husk Extract which might be via the inhibition of CRP.

Keywords: Arthritis, Citrus aurantifolia, Inflammation, Maize husk

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Introduction

Lime (*Citrus aurantifolia* (Christm)) is a genus of flowering plants in the family Rutaceae (orange family). It is a fruit that originated from the Himalayan region of India. Studies have shown that *Citrus aurantifolia* (Christm) has both medicinal and cosmetic values, de Castillo *et al.,* 2000 [1] observed from their study that it exhibited antimicrobial activity against vibro stains. Information from many literatures have shown that it can be used as antiproliferative substance against tumor cell lines [2], cytoxin [3] and antimicrobial against upper respiratory tract pathogens [4]. The medicinal importance of *Citrus aurantifolia* has been linked to the presence of important phytochemicals such as β-pinene, limonene, γ-terpinene, terpinolene, α-terpineol and citral [5].

Maize (*Zea mays* L.) is an edible grain which originated from South America and it is now a common masticatory in Nigeria. Every part of *Zea mays* (grains, leaves, corn silk, stalk and inflorescence) has been observed to be of high medicinal importance. The corn silk is used as antidiabetic or diuretics and treatment of urinary troubles and gallstones [6]. The ash of the cob was used for treatment of cough [7] and the husk has been reported to have immunological effects because it contains arabinoxylan [8] and anti-inflammatory activities [9].

Arthritis is a group of conditions involving inflammation of the joints of the body. Inflammation is a complex immune response intended to protect the body from various harmful agents (e.g. microbes and toxins) which is characterized by four cardinal features; heat, redness, swelling and pain. All these result in loss of function which refers to either a simple loss of joint mobility due to oedema and pain, or to the replacement of functional cells by scar tissue [10].

The present study was undertaken based on reports from some traditional medicine users in south-west of Nigeria indicating that lime with maize husk is an effective remedy for the treatment of arthritis. It was also undertaken as a follow up on the previously published research on the anti-inflammatory effect of maize husk [10]. It is also worthy to carry out this

research because arthritis is a common condition that affects many people especially in the old age, it is pertinent to explore the effects of some common medicinal plants (such as Lime with maize husk extract) on formaldehyde-induced arthritis in male Wistar rats. Furthermore, some other biomarkers of inflammation such as sera C-reactive protein, Creatinine and Urea were measured so as to corroborate the effects of these plant extracts on arthritis. Serum ferritin was measured to know the level of Iron storage in Wistar rats. The extent to which the plant extracts are able to reduce arthritis was compared to a well known non steroidal anti-inflammatory drug (NSAID), Indomethacin so as to know the efficacy of the LJ and LJMHE on arthritis reduction.

Materials and Method

Preparation of lime and *Zea mays* husk extract

The lime was obtained commercially from the market at Post office, Ilorin, Kwara State, Nigeria. The lime was washed and sliced into two halves, squeezed into a clean container, filtered and kept in an air-tight bottle.

Fresh maize was purchased from Ajase-ipo market, Kwara State and the husks were removed and gathered together. The husks were thereafter air-dried for two weeks. The powdery sample (120g) was exhaustively extracted with 1.5 litres of distilled water. The resulting solution was evaporated in water bath to give a light brown extract weighing 13.5g.

All drugs and the extract used were dissolved in normal saline. Hence, normal saline served as the vehicle for administration apart from lime which was already in liquid form.

Animal selection and care

Thirty male Wistar rats weighing between 170 ± 7g were used. They were bought, kept in well ventilated cages and allowed to acclimatize in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin, Kwara state for a period of two weeks. Food and water were provided for them *ad libitum*.

Experimental procedure

Rats were divided into six (6) groups of five (5) rats each with each group treated as follows:

Group 1 (the control group) received normal saline (10 ml/kg), while the reference group, (Group 2) was given Indomethacin (5 mg/kg). Groups 3 and 4 received lime juice (LJ) at different doses 2.5 ml/kg and 5 ml/kg respectively. Group 5 was given the maize husk extract (50 mg/kg) together with 5 ml/kg of lime (LJMHE). Group 6 received 100 mg/kg of vitamin C.

Chemicals

Formalin was a product of Klincent Laboratory PVT LTD, Mumbai, India, while Indomethacin was manufactured by Tuyil Pharmaceutical industry, Ilorin, Nigeria.

Animal sacrifice and blood collection

Animals were sacrificed on day 10 after administration of the respective extracts, drugs or saline, using chloroform as the anaesthetic agent. Blood samples were collected into EDTA bottles through cardiac puncture. Blood samples were centrifuged at 3000rpm and plasma collected into plain bottles for analyses.

Evaluation of Arthritis

Paw oedema was induced by injecting 0.1ml of 2% formaldehyde into the right hind paw of each rat under the subcutaneous layer of the subplantar aponeurosis of each rat according to the method described by Olajide *et al.,* [11].

The paw size was measured by wrapping a piece of cotton thread round the paw to measure the circumference before and after formaldehyde injection. The measurement of paw size after lime only and lime with extract, control and drug administration was repeated for 10 consecutive days with formaldehyde induced paw oedema repeated on day 3 of the experiment.

The inhibitory activity was calculated according to the following formula;

$$\text{Percentage inhibition} = \frac{(\text{Pf} - \text{Po})_{\text{control}} - (\text{Pf} - \text{Po})_{\text{treated}}}{(\text{Pf} - \text{Po})_{\text{control}}}$$

Assessment of serum parameters

Measurements of C-reactive protein and ferritin

Microplate wells were formatted for each serum reference, control and test specimen to be assayed in duplicate. The manufacturer's procedures for assay were strictly followed, starting with 0.100ml of the working CRP enzyme reagent for CRP and 0.100ml of the working ferritin enzyme reagent for ferritin. After the whole procedure, absorbance for each index was read in

each well at 450nm (a reference wavelength of 620-630 nm was used to minimize well imperfection) in a microplate reader.

Measurement of Creatinine

The optical density was mixed and read for 60 seconds after the sample and standard addition, the second reading was taken at exactly 60 seconds after the first reading.

Measurement of Urea

The absorbance of the sample and that of the standard were measured against the reagent blank. The concentration of the urea was measured as;

$$\text{Urea}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 40$$

Statistical Analysis

All values were expressed as mean ± standard error of mean (SEM). The level of significance was determined using one way ANOVA. $p<0.05$ was considered statistically significant using statistical package for social sciences (SPSS) version 20.0.

Results

Effect of lime juice and maize husk on formaldehyde induced arthritis

From figure 1, a significant reduction in paw size was observed in groups 3, 4 and 5 when compared with the other groups starting from day 4. This implies that lime alone and combined with *Zea mays* husk extract have high anti-arthritic effects. This was further proved on days 9 and 10 when a greater level of paw reduction was observed.

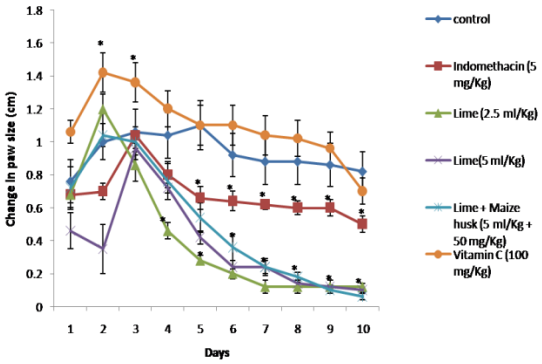

Fig 1.Effect of maize husk extract and lime singly or in combination on formaldehyde induced Arthritis in rats. Each value is the mean ±S.E.M. of 5 rats. *p< 0.001 compared with control. ANOVA was used followed by a post hoc test to compare between groups.

Effect of lime juice and maize husk on C reactive protein

Table 1 shows the result of the CRP, a significant ($p<0.05$) decrease was observed in the serum C - reactive protein in all the treated groups (2 - 6) in comparison to group 1 (the control

group). Also, a significant decrease was observed in the serum concentration of CRP in groups 2, 3 and 4 when compared to group 6.

Effect of lime juice and maize husk on ferritin

The results in figure 3 showed that there were no significant changes in the SF in groups treated with LJ (2.5mg/Kg), LJMHE and Indomethacin compared with control group (13.80 ± 0.60) whereas SF was increased in the groups treated with LJ (5mg/Kg) and ViC (21.12 ± 0.78 and 22.38 ± 0.10 ng/ml respectively). Also a significant ($p<0.05$) increase was observed in groups 3 and 4 when compared to group 2 only.

Effect of lime juice and maize husk on creatinine

Figure 2 showed the serum concentration of creatinine and from the result, it was observed to be significantly ($p<0.05$) higher in groups 3, 4 and 5 when compared with those of groups 1, 2 and 6. The trend of the result also showed that groups 3 and 4 were significantly ($p<0.05$) increased when compared to group 5.

Effect of lime juice and maize husk on urea.

A significant ($p<0.05$) increase was observed in serum concentration of groups 3, 5, and 6 when compared to group 1 as shown in figure 4. Also, groups 2, 4 and 5 decreased significantly ($p<0.05$) in comparison to group 6.

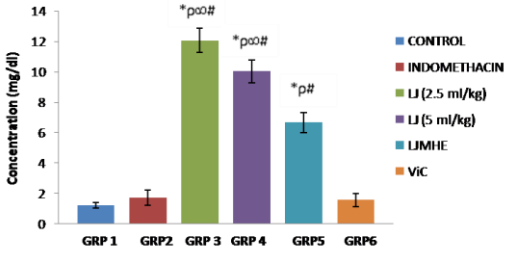

Figure 2: Changes in the serum concentrations of creatinine of control and experimental rats

* means significantly different from group 1

ρ means significantly different from group 2

∞ means significantly different from group 5

means significantly different from group 6

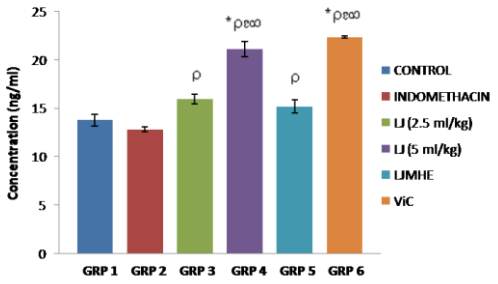

Figure 3: Changes in the serum concentrations of ferritin of control and experimental rats.

* means significantly different from group 1

ρ means significantly different from group 2

ε means significantly different from group 3
∞ means significantly different from group 5
* means significantly different from group 1
ρ means significantly different from group 2
means significantly different from group 6

DISCUSSION

The formaldehyde model of arthritis used in this study has been shown to be useful for detecting antiinflammatory and anti-arthritic effects of phlogistic substances. It has also been shown to partially correlate with human arthritic conditions [12, 13, 14]. Results obtained from this study shows that LJ at both doses and LJMHE inhibited formaldehyde-induced arthritis in rats significantly especially starting from day 4 to the last day of administration. The two LJ groups and the LJMHE produced better anti-arthritic effect compared with the indomethacin (5mg/Kg) as well as Vitamin C. The LJMHE seems to be the best of all the groups at 9 and 10th days of treatment.

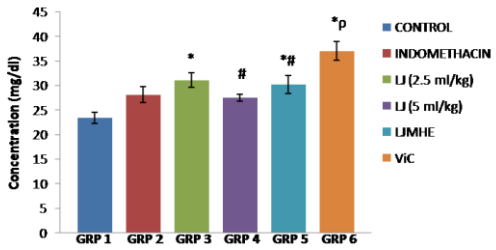

Figure 4: Changes in the serum concentrations of urea in control and experimental rats.

Though, this effect has not been associated to a specific mechanism of action, it can be assumed that they have similar mechanism as the non-steroidal anti inflammatory drugs (NSAIDs) by inhibiting cycloogenase 2 mediated prostaglandin biosynthesis [15]. It could also be due to its inhibitory action on prostaglandin E2 which tends to exert catabolic effect on osteoarthritic cartilage [16].

CRP has been suggested to be a marker of inflammation in several conditions including psoriasis, rheumatoid arthritis, tuberculosis, cancer, and myocardial infarction [17]. CRP has been recently recognized as an independent predictor of future coronary heart disease [18, 19] and also associated with the metabolic syndrome and diabetes mellitus. The result of this work showed that Indomethacin, lime at both doses (2.5 ml/kg and 5 ml/kg) and maize husk extract together with lime juice reduced the level of CRP. This implies that these substances have a way of reversing or protecting the body from arthritis and other adverse effects

that increase the concentration of CRP in the body. This work is in line with the work done by Yunsheng Ma *et al.,* [20] since maize husk is very rich in dietary fibre [21]. They observed that increased consumption of dietary fibre appears to be strongly associated with lower CRP concentrations and thus suggested that a diet high in fibre may play a role in reducing inflammation and, thus, the risk of cardiovascular disease and diabetes. Also, maize husk has been reported to have anti-inflammatory properties according to the work done by Owoyele *et al.,* [9].

GROUPS	TREATMENT	CONCENTRATION (mg/ml) MEAN ± SEM
1	Control	32.15 ± 2.21
2	Indomethacin	2.12 ± 0.23*α
3	LJ (2.5ml/kg)	3.81 ± 0.40 *α
4	LJ (5ml/kg)	3.81 ± 0.70 *α
5	LJMHE	6.35 ± 1.06 *
6	ViC (100mg/kg)	13.41 ± 3.20*

* means significantly different from group

*α means significantly different from group 6

In this study, it was observed that serum creatinine level was greatly increased in the groups that received both doses of lime juice as compared with other groups and relatively increased in group that received LJMHE. The urea level was also highly increased in groups that received lower dose of LJ, LJMHE and vitamin C relative to other groups especially the control group. Since urea and creatinine are indicators of kidney functions and are chemical products of protein and muscle metabolism respectively [22, 23], it can be deduced from this study that these two substances increased in some groups as a result of increased metabolism under the influence of the substances administered.

Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion; hence it makes iron available for absorption in the body [24]. It is also a marker of cellular damage and inflammation [25, 26]. It was observed in this study that vitamin C and a higher (5 ml/kg) dose of LJ increased serum ferritin level compared to every other group this implies that there is a tendency of these substances to help relieve iron-deficiency anaemia. Although, a little bit increase in serum ferritin level was

also observed under the influence of lower dose (2.5 ml/kg) of LJ and LJMHE. Mission Pharmacal Company [27] recorded that a cup of lime juice contains 0.22 mg of Iron; this could be one of the reasons for increased serum ferritin level observed in this study rather than an indication of cellular damage or widespread inflammation. A study done by Goralska *et al.,* [28] recorded that the ascorbic-acid induced increase in ferritin concentration is due mainly to an increase in ferritin synthesis at the translational levels which supports the finding of this work. The higher levels of ferritin observed in groups 4 and 6 may also be due to similar effects of ascorbic acid since lime at the higher dose would also contain high amount of vitamin C. On the other hand Garcia *et al.,* [29] observed that increasing dietary ascorbic acid by 25 mg at each of 2 meals per day did not improve iron status in iron-deficient women consuming diets high in phytate and non-heme iron. The contrast between this study and that of Garcia *et al.,* [29] could be as a result of many factors bothering on methodology, constituents of diets and extract as well as species specific mechanisms. More research work can still be designed in this area.

Conclusion

In conclusion, it can be deduced from this study that LJ and LJMHE have anti-arthritic activities and not only that, LJ can serve as a good source of iron supply in the body.

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CONFLICT OF INTEREST

The authors declare no conflict of interests

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