Comparative Studies on Proximate and In Vitro Anti-Inflammatory Activities Turmeric Methanol Extract in Albino Wistar Rats

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ABSTRACT
All throughout the world, turmeric has attracted the interest of scientists and medical specialists. The goal of this study was to pinpoint turmeric methanol extract's immediate proximate and anti-inflammatory activities. A spectrophotometric approach was used to choose the phytochemical screens (both qualitative and quantitative). The recent approach (standard method) was used to research the anti-inflammatory effects and proximate components of turmeric. The qualitative phytochemical screening revealed a substantial amount of turbid alkaloid (++) but no steroid (-). Phenol (151.34±1.01 mg/100g) had the highest concentration (5.83%), while flavonoid (22.30±0.62 mg/100g) had the lowest (5.83%), according to the quantitative screening. The anti-inflammatory activity of the turmeric methanol extract in the treated groups (150, 300, and 600 mg/kg body weight) and the positive control group demonstrated a valuable value (inhibitory values). Paw thickness was also significantly different (reduced) between the 600 mg/kg and positive control groups promising anti-inflammatory effects. These findings demonstrated that inflammation can be treated using turmeric's anti-inflammatory and adjuvant capabilities.

Keywords: *Curcuma longa; Inflammation; Phytochemical*

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INTRODUCTION

Turmeric is a topic of interest to the scientific, medicinal, and culinary sectors. Turmeric comes from a perennial herbaceous plant called Curcuma longa, which belongs to the ginger family (Priyadarsini, 2014). Despite the fact that curcumin's health benefits have been known for thousands of years, only recent research has been able to pinpoint its exact mechanism(s) of action and bioactive ingredients (Gupta et al., 2013). Curcuma longa (turmeric) and other Curcuma species' rhizomes contain a naturally occurring polyphenol called curcumin, also known as diferuloyl methane/1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Aggarwal et al., 2003). C. longa has been used as a medicine in Asian countries for generations due to its antioxidant, anti-inflammatory, antimutagenic, antibacterial, and anticancer properties (Mahady et al., 2002; Reddy et al., 2005; Lestari et al., 2014; Vera-Ramirez et al., 2013). A polyphenol called curcumin has been shown to target a number of signaling molecules while also exhibiting cellular activity (Gupta et al., 2013). It has been demonstrated to be effective in treating pain, metabolic syndrome, and inflammatory and degenerative eye conditions (Aggarwal et al., 2009; Panahi et al., 2016; Kuptniratsaikul et al., 2014; Azzolani et al., 2013; Allegri et al., 2010). It has also been demonstrated to be beneficial for the kidneys (Trujillo et al., 2013). While curcumin supplementation appears to provide a variety of therapeutic benefits, the majority of these are related to the anti-inflammatory and antioxidant properties of the compound (Gupta et al., 2013; Aggarwal et al., 2009). Although curcumin is thought to have anti-inflammatory and antioxidant properties, one of its biggest drawbacks when taken alone is that it has a low bioavailability

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(Anand et al., 2007).

It is thought that inflammation is a sophisticated biological process that causes tissue homeostasis to break down. Numerous biological, pharmacological, or physical stressors can cause inflammation, and depending on the stimulus, it may be acute or chronic. An inflammatory cascade that can lead to a range of disorders, including chronic asthma, rheumatoid arthritis, inflammatory bowel disease, and psoriasis, is triggered when the immune system's persistent attempts to reverse the detrimental effects of acute inflammation are unsuccessful. Chronic infections and inflammation have been revealed to be important cancer risk factors in experimental research and clinical epidemiological data (Ferlazzo et al., 2015). In the past, it was thought that underlying infections and inflammatory reactions were responsible for 15–20% of all cancer-related deaths (Ferlazzo et al., 2015). While many different therapeutic modalities, such as corticosteroids, nonsteroidal anti-inflammatory medications (NSAIDs), and biologic medicines, are routinely used to treat inflammation, they are both costly and have a number of side effects. In order to decrease the downsides of both synthetic and biologic medications, phytocompounds derived from diverse medicinal plants have been used as viable replacements. Significant research on medicinal plants supports the notion that plant extracts can be used as therapeutic agents due to the synergistic and cumulative effects of many components (Ferlazzo et al., 2015). This study will clarify the relationship between the proximate and anti-inflammatory capabilities of turmeric because there are little records of it.

MATERIALS AND METHODS

Sample

The turmeric (Curcuma longa), which was purchased in February 2021 from the kure market in the Bosso Local Government Area of Niger State, Minna, was confirmed for its safe use in research by the Department of Plant Biology at the Federal University of Technology in Minna with batch number 00020.

Test Animal

The rats used in the investigation on inflammation were donated by the National Institute for Research in Kaduna State, Nigeria. The rats, whose weights ranged from 154.89 to 3.45 g, had a week of acclimation at FUTMINNA's Animal Housing Unit, Department of Biochemistry, before being used. All animal experiments were carried out in accordance with recognized guidelines for the treatment of lab animals.

Chemicals and Reagents

Phenol solution, egg albumin, distilled water, 10% Folin-Ciocalteu, 7.5% Sodium Carbonate, 50% Methanol, 10% Aluminum Chloride, 10% Sodium Acetate, 96% Ethanol, 20% Sulfuric Acid, 60% H2SO4, 0.5% Formaldehyde, and 1.0% Hydrochloric Acid, Ethanol, ferrous sulfate, dimethylsulfoxide (DMSO), and regular saline are a few examples of chemical used. All of the ingredients and reagents on this list are of analytical quality.

Methods

Preparation of Turmeric

The fresh turmeric sample was cleaned, divided into slices, and dried for around five (5) days in the open air before the top layer was removed. Using an electric blender, the dried turmeric was ground into a powder. Prior to the extraction process, the crushed turmeric was cooled (Gupta et al., 2015).

Turmeric Extract Preparation

1.5 liters of methanol were added to two 1 liter beakers that held 1.5 grams (g) of dried turmeric each. The two beakers were then covered with aluminum foil or paper and let to stand for 72 hours. After 72 hours, the mixture was strained through filter paper and heated to 47 degrees Celsius to remove the methanol from the extract (Luckins, 2002). Following that, the extract was weighed, quantified, and saved for later analysis.

Inoculum Preparation

An uncooked chicken egg was used to create an egg albumin solution. A 0.1 mL solution made from egg albumin (the egg's white after it's been cracked) and distilled water was utilized to incite inflammation in albino rats. The solution's concentration was set at 1%.

Phytochemicals Screening

The methods for screening phytochemicals utilized in this work were discussed by Parekh and Chanda (2007), Evans (1996), and others. To ascertain whether phytoconstituents were present in the turmeric powder sample, examinations were carried out by the Center for Genetic Engineering and Biotechnology.

Qualitative Analysis

Tannin analysis

Two milliliters of the extract and two milliliters of the 10% alcoholic ferric chloride were mixed. Tannin was present as indicated by the dark blue color (Kumar et al., 2007).

Steroid test

A 2 mL of acetic anhydride and 2 mL of sulfuric acid were added to 1 mL of the extract. Steroids were present because the extract's color altered from blue to a dark green shade (Edeoga et al., 2005).

Flavonoid detection

A 10 mL of ethyl acetate were added to a 10 g extract, which was then cooked in a water bath for 5 minutes at 50 degrees Celsius. After adding 1 mL of diluted ammonia to the filtrate, a yellow tint appeared, suggesting that the test for flavonoid was successful (Harborne, 2005).

Terpenoid analysis

A mixture of 5 mL of the extract, 3 mL of sulfuric acid, and 2 mL of chloroform were added. The combination formed a layer, and the appearance of a reddish-brown color showed the existence of terpenoids (Edeoga et al., 2005).

Saponin test

A 20 mL of distilled water and 2 g of the extract were heated in a water bath to 45 °C. Following a filtering process, the mixture was mixed with 5 mL of distilled water to make 10 mL of the filtrate. An emulsion formed after the liquid was violently shaken and 3 drops of olive oil were added to the stable foam, indicating that the saponin test was successful (Edeoga et al., 2005).

Alkaloid detection

A 1 g extract was mixed with 5 mL of 2N hydrochloric acid and 5 mL of methanol. The mixture was filtered, and the filtrate was then treated with Meyer's and Wagner's reagents. Turbidity suggested a positive alkaloid test result (Harborne, 2005)

Phenol analysis
When 2 mL of phenol extract and 1 mL of ferric chloride were mixed, a reddish-brown color emerged, showing the presence of phenol, however it was not a strong or deep color.

Ascorbic test
A 2 mL of the extract, which contained 2% by weight of ferrous sulfate, sodium bicarbonate (0.1 g), and purified water were combined. After giving the combination a good shake, it was given a brief period of time to stand. The presence of 5 mL of 1 M sulfuric acid resulted in the appearance of a dark violet colour, which then vanished. The disappearance of the violet hue revealed the presence of ascorbic acid in the sample (Ganesan and Bhatt, 2008).

Free reducing sugar detection
A crimson cuprous oxide precipitate formed when Fehling reagent was applied to 1 mL of turmeric extract, indicating that the test for free reducing sugar was effective (Sofowora, 1993).
Quantitative analysis

Phenol determination
A 0.1 g of the turmeric extract was mixed with 10 mL of distilled water. In addition, 0.5 mL of the extract solution was oxidized using 2.5 mL of the 10% Folin-Ciocalteau reagent. Next, 2 mL of sodium carbonate solution at 7.5 percent was used to neutralize it. The mixture was incubated for 40 minutes at 45 degrees Celsius. The absorbance at 765 nm was then measured using a UV-spectrophotometer (UV-1800) (Singleton et al., 1999).

Saponin test
A 0.5 g of the extract was dissolved in 20 mL of 1N HCl, and the mixture was then heated for 4 hours in a water bath. But after filtering and letting the liquid cool, 50 mL of petroleum ether was added. The supernatant was combined with 6 mL of ferrous sulfate, 2 mL of H_2SO_4, 5 mL of acetone:ethanol, and left to stand for 10 minutes after being evaporated (1:1). After that, the absorbance at 490 nm was discovered using a UV spectrophotometer (Oloyed, 2005).

Tannin analysis
A 0.2 grams of extract were dissolved in 20 mL of 50% methanol, which was then parafilm-wrapped and heated for an hour in a water bath at 80 degrees Celsius. After filtering the mixture, 20 mL of Deni's distilled water, 10 mL of sodium carbonate, and 2.5 mL of Folin-reagent were added. After giving the mixture a good shake, it was allowed to develop a bluish-green hue for 20 minutes. A UV spectrophotometer (UV-1800) was used to measure the absorbance at 760 nm (Auta et al., 2011).

Flavonoid determination
A 0.5 mL of the extract was mixed with 0.1 mL of 1 M sodium acetate, 0.1 mL of pure methanol, 1.5 mL of the extract, 2.8 mL of distilled water, and 0.1 mL of 10 percent aluminum chloride. A 30-minute room-temperature incubation time came next. Utilizing a UV spectrophotometer with a

wavelength of 415 nm, the absorbance was measured (Cheng et al., 2002).

Alkaloid test

Before filtering using whatman No1 filter paper, a 0.5 g extract was diluted in 5 mL of a 1:1 solution of 96 percent ethanol and 20 percent H2SO4. 1 mL of the filtrate was then allowed to stand for 5 minutes before being combined with 5 mL of 60 percent H2SO4. After 5 minutes, 5 mL of 0.5 percent formaldehyde was added, and it was allowed to rest at room temperature for 3 hours. A UV spectrophotometer was used to detect the absorbance at 565 nm (Oloyed, 2005).

Proximate Analysis of Raw Turmeric

Moisture content determination

Using the oven drying procedure, the moisture content was determined by precisely weighing 2 g of well-mixed material in a cleaned, dried moisture container (W1). The moisture container containing the sample was heated to 103.5°C for 4-5 hours to ensure a constant weight. The can was then allowed to cool in the desiccators for 30 minutes. After cooling, it was weighed a second time (W2). Sharoba (2009) and Aoac (2005) stated that the formula for calculating the % moisture is as follows,

$$\%\text{Moisture} = \frac{W_1 - W_2 \times 100}{\text{Wt. of sample}} \qquad (1)$$

Where: W1= Initial weight of can + Sample and W2= Final weight of can + Sample (Aoac, 2005)

Determination of total ash content

After being cleaned and heated to 600°C for an hour in a muffle furnace, an empty crucible was weighed. Desiccators were used to reduce the temperature (W1). Each sample weighed 2 g, which was put in a crucible. A blowpipe was used to burn the sample over a burner. The crucible was then heated to 600°C in a muffle furnace for three hours. The sample's organic makeup has completely oxidized, as evidenced by the presence of gray-white ash. The ashing furnace was then turned off. After cooling in desiccators, the crucible was weighed (W3). The following calculation was used to determine the percentage of ash: (Aoac, 2005).

$$\%\text{Ash} = \frac{\text{Difference in Wt. of Ash} \times 100}{\text{Wt. of sample}} \qquad (2)$$

Difference in wt. of Ash = W3-W1

Crude protein test

The protein content of the sample was determined by the Kjeldahl technique. A digestion flask received between 0.5 and 1 g of dry ingredients. In addition, 0.05 g of copper tunings, 10-15 mL of concentrated H2SO4, and 2 g of Na2SO4 were added. Before beginning the digestion process, the flask's contents were thoroughly mixed and heated until the mixture became transparent (blue-green in color). It took me two hours to absorb everything in my head. After chilling, the liquid was transferred to a 100 mL volumetric flask and the required volume of distilled water was added. The digest was distilled using the Markam Still Distillation Apparatus. Following an additional 10 minutes of distillation, the NH3 generated up to 75 mL was collected as NH4OH in a conical flask with 20 mL of a 2 percent boric acid solution and a few drops of

methyl red and bromo-crysol green indicator. The NH4OH causes the distillation to have a yellow tint. The distillate was titrated with conventional 0.01NHCl solutions until a pink tint appeared. All of the earlier steps were performed with a blank as well. The sample's crude protein content was calculated using the formula below: (Aoac, 2005).

$$\%N = \frac{(S-B)xNx\ 0.014xDx100}{Wt.of\ the sample\ xV} \quad (3)$$

%Crude Protein = 6.25* x %N (*Correction factor)

Where: S= Sample titration reading, B= Blank titration reading, N= Normality of HCl,
D= Dilution of sample after digestion, V= Volume taken for distillation, 0.014= Mill equivalent weight of Nitrogen

Crude fat content analysis

Crude fat content was computed using an intermittent soxhlet extraction device. Crude fat was detected using the ether extract method and the soxhlet equipment. A 2 g dry sample was inserted into the extraction tube after being placed in a fat-free thimble and coated with filter paper. The round bottom flask was placed into the apparatus after it had been dried, weighed, and filled with petroleum ether. The extraction took 6 hours to complete. The ether was then allowed to vanish. For ether cleaning, the extract was transferred to a fresh glass dish, and the ether was then evaporated over a water bath. It was then baked for 30 minutes at 105°C before being placed in a desiccator. The following formula was used to determine the crude fat percentage: (Aoac, 2005).

$$\frac{\text{Weight of flask with oil – Weight of empty flask x 100}}{\text{Weight of sample}}$$

Determination of carbohydrate content

Difference was used to determine the sample's carbohydrate content. The carbohydrate content is the sum of all previously determined proximate factors deducted from 100. (Aoac, 2005).

100 – (%Moisture content + %Ash Content + %Crude fiber + %Crude protein + %Crude fat).

In vivo screening for inflammatory activity

The method described by Ahmed et al. was used to investigate the anti-inflammatory effects of the methanol extract of turmeric (2010).

Infection of Animals

The inoculums, which were artificial egg albumin at 1%, were administered to all of the experimental rats in order to elicit inflammation. 0.1 mL of the egg albumin solution was injected intraperitoneally into each rat, weighing between 105.94 and 183.17 g.

Administration of extract to infected rats

Using the methodology outlined by Sanchez-Maeto et al., the anti-inflammatory effect of The methanol extract of turmeric was studied (2006). Fifteen (15) healthy albino rats were divided into five (5) groups of three (3) each. The positive and negative control groups received distilled water and sodium diclofenac (100 mg/kg body weight; from a stock solution of 50 mg/mL), respectively.

Groups 1, 2, and 3 received oral dosages of 150, 300, and 600 mg/kg bw of methanolic turmeric extract (100 mg/mL), respectively. This was completed an hour before all of the rats' right hind paws were administered intraperitoneally with 0.1 mL of prepared, 1 percent egg albumin to induce inflammation. The back paw started to swell and turn red after around 40 minutes. The thickness of the injected paw was then measured at 0, 1, 2, 3, 4, and 6 hours following the injection of egg albumin using a vernier caliper (Ahmed et al., 2010).

Level of inflammation in rats

The thickness of the paws of wistar rats that had been administered egg-albumin to excite them was tested to ascertain the effects of turmeric methanol extract. This was accomplished by utilizing a vernier caliper, as described by Ahmed et al (2010). Rat paws were placed on the vernier caliper and the quantity of edema and redness was properly measured. The procedures for handling experimental animals were followed during this.

Analysis of data

Bar graphs and analysis of variance (ANOVA) were used to analyze the results of the analysis. The connection between the mean variables was evaluated using the Duncan test. The outcomes were also shown in tables as Mean Standard Deviation. Version 23 of IBM SPSS (Statistical Package for Social Science) was used to carry out the investigation. While P values of 0.05 or lower were not considered statistically significant, those of 0.05 or higher were.

RESULTS

Percentage yield of turmeric extract

This table shows the yield of turmeric's aqueous extract as a percentage. From a sample of 150 g of garlic, 10.72 g of extract was obtained, or 7.15 percent of the total extract.

Table 1. Yield of Methanol Extract of Curcuma longa in Percentage

Plants	Powdered turmeric (g)	Extract yield (%)
Turmeric	150	7.15

Qualitative phytochemical screening of turmeric

The phytochemical results of the turmeric methanol extract are displayed in table 2.

Table 2. Qualitative Phytochemical Analysis of Curcuma longa

Phytochemicals	Status/Results	Inferences
Tannin	+	Dark-blue color
Ascorbic acid	-	Violet to colorless
Alkaloid	++	Turbidity present
Saponin	+	Froth emulsion
Reducing sugar	-	Red precipitate
Terpenoid	+	Reddish-brown color
Flavonoid	+	Yellow color
Steroid	-	Blue to dark-green color
Phenol	+	Red-brown color

Keys: (-) absent, (+) present, (++) highly present

Quantitative phytochemical screening of turmeric

The results for the number of phytochemicals found in the C. longa methanol extract as well as the % amount for each parameter are shown in table 3.

Table 3. Quantitative Phytochemical Analysis of C. longa

Phytochemicals	Results(mg/100g)	Percentage (%)
Phenol	151.34±1.01^{e}	39.54
Saponin	145.11±1.79^{d}	37.94
Tannin	35.50±1.19^{c}	9.28
Flavonoid	22.30±0.62^{a}	5.83
Alkaloid	28.26±1.05^{b}	7.39

Values represent the mean and standard deviation of three independent determinations. $p<0.05$ denotes superscripts with various values within the same columns (significantly different).

Proximate analysis of turmeric extract

The results of the proximate components of the turmeric extract sample are displayed in the table below.

Table 4. Proximate Compositions of Methanol Extract of Turmeric

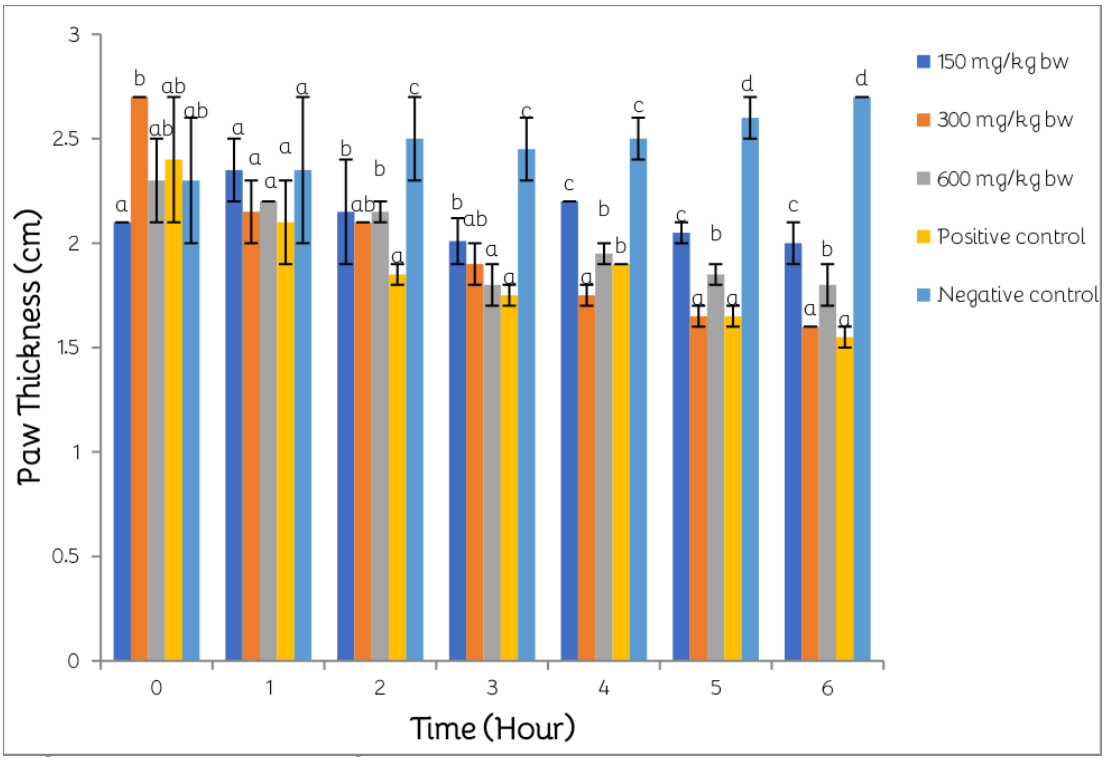
Parameters	Turmeric sample (%)
Moisture content	11.35±0.05
Ash content	3.50±0.05
Protein content	5.26±0.04
Fiber content	1.31±0.05
Lipid content	5.62±0.13
Carbohydrate.	73.42±0.18

Values represent the mean and standard deviation of three independent determinations. $p<0.05$ denotes superscripts with various values within the same columns (significantly different).

Anti- inflammatory activity of C. longa extract

Paw thickness measurement

Figure 1 shows the findings of paw thickness measurement performed using a calipher. When compared to the treatment groups and the positive control group, there was a statistically significant decrease in swelling in the treatment groups (150, 300, and 600 mg/kg bw), while the infected but untreated group showed a significant increase ($p<0.05$).



DISCUSSIONS

The amount of turmeric extract used in this investigation was equal to the 7.15 percent methanol extract that 150 g of powdered turmeric produced (Table 1) (Table 1). This was computed by dividing the weight of the turmeric powdered by the weight of the acquired extract, which weighed 150 g. (100). Tannins (+), phenol (+), and other phytochemicals were discovered throughout the study's qualitative phytochemical screening. With phenol having a high concentration (++) and other compounds having a lesser concentration (+), the alkaloid test revealed a very deep turbidity (Table 2). The steroid test's color changed from blue to dark green, while the ascorbic acid test's color changed from violet to colorless, all of which indicated a positive result. Additionally, Chanda, 2007, and Evans, 1996, both offered a typical technique for doing so. The data that follow are based on research by Mann et al. (2010), who detected flavonoids and tannins in an ethanol extract of turmeric.

Phenol had the highest concentration of phytochemicals (151.34±1.01 mg/100g) and flavonoid had the lowest concentration (22.30±0.62 mg/100g) in this study's quantitative phytochemical screening. Of the total parameters evaluated, flavonoid and phenol were found in 5.83 percent and 39.54 percent, respectively. According to Table 3, saponin made up 37.94% of the total, whereas alkaloid and tannin made up 7.39% and 9.28%, respectively. The examined traits did, however, differ significantly from one another ($p>0.05$).

Table 3 provides a close study of turmeric extract. The parameters moisture content, ash content,

crude fiber, and crude protein were among those looked at. The bulk (73.42±0.18 percent) of the metrics examined were carbohydrates, while fiber accounted for the least proportion (1.31±0.05 percent). Ash, moisture, and lipid content were all calculated to be in the range of 11.35±0.05, 3.50±0.05, and 5.62±0.13, respectively.

There is a statistically significant difference between the treatment and control groups in the ability of the turmeric methanol extract to reduce inflammation in Figure 1. ($p<0.05$). From the first hour to the final hour (six) of recording, the rat paw thickness (swellings) in the negative control group increased steadily. The treated and untreated groups did not differ substantially after 1 hour ($p<0.05$), whereas the 600 mg/kg body weight (bw), positive and negative control groups did not differ significantly at 0 hours ($p>0.05$) (Figure 1). The treatment groups (150, 300, and 600 mg/kg bw) also experienced small declines, with the 600 mg/kg bw group showing the most decline. The positive control group, often known as the "standard group," experienced a considerable decrease in paw thickness. The fact that there was a considerable drop in 600 mg/kg body weight also supports the idea that methanol extract treatment of inflammation is dose dependent. In the hours 0 through 4, there was no significant difference ($p>0.05$) between the 600 mg/kg body weight and positive control groups. However, in the hours 5 and 6, there was a significant difference ($p<0.05$). The decrease in 600 mg/kg bw may therefore be due to the bioactive components of turmeric, such as curcumin and its derivatives, as well as phytochemicals in the methanol of the extract, particularly phenol.

Curcumin and other substances have the ability to inhibit cyclooxygenase-2, an enzyme involved in the production of prostaglandins. Reduced paw thickness in groups receiving lower doses of the turmeric extract—150 and 300 mg/kg bw—could be the result of an insufficient dose, poor absorption, or more crucially a postponed start to therapy. It is recommended to begin treatment before the condition progresses to a chronic stage and to adjust the dosage as necessary.

CONCLUSION

Due to its numerous benefits, which appear to mostly be accomplished through anti-inflammatory mechanisms, C. longa has attracted attention from all around the world. The analysis found that turmeric's anti-inflammatory activity was dose-dependent due to its nutritional composition and phytoconstituents (bioactive compounds). The analysis found that turmeric is both healthful and a respectable source of carbs. Since high anti-inflammatory activity was observed at 600 mg/kg body weight as found in the positive control group, it is proposed that more scientific research be done on other turmeric plant components such as roots, stems, leaves, etc. for effective anti-inflammatory action.

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