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Article

Biochemical and Redox Activity of Curcuma Longa Leaves Naturally Grown in Rodent Model

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Abstract: Turmeric, with the Latin name Curcuma longa, is widely known for its extensive medicinal properties, most of which originate from a class of bioactive components inherently contained in the plant itself. A study focused on this plant specifically selected rodents as experimental models, with two items to be detected: one was the various biochemical components contained in the leaves of Curcuma longa, and the other was its antioxidant activity — that is, the redox regulatory capacity mentioned in the original text. The experiment specifically used Wistar strain rats. The researchers divided all the rats participating in the experiment into two equal groups: one was the control group receiving no additional intervention, and the other was the experimental group to observe changes. The daily diet of the experimental group's rats was additionally supplemented with powder ground from Curcuma longa leaves, creating a distinction from the regular diet of the control group. Subsequent biochemical test results showed that these turmeric leaves contained large amounts of phenols, flavonoids and curcuminoids. To further verify its antioxidant capacity, the study also adopted three specialized antioxidant assay experiments: DPPH, ABTS, and FRAP. The final measurement revealed that the turmeric leaves had a strong free radical scavenging ability — and scavenging free radicals is precisely one of the core manifestations of antioxidant effects. Oxidative stress markers, including malondialdehyde, and superoxide dismutase, catalase, and glutathione peroxide assessed in the blood and liver tissue. The experimental group showed low levels of MDA and an increase in SOD, CAT, and GPx activities compared to the control group. The statistical calculations verified the distinguishing difference between the groups with $p<0.05$. The outcomes of the research empower the value of antioxidant properties and the ability of Curcuma longa leaves to address issues related to oxidative stress.

Keywords: Redox Activity, Curcuma Longa, turmeric, Antioxidant.

Introduction

As a typical annual compound-leaved herbaceous plant of the ginger (Zingiberaceae) family, the turmeric stem at its root is edible. It is widely cultivated in tropical regions and serves as a staple in both traditional Chinese medicine and as an Ayurvedic home remedy for wound healing. The well-documented rhizomes of Curcuma longa are known for their therapeutic actions and include anti-inflammatory, anti-oxidant, anti-cancer properties [1], [2]. By contrast, despite their enormous possible

for producing biologically active substances [3], the leaves received relatively little attention until recent years. In its leaves are Secondary metabolites and these active compounds primarily include the complex of curcuminoids consistent of demethoxycurcumin, curcumin and bisdemethoxycurcumin. These agents increase the bioactivity of the given drug significantly. It is closely related to the pathogenesis of the disease, especially the role of oxidative stress in the development of many chronic diseases including cardiovascular disease, diabetes and neurodegenerative illnesses [4]. From this foundation, it can be seen how natural anti-oxidants such as what is found in Curcuma longa leaves might be beneficial for health [5].

Previously, the work of other studies mostly focus on Curcuma longa rhizomes and leaves are left relatively little researched. They found a wide range of phenolic and flavonoid compounds from those parts [4], [5], [6]. This trial has main goals is to speculate on the biochemical composition of the leaves of Curcuma longa, examine their antioxidant action using a range of assays, and to see how these leaves affect various markers of oxidative stress in rodent model.

Materials and Methods

Plant Material

Leaves of Curcuma longa were collected from naturally grown plants. The leaves were thoroughly cleaned, dried and powdered using a mechanical grinder. These powdered leaves were stored in an airtight container at room temperature till further use.

Animal Model

A total of forty Wistar rats weighing 150-200 grams of both sexes were obtained for the study. The rats were kept under laboratory conditions with a 12-hour light period and a 12-hour dark period and the rats were given food pellets and water ad libitum. The study was conducted as, per the guidelines of the Scientific Research Ethical Committee (SREC) of Pharmacy College – Tikrit University No. 16 in 7/4/2024.

Experimental Design

The present study was designed by selecting 20 Rats were divided randomly into two groups with 10 rats, in each group. The first group of rats is called the Control group and were fed with normal diet. The second group of rats were the experimental group which were fed with normal diet containing Curcuma longa Leaf powder 2% w/w. The study of the effect of Curcuma longa leaf powder supplementation was conducted for one month. The rats were periodically observed weekly for recording their body weight and food intake.

Biochemical Analysis

In order to evaluate the biochemical composition of Curcuma longa leaves, a series of spectrophotometric assays were applied in order to measure the total phenolic, flavonoid, and curcuminoid contents. The total content of phenols were estimated by the reaction of the Folin-Ciocalteu reagent with phenolic compounds, as reported by Singleton et al. [7]. As the reaction product is a blue complex that is able to be measured spectrophotometrically at 765 nm, the results were expressed as milligrams of gallic acid equivalents, per gram of weight. On the other hand, the total flavonoid content was measured through the formation of a flavonoid-aluminum complex, followed by measuring the absorbance of the previous solution at 415 nm, according to Chang et al. [8]. Moreover, the results were expressed as milligrams of quercetin equivalents per gram of dry weight. Another bioactive compound is curcuminoid, which includes curcumin, demethoxycurcumin, and bisdemethoxycurcumin and was determined by the high-performance liquid chromatography technique, according to Jayaprakasha et al. [9]. The HPLC analysis was done by passing the solution through a reverse-phase C18 column, by setting the mobile phase as acetonitrile and water, and measuring at 425 nm, the results were expressed as milligrams per gram of dry weight.

Antioxidant Activity

We investigated the antioxidant activities present in Curcuma longa leaves using a comprehensive approach that examined multiple dimensions of their protective capacity.

A triple antioxidant activity analysis were employed including: DPPH radical scavenging activity, ABTS radical cation decolorization assay, and the ferric reducing antioxidant power (FRAP) assay iron ion reduction antioxidant capacity—fully revealed its powerful antioxidant activity. Using commonly used reagents for studying the stability of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals in antioxidant studies, a preliminary qualitative study of the antioxidant activity of turmeric leaf extract was conducted, demonstrating its ability to scavenge DPPH free radicals. [10].

The ABTS supercationic decolorization tests did not cause significant color pull on the samples. The degree of decolorization by the fluorescence inducer could be preliminarily determined by measuring the relative attenuation of the fluorescence in the control group using ABTS base (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as a substrate. The degree of decolorization was expressed as the percentage of inhibition by spectrophotometric measurements, while also considering the antioxidant components contained in this chemical method. The decolorization ability was further tested through fluorescence experiments using the fluorescence inducer. [11]. Lastly, the FRAP assay was performed with the leaf extract to determine its reducing power.

This assay measures the reduction of ferric (Fe^{+3}) to ferrous (Fe^{+2}) ions in the presence of the extract, with the results expressed in micromoles of Fe(II) equivalents per gram dry weight [12]. All these tests combine to give a comprehensive indication of the antioxidant potential of Curcuma longa leaves.

Measuring Oxidative Stress

By collecting blood samples from animals on a high-fat diet and comparing their activity against various oxidative stresses, we can preliminarily reveal the possible pathogenic mechanisms in humans. Utilizing the specific properties of malondialdehyde (MDA), specifically its reaction with thiobarbituric acid to reduce its original concentration, we can indirectly provide a reliable estimate of its concentration. Through the reaction of MDA with a series of typical chromogenic complexing agents such as thiobarbituric acid, and by measuring the intensity of the reaction using spectrophotometry, we study the cost-effectiveness of spectrophotometry. [13].

The activity of the lawn can be preliminarily determined by colorimetric analysis. A preliminary assessment of its activity can be made by accurately detecting the formation of superoxide and its reduction effect on nitroblue tetrazolium. [15]. Its activity can be indicated by detecting the decrease in the absorbance of hydrogen peroxide at 240 nm, thus indirectly demonstrating its catalytic activity in decomposing hydrogen peroxide into water and oxygen [15].

Finally, by measuring the specific activity of glutathione peroxidase and its peroxides (hydrogen peroxide and organic hydrogen peroxide) using a colorimetric method, we were able to preliminarily reveal its general activity. A more accurate assessment of GPx activity can be made by measuring the decrease in NADPH absorbance at 340 nm. [16].

Together these tests are a comprehensive assessment of not only rat liver cell oxidative stress but also how animals defend themselves against such damage.

Results

Biochemical Composition

The chemical analysis of Curcuma longa leaves indicated an existence of high levels in the total phenolic contents, flavonoids and curcumimiods. A concentration of 52.3 ± 2.1 mg GAE/g was deduced as the total phenolic content. Total flavonoid content measured at 27.8 ± 1.5 mg QE/g, as indicated in the table 1. The curcuminoid content was further quantified as 16.2 ± 0.8 mg/g.

Table 1. the biochemical composition of the plants.

Parameter	Content (mg/g)
Total Phenolic Content	52.3 ± 2.1 mg GAE/g
Flavonoid Content	27.8 ± 1.5 mg QE/g
Curcuminoid Content	16.2 ± 0.8 mg/g

Antioxidant Activity

In the antioxidant assays, radical scavenging activities were significantly higher in experimental group than from those of control Group. In the experiment group a meaningful antioxidant activity in DPPH radical scavenging assay was seen (81,5% ± 3.2%) compared to control group as well where only showed about 22.7% ± 2.8%. The percentage of inhibition for the experimental group against ABTS radical cation decolorization assay was 72.6 % ± 2.9, while it showed a significantly lower ($p < 0.001$) value for control. The ferric reducing antioxidant power (FRAP) assay also showed increased antioxidative activity in the experimental group compared with that of the control, where it exhibited a FRAP value corresponding to 512 ± 25 μmol Fe(II)/g and this was significantly higher than for those recorded to be105 ±18μ molFe(II)/g at $p<0.05$ (Table 2).

Table 2. radical scavenging activities of the study.

Assay	Control Group	Experimental Group	*p*-value
DPPH Radical Scavenging Activity	22.7% ± 2.8%	81.5% ± 3.2%	<0.05
ABTS Radical Cation Decolorization	18.4% ± 2.3%	72.6% ± 2.9%	<0.001
FRAP Assay	105 ± 18 μmol Fe(II)/g	512 ± 25 μmol Fe(II)/g	<0.05

Oxidative Stress Markers and antioxidant enzyme activities:

Independent-samples t-tests indicated statistical significance between control and experimental for all oxidative stress markers, as well antioxidant enzyme activities at $p<0.05$. all of the studied parameters showed a significant improvement in the experimental group as compared to control. The experimental group had lower malondialdehyde levels significantly comparing to the control group ([2.8 ± 0.3] vs [4.5 ± 0.4] nmol/mg protein, respectively). Lower level of MDA indicated less lipid peroxidation in the group subjected to treatment as depicted. The activity of superoxide dismutase (SOD) which converts superoxide radicals to oxygen and hydrogen peroxides was determined in experimental group; it showed significantly higher production compared with control group that rendering SOD as U/mg 45.6 ± 3.2 vs 36.2 ±2.8. Conversely, an increase in catalase activity (decomposing hydrogen peroxide to water and oxygen) was found in the experimental group: 29.4 ± 2.1 U/mg protein compared with that of control rats (24.5±1.9 CSAA unit). The activity of glutathione peroxidase (GPx) was also higher in the experimental group as presented at 18.6 ± S.E3 U/mg protein compared to that observed in pooled cytosol from control animals, which measured activation level 14.8 ± S.DP2 U/mg protein against hydrogen and low organic hydroperoxides.

Table 3. Oxidative stress markers and antioxidant enzyme activities

Marker	Control Group	Experimental Group	*p*-value
MDA Levels	4.5 ± 0.4 nmol/mg	2.8 ± 0.3 nmol/mg	<0.05
SOD Activity	36.2 ± 2.8 U/mg	45.6 ± 3.2 U/mg	<0.05
CAT Activity	24.5 ± 1.9 U/mg	29.4 ± 2.1 U/mg	<0.05
GPx Activity	14.8 ± 1.3 U/mg	18.6 ± 1.5 U/mg	<0.05

Discussion

This study provides strong evidence that leaves from C. longa exert potent antioxidant effects. The biochemical screening showed the presence crude phenolic, flavonoid and curcuminiods with their antioxidant activity Our findings were consistent with the previously reported literature that demonstrated profound bioactive potential associated with Curcuma longa [17].

The antioxidants in turmeric, we were not only surprised to discover its powerful ability to scavenge free radicals in the body, but also revealed its many other health benefits for the human body. Results from DPPH, ABTS and FRAP assay revealed greater antioxidant activity in the experimental group than control. Thus, these results confirm the previous findings indicating that leaves have the ability to scavenge free radicals and can be effective in counteracting to diseases related to oxidative stress [5].

Oxidative stress markers also just showed the antioxidant potential of Curcuma longa leaves. In relation to the control group, we observed a reduction in MDA levels and an increase of SOD, CAT and GPx activities were found for experimental group. MDA is associated with lipid peroxidation which when reduced means that real oxidative damage. The SOD, CAT and GPx are important antioxidant enzymes to protect cells against oxidative damage. Enhanced antioxidative defense mechanisms can be inferred by the increase in activities of these enzymes in experimental group for example, [6].

In another study, the correlation of investigated parameters analysis had proven significant differences between control and experimental groups. Through research on turmeric supplements, we can easily find that its multi-dimensional adjustment of the salty flavor, a comprehensive dependent variable, plays a significant role in increasing the overall content of antioxidants [18], [19].

The discovery of the potential therapeutic value of turmeric leaves has significant practical and theoretical value. Its outstanding in vitro antioxidant activity and ability to significantly reduce the levels of in vivo oxidative stress markers make it a promising candidate drug for preventing or improving various diseases caused by redox imbalances. Its pathogenic effect on oxidative stress is particularly prominent in patients with chronic diseases, such as cardiovascular disease, diabetes, and other neurodegenerative diseases [4], [20], [21].

Conclusion

Comprehensive research has revealed that turmeric extract not only possesses significant biochemical effects but also exhibits good antioxidant potential. Its ability to reduce oxidative stress in rodents and enhance their antioxidant defense mechanisms demonstrates its strong antioxidant properties. In conclusion, our research preliminarily confirms that turmeric leaf extract has excellent therapeutic effects, making it worthy of further research and application in clinical practice.

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Conflict of Interests

The authors declare no conflict of interest during this study.

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