Effects of Aqueous Extract of *Curcuma Longa* Rhizome On Motility of Isolated Rabbit Jejunum

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*Accepted December 2019, and Published December, 2019*

**ABSTRACT**

*Turmeric (curcuma longa) is a rhizomatous herbaceous perennial plant of the ginger family and the order Zingerberales. It is widely cultivated and used in the treatment of various ailments. In this study, the effect of aqueous extract of C. longa on isolated rabbit jejunum was investigated in vitro using Physiograph (Meditech, India). The rhizome of Curcumin was extracted using Soxhlet extraction method and distilled water was used as a solvent. The elemental analysis was determined using AAS and the result revealed the presence of Potassium, Magnesium, Iron and Nitrogen. The percentage concentrations of trace elements in the aqueous Curcumin rhizome were within the WHO standard limit. The aqueous extract at concentration tested (100 mg/ml) significantly decreased (p<0.05) jejunum smooth muscle contraction. Addition of Atropine (1mM) or Propranolol (1mM) further decreased the amplitude of jejunum smooth muscle contraction. Curcumin rhizome (100 mg/ml) blocked contraction induced by Ach (0.001µg/ml). The result of this work has shown that rhizome of C. longa produced jejunum smooth muscle relaxation, plant extract with antispasmodic activity may reduce gastrointestinal motility thereby delay gastric emptying and may be important in treatment of disease ailments like diarrhoea and colic.*

**Key Words:** Curcumin, Relaxation, Physiograph, Rabbit, *in vitro*. 

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INTRODUCTION
Turmeric (curcuma longa) is a rhizomatous herbaceous perennial plant of the ginger family and the order Zingerberales. The part of turmeric used in food and medicine is the rhizome [1]. This rhizome has a long history of used in herbal remedies particularly in China and India. The uses of turmeric dated back nearly 4000 years to the Vedic culture in India where it was used as a culinary spice and has some religious significance [2]. Today, turmeric is widely cultivated in the tropics and goes by significant names in different cultures and countries. The species of C. longa known as golden spices of India is a native to tropical South Asia and have been in existent for over 500 years. The main component in turmeric is Curcumin, which is a powerful antioxidant. It has been reported to have cancer preventive effect as well as anti-inflammatory properties [3]. The Arabians introduced it to Europe in the 13th Century and owed to the recent research that highlighted its therapeutic properties [3].

Turmeric comes from the root of C. longa plant and has tough brown skin with deep orange colour. This herb has a peppery warm and bitter flavour, the mild fragrance slightly reminiscent of orange and ginger which relayed to the rhizomes or underground stem, has a round segmented skin [1]. The length of the main rhizomes is approximately 2.5 cm – 7 cm in length, with a diameter of 2.5 cm, with distinct nodes and smaller tuber branches [Reference].

The preventive and therapeutic effect of turmeric has been examined in animal models. Previous study reported it to exhibit anticancer activity [4] and also reported to have hepatoprotective [5], Cardioprotective [6], hypoglycemic [7], and antiarthritic activities [8]. In various experimental models, turmeric has also been reported to exhibit activity against the development of skin cancer [9], breast cancer [10], and oral cancer [11]. It prevents carcinogenesis at various steps, including inhibition of mutation [12] detoxifying carcinogen [13], decreasing cell proliferation and induced apoptosis of tumor cells [4]. Turmeric extract prevents animals tumor induced by Dalton's lymphoma [14]. Topical application of turmeric was found to decrease multiplicity and onset of skin tumors [9].

The aim of this study is to examine the pharmacological effect of the aqueous extract of C. longa (rhizome) alone and in combination with standard drugs (Acetylcholine, Atropine and Propranolol) on the isolated rabbit jejunum in vitro.

MATERIALS AND METHODS
Plant Collection and Identification
The plant turmeric (C. longa) was purchased from Monday market, Maiduguri, Borno State, Nigeria. The turmeric species was identified by a botanist at the Department of Biological Sciences, University of Maiduguri.

Elemental Analysis
Elemental analysis was determined using AAS, (Buck Scientific, 210 VGP system, USA) as described by the manufacturer.

Extract Preparation
Six hundred and seventy grams of C. longa was weighed and placed in round bottom flask of Soxhlet extractor apparatus and two litres of distilled water was added, condenser was then attached to the flask fitter with rubber tube for water to circulate the mixture for about 2 hrs.
Six (6) adult rabbits (*Oryctolagus cuniculus*) were purchased from Maiduguri, Monday Market. They were acclimatized to laboratory conditions for 14 days before the commencement of the experiment. They were deprived of feed overnight and water provided *ad libitum* before the experiment. The animals were managed according to the International Guiding Principles for Biomedical Research [15].

The preparation was allow to equilibrate for 30 min as the tissue exhibited stable spontaneous contraction. The initial reading was taking as a baseline. The segment that did not show spontaneous contraction was discarded from the experimental protocol. Three (3) experiments were carried for the same jejunum which always received the same extract/or drug treatment.

**Experimental Animals**

Six (6) adult rabbits (*Oryctolagus cuniculus*) were purchased from Maiduguri, Monday Market. They were acclimatized to laboratory conditions for 14 days before the commencement of the experiment. They were deprived of feed overnight and water provided *ad libitum* before the experiment. The animals were managed according to the International Guiding Principles for Biomedical Research [15].

**Preparation of Physiological Solution**

The Tyrode’s solution was prepared by dissolving the salts in distilled water and had the following Components (g/L) Calcium Chloride (anhydrous) 0.2, Magnesium Chloride (anhydrous) 0.1, Potassium Chloride 0.2, Sodium Chloride 8.0, Sodium Phosphate Monobasic 0.05 (anhydrous) and D-Glucose 1.0. The salts were weighed using a sensitive weighing balance (ADA120/C chemical balance). To prevent the precipitation of the solution, calcium chloride was also added. The solutions were prepared fresh on the day of each experiment.

**Experimental Protocol**

Assessment of pharmacological effect of *C. longa* aqueous extract on rabbit jejunal segment was performed. Each rabbit was humanely sacrificed and exsanguinated by severing the jugular vein and carotid artery in the neck region. It was then immediately dissected to locate the jejunum situated between the duodenum and ileum. The jejunum was excised and immediately placed in a Petri dish containing Tyrode’s solution maintained at room temperature. Subsequently the jejunum was cut into 2 – 3 cm segment used to test the effect of the aqueous extract on the contractile activity of the gut smooth muscle according to [16] with slight modification, each segment was cleaned off ingesta and omental tissues, and thread was carefully attached to each of the jejunal segment without occluding the lumen. The segment was then mounted in a 75 ml organ bath containing oxygenated (95% oxygen and 5% carbon dioxide), Tyrode’s solution maintained at 37°C. The tissue was linked to a physiographic machine which is a modified Kymograph method.

A stock solution of 100 mg/ml of the extract was prepared just before the experiment. To obtain the response of the segment to the extract, they
were exposed to concentration of the extract (5 mg/ml) in the organ bath and the contraction recorded in the Digital student Physiograph, channel 1 (Medicaid system, India) with speed of 0.2 mm/sec. The contraction of the segment following each dose exposure (starting with lowest dose) were observed for at least 20 second after which the organ bath was drained and the mounted tissue washed twice with Tyrode's solution before the next dose was administered. The procedure was repeated with different rabbit jejunum, three times under same experimental procedure. The height (amplitude) of contraction/or relaxation was determined from the Physiograph using a standard procedure.

**Data Analysis**
All results were presented as Mean ± S.E.M, the comparison between pretreatment and various groups were analyzed using student’s T-test and P value less than or equal to 0.05 was considered to be significant.

**RESULTS**

The result of the proximate analysis of *C. longa* (rhizome) is shown below (Table I), the results indicate high percentage of dry matter

| Table I: Results of proximate analysis of rhizome from *C. longa* |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Extract Sample    | %Dry matter     | %Moisture content | %Crude protein | %Ether extract  | %Crude fiber    | %Ash |
| Curcumin          | 92.2            | 7.8              | 4.02           | 2.0             | 4.0             | 1.0  |

**Table II: Results of macro and micro elements analysis of rhizome from *C. longa***

<table>
<thead>
<tr>
<th>Macro Elements</th>
<th>N</th>
<th>P</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>% in the sample</td>
<td>1.40</td>
<td>0.24</td>
<td>2.00</td>
<td>0.70</td>
<td>1.46</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Micro Elements</th>
<th>Cd</th>
<th>Zn</th>
<th>Fe</th>
<th>Ni</th>
<th>Cr</th>
<th>Pb</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>% in the sample</td>
<td>0.08</td>
<td>0.04</td>
<td>9.41</td>
<td>1.21</td>
<td>0.27</td>
<td>4.49</td>
<td>0.62</td>
</tr>
</tbody>
</table>

The results revealed that turmeric possesses electrolytes such as potassium (K⁺) and sodium (Na⁺), calcium (Ca²⁺) and phosphorus (P) in a trace amount. The primary (macro) nutrients are nitrogen (N), magnesium (Mg²⁺), and potassium (K⁺), (Table II).

The result for micro element analysis has shown in table II, which are within the WHO acceptable limits (Table II).

**Cholinergic Antagonist effect**
Acetylcholine (Ach) is used as an agonist to stimulate the jejunum smooth muscle contraction; the extract of *C. longa* rhizome (100 mg/ml) significantly reduced the contraction induced by Ach (0.001 μg/ml), p<0.05, with maximum relaxation of 58.8±1.6% (Figure 1a and 1b).
Figure 1a: A representative Physiograph plot. Green arrow indicate baseline (control) plot with the physiological solution, Blue arrows are Ach plot, brown arrow is the Ach with Curcumin and white arrow indicate Curcumin alone.

Figure 1b: Effect of *C. longa* rhizome on jejunum smooth muscle induced contractility by Ach. N=3. Data are expressed as Mean ± S.E.M.

Atropine which is a well-known anti-cholinergic agent at concentration of (0.01 μg/ml) further decreased amplitude of smooth muscle relaxation in the presence of *C. longa* rhizome (p<0.05) Figure 2a and 2b. The maximum relaxation was (31.3±1.6%) figure 2b.
**Figure 2a:** A representative Physiograph plot. Green arrow is the baseline (Control); Blue arrow is the treatment with Curcumin. Brown indicates curcumin with atropine and white arrow indicates Atropine alone.

**Figure 2b:** Effect of *C. longa* rhizome on jejunum smooth muscle relaxation in presence or absence of Atropine. N=3. Data are expressed as Mean ± S.E.M.

Figure 3a and 3b indicate that Curcumin rhizome has no effect on contraction induced in the presence or absence of Propranolol (0.01 μg/ml).

**Figure 3a:** A representative plot on the physiograph, Green arrow indicates the baseline (control), Blue indicate Propranolol alone. Brown and white arrows indicate curcumin in absence or presence of Propranolol respectively.
DISCUSSION
In this study, the effect of Curcumin on the isolated rabbit jejunum smooth muscle relaxation was investigated using a Physiograph. The results from elemental analysis revealed that the aqueous extract of Curcumin rhizome contain high percentage of Potassium (2.0 %), Magnesium (1.46%) and Nitrogen (1.4 %). Magnesium was earlier reported to cause full relaxation of airways smooth muscle [17]. Magnesium can inhibits $\text{Ca}^{2+}$ influx by blocking the voltage-dependent calcium channels hence relaxation of the smooth muscle. Potassium was reported to $\text{K}^{+}$ produce increase of $\text{K}^\text{efflux}$ from myometrium which decrease excitation and subsequent loss of contraction of uterual smooth muscle [18].

Curcumin itself had shown significant concentration-dependent relaxation effects on rat tracheal smooth muscle with both KCL and Methacholine induced contraction [19]. Therefore, elements found in the plant extract could be responsible for the relaxant property.

In intact body, the process of smooth muscle cell contraction was regulated principally by receptor and mechanical (stretch) activation of the contractile proteins myosin and actin [20]. Also, the change in membrane potential, by firing of action potentials or by activation of stretch-dependent ion channels in the plasma membrane, can also trigger contraction. The lower percentage of $\text{Ca}^{2+}$ in Curcumin could be an attributing factor for relaxation effect on the jejunal smooth muscle, $\text{Ca}^{2+}$ normally binds to calmodulin and this complex binds to activates myosin light chain kinase (MLCK). The activated $\text{Ca}^{2+}/\text{calmodulin}/\text{MLCK}$ phosphorylates the regulatory light chain subunit of myosin, which leads to an increase in actin-activated myosin MgATPase activities [21], [22].

The low percentage of proteins and crude fiber in Curcumin extract could be some of the leading factors in relaxant properties, report was established that thin-filament proteins have been proposed as secondary sites of regulation of contractile elements [20] but, additional studies are needed to establish the physiological roles in extract. Changes in the $\text{Ca}^{2+}$ sensitivity of smooth muscle contractile elements with different modes of cellular stimulation may be related to inactivation of myosin light chain kinase or activation of protein phosphatase activities. Although, the contractile elements in smooth muscle cells were not dependent solely on $\text{Ca}^{2+}$ [21] [23], but also used additional regulatory mechanism.
The results suggest that the Curcumin caused relaxation of jejunum smooth muscle by low influx of \( \text{Ca}^{2+} \) in the muscle cells as well as non-release of sufficient calcium from intracellular stores.

The result of this study is in agreement with the previous work which reported the antispasmodic effect of plant extract on isolated jejunum segment in laboratory animal, shown to inhibit the contraction of isolated rat aorta [24], Guinea pig ileum and rat uterus [25], rabbit jejunum and trachea [26], Curcumin has also shown to inhibit contractility of isolated urinary bladder in two studies [27] and [28], turmeric (\textit{Curcumin longa}) was also reported to have inhibitory effect on vascular smooth muscle which were similar to our findings on the isolated rabbit jejunum.

The results of responses of the rabbit (\textit{Oryctolagus cuniculus}) jejunum segments were presented in Fig (1a, 1b, 2a, 2b, 3a and 3b). The decreased amplitude of contractility seen in figure (1a, 1b, 2a and 2b) is not surprising because gastrointestinal tract (GIT) motor tone was reported to occur through multiple mediators which include neurotransmitters, inflammatory mediators and oxidative metabolites [29]. Curcumin has shown to blocked contraction induced by Ach (Figure 1a and 1b). Acetylcholine induced contraction of smooth muscle results from activation of muscarinic receptors and differences in muscarinic receptor are now known to exist [30]. The extract may produce its effect through \( \alpha_2 \)-adrenoceptor or through acetylcholine antagonism since the extract relaxed acetylcholine induced contractions. Atropine a muscarinic antagonist further increased the relaxant effect in presence of Curcumin (Figure 2a and 2b), the resulting membrane depolarization results in a large transient relaxation response from the intestine. Curcumin also produced similar effects with propranolol, a non-selective beta-blocker on rabbit jejunum smooth muscle (Figure 3a and 3b). Curcuminoids was earlier reported [25] to produce a relaxant effect on smooth muscle in isolated guinea-pig ileum and rat uterus via receptor-dependent and independent mechanisms.

**Conclusion**

The results of \textit{in vitro} study revealed that Curcumin (rhizome) decreased the jejunum smooth muscle spontaneous contractions. The decrease could be due to antispasmodic properties of the Curcumin extract which could have resulted from low calcium release from intracellular stores or none calcium feed-back against its concentration gradient initiated by the extract. It could also be due to anti-cholinergic mechanism of action. The results suggest that properties such as these may reduce gastrointestinal motility and delay gastric emptying which may be important in the treatment of some disease conditions such as diarrhea and colic.

**Acknowledgment**

The authors would like to acknowledge the technical support by the technologist Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri. We are also grateful to the Department for the financial support.

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