

## Investigating Anti-diabetic Effects of Autoclaved *Curcuma longa* Linn (turmeric) Extracts using Mouse Tissues in vitro

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### Abstract

Curcumin, the active compound found in turmeric, is believed to delay the development of diabetes through several mechanisms. This study aimed to investigate if an aqueous extract of turmeric can improve glucose uptake and uric acid in mouse tissues in vitro, after inclusion of turmeric in the diet for four weeks. Fourteen adult male Swiss mice were divided into three groups. The first group was the control (n=6) that was given clean water, the second group of mice (n=4) was given 5% autoclaved turmeric extract in drinking water, and the third group (n=4) was given 5% non-autoclaved turmeric extract in drinking water. After four weeks, the cardiac muscle, skeletal muscle, pancreas, and liver tissues were dissected and used for analysis. The results showed that the aqueous 5% turmeric extract reduced glucose in cardiac tissues while the plasma glucose was not changed. Cardiac muscle, liver, pancreas, and skeletal muscle showed glucose absorption after the 5% turmeric treatment. This research shows that turmeric did improve glucose uptake in most tissues, although it was not significant due to the limitations of this study. Tissues may need to be cultured longer and media processed quicker to prevent evaporation. Turmeric continues to show great potential in the treatment of type 2 diabetes and may present an alternative way of treating diabetes.

**Keywords:** Anti-diabetic; *Curcuma longa* Linn; In vitro; Turmeric

## Introduction

There is historical interest in the use of traditional medicines for the management of different diseases. Understanding traditional medicines may be a basis for the development of newer, safer medicines. However, there is a lack of evidence-based data about this medical practice, and scientific examinations are required to determine the physiological mechanisms of these medicinal plants to obtain scientific data and improve the development of novel medicinal treatment options.<sup>1,2</sup>

Turmeric is traditionally used for spicing and colouring food.<sup>3</sup> Researchers have reported several medicinal properties of turmeric when it is ingested, which include it being anti-diabetic, hypolipidemic, anti-inflammatory, anti-diarrhoeal, and even anti-cancerous.<sup>4</sup> The active compound found in turmeric thought to produce these pharmacological effects is called curcumin. Curcumin is believed to delay the development of diabetes through several mechanisms associated with protein oxidation, lipid peroxidation, and anti-inflammatory factors, such as tumour necrosis factor-alpha, nuclear factor-kappa B, peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ). Moreover, curcumin may also increase the plasma insulin level, the activity of lipoprotein lipase (LPL), and be involved in the activation of liver enzymes linked with glycolysis, gluconeogenesis, and lipid metabolism.<sup>5</sup>

Curcumin, demethoxycurcumin, bisdemethoxycurcumin and ar-turmerone have been reported as the dominant anti-diabetic pharmacological compounds of *Curcuma longa* ethanol extract.<sup>6</sup> There is evidence that *Curcuma* has the ability to decrease the level of blood glucose in different in vivo models and that it has other pharmacological effects, such as anti-inflammatory, anti-carcinogenic and antioxidant effects. *Curcuma longa* has

gained interest as a novel alternative in anti-diabetic therapy.<sup>5,6</sup> The purpose of this study is to understand the effect of filtered aqueous 5% *Curcuma longa* (turmeric) extract on glucose uptake in mice and its insulin-like properties over a period of four weeks. Tissues from the liver, pancreas, cardiac muscle, and skeletal muscle of treated mice were tested in vitro using tissue culture.

Previous work that was carried out using in vitro tissue cultures found that 10uL/mL of aqueous extract of *Curcuma longa* (AEC) had a significant effect on insulin release with reduced or no toxicity.<sup>1</sup> This study led to the suggestion that water-soluble components of turmeric stimulate the secretion of insulin from the pancreas and increase glucose uptake in in vitro muscle tissues. AEC may act through an insulin-mediated pathway resulting in enhanced peripheral glucose uptake.<sup>1</sup> However, autoclaved AEC has not previously been studied. Traditionally boiling or cooking turmeric is similar to autoclaving; the heating process generally denatures proteins.

The aim of this study is to understand if *Curcuma longa* bioactive compounds may be useful in the regulation of high blood glucose levels and to understand if non-protein molecule/s in aqueous turmeric extract that withstand the autoclaving process may improve insulin release and have insulin-like properties. To do this, we prepared a 5% autoclaved aqueous extract of *Curcuma longa* and a 5% non-autoclaved aqueous extract and tested them on mice.

## Methods

### Materials

All chemicals and products used were of analytical quality and were purchased from the following manufacturers: Austral Herbs (Uralla – NSW 2358, Australia): *Curcuma longa* Linn (CL) rhizome; Sarstedt Australia

Pty Ltd (South Australia): Dulbecco's modified Eagle's medium (DMEM).

**Preparation of *Curcuma longa* Linn Extracts**  
Curcuma extracts were made by soaking 10g of CL powder in 1L distilled water for 10 days at room temperature.<sup>6,7</sup> The root (rhizome), which can be steamed or boiled, dried, and ground into a powder to create an extract, is the plant portion that is employed.<sup>3</sup> Curcumin is the name of the active ingredient in turmeric that is known to have pharmacological effects. It was established that curcumin has a diferuloylmethane chemical structure.<sup>8</sup>

Tumor Necrosis Factor Alpha (TNF-  $\alpha$ ) and free fatty acids (FFA) are thought to be weakened by curcumin. Additionally, nuclear factor-kappa B activation is inhibited by curcumin. Peroxisome proliferator-activated receptor gamma has been demonstrated to be activated by curcumin. Additionally, lipoprotein lipase activity and plasma insulin levels may both rise as a result of curcumin. Additionally, there is proof that curcumin plays a role in the activation of liver enzymes related to lipid, glycolysis, and gluconeogenesis metabolism.

Whatman no. 1 filter paper was used to filter the aqueous extract. To eliminate smaller particles, the extract was centrifuged at 7000 G for 15 minutes before being kept at -20C. Two 5 percent CL extracts were made, one of which was autoclaved. The 10uL/mL AEC had a considerable impact on insulin release with little to no damage, according to earlier research employing in vitro tissue cultures. This research raised the possibility that the water-soluble turmeric compounds stimulate insulin release from the pancreas and boost glucose uptake in in vitro muscle tissues.<sup>1</sup>

A 5% autoclaved AEC extracts was separated based on molecular size and analyzed

individually. A 5% extract was chosen as it has less or no toxicity during treatment<sup>8,9</sup> *Curcuma longa* ethanol extract was found to have PPAR-ligand-binding activity, to induce the differentiation of adipocytes in a dose-dependent manner, and to have a persistent hypoglycemic impact on genetically diabetic KK-Ay mice.<sup>6</sup>

Curcumin, demethoxycurcumin, bisdemethoxycurcumin, and arturmerone were shown to be the main anti-diabetic pharmacological components of *Curcuma longa*, in addition to conducting the extraction of curcuminoids and sesquiterpenoids from the ethanol extract of *Curcuma longa*.<sup>6</sup> In an in-vitro tissue culture experiment, it was previously demonstrated in our lab that water-soluble turmeric components stimulate insulin release from the pancreas and boost glucose absorption in muscle tissues.

AEC at lower concentrations of 0.1uL/ml had little effect, but at 10uL/ml, insulin release was significantly affected with little to no tissue harm.<sup>1</sup> AEC may work through an insulin-mediated route, resulting in increased peripheral glucose absorption, according to a 2011 hypothesis made by Mohankumar and McFarlane. Traditionally, turmeric is consumed with foods that have been boiled or cooked in an autoclave. But prior research on turmeric extracts that had been cooked or autoclaved was lacking. We predict that non-protein molecules that withstand the autoclaving procedure in aqueous turmeric extract may enhance insulin release and/or contain insulin-like characteristics. The heating technique typically denatures proteins. These bioactive compounds might help control and/or decrease hyperglycemic diseases like diabetes.

#### *Animal Experiments and Preparation of Animal Tissues*

Fourteen adult male Swiss mice (18–22 g)

were acquired from the Small Animal House, UNE, in accordance with the approval of the University of New England Animal Ethics Committee and NH & MRC guidelines. The mice were housed in suitable conditions and were kept at 21°C in a light-controlled environment (light off at 7pm and on at 7am every day). Mice were randomised into one of three groups: i) control, given clean water (n=6); ii) given 5% autoclaved turmeric extract in drinking water (n=4); iii) given 5% non-autoclaved turmeric extract in drinking water (n=4). Mice had access to their respective drinking water during the experiment.

#### *Blood/Organ Collection*

Blood samples were collected using heart puncture after four weeks. Plasma was collected after samples were taken in heparinized tubes and centrifuged at 1000 G for 20 minutes. Plasma was then kept at -20°C in 5mL vials. The pancreas, heart, abdominal muscle tissues, and liver were directly collected and put in ice-cold phosphate-buffered saline after mice were euthanized by CO<sub>2</sub> asphyxiation (phosphate buffered saline (PBS), pH 7.4). Tissues were collected and cut into 2mm3 pieces, which were then weighed.

#### *In vitro Bioassay - Tissue Culture*

Before incubation, tissues were cleaned in PBS and five pieces of each tissue were inserted in each well of a 48-well tissue culture plate.<sup>10</sup> To simulate normal glycaemic circumstances, each well was filled with 1 mL of DMEM and 5 mM glucose solution. As a positive control, insulin was administered to wells cultured with skeletal muscle tissue.

The incubation took place for 3 hours at 37°C in a humidified environment with 5% CO<sub>2</sub>. The tissue samples were weighed again after incubation, and the culture media and tissue samples were stored at -20°C until analysis.

Plasma glucose analysis and uric acid analysis At room temperature,<sup>11</sup> frozen mouse plasma and tissue culture medium were thawed. Glucose and uric acid concentrations were measured in 100uL samples using a DADE clinical analyser (DADE-XL, USA) according to the manufacturer's instructions.

#### *Data Analysis*

To assess metabolic uptake by concentration, tissue weights were utilized. The data from serum glucose secretion and uptake as well as tissue culture glucose secretion and uptake were statistically analyzed using SAS statistical software (SAS Institute Inc. Cary, NC, USA) and evaluated using a two-way ANOVA followed by the Student-Newman Keuls post hoc test. The results were provided as mean standard deviation (SD), and p≤0.05 was used to determine significance.

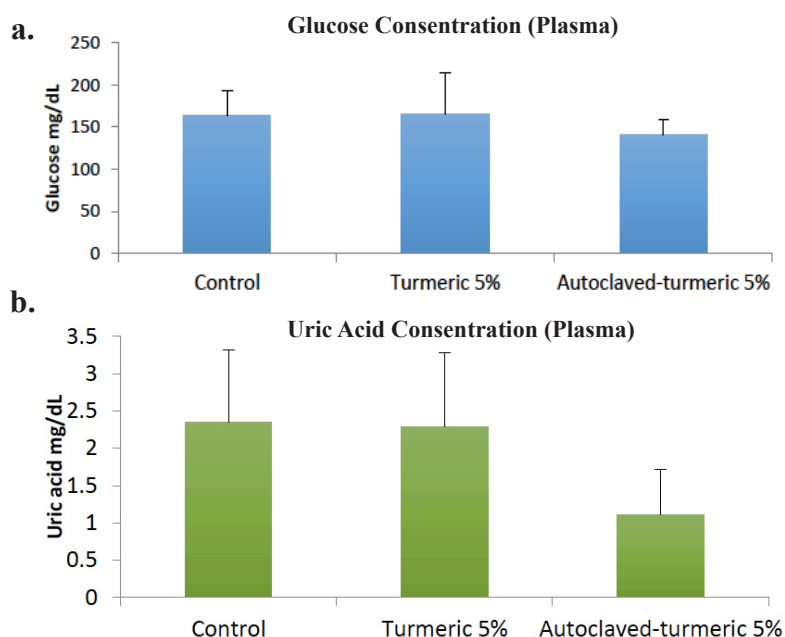
### **Results and Discussion**

#### *Glucose and uric acid analysis in plasma*

The glucose concentration of the autoclaved turmeric treatment group was 140.84 mg/dL and that of the 5% turmeric treatment group was 164.90 mg/dL, compared to the negative control group, which was 164.37 mg/dL. There was no significant difference between the treated groups (Figure 1a).

Plasma uric acid was also measured in all the treatment groups. Uric acid concentration in the control was 2.35 mg/dL, 2.29 mg/dL in the 5% turmeric treatment group and 1.11 mg/dL in the autoclaved turmeric treatment group. There was no significant difference among groups (Figure 1b).

Plasma glucose analysis in mice treated with 5% turmeric showed glucose secretion while the autoclaved 5% turmeric treatment indicated that glucose was absorbed in plasma. Glucose transporters (GLUT) 1 and GLUT 3 proteins are found abundantly in the plasma membrane



**Figure 1. Glucose and Uric Acid analysis in Plasma**

a) The plasma glucose concentrations after treatment of mice for four weeks in the control, 5% turmeric and 5% autoclaved turmeric treatment groups. b) The uric acid concentrations in plasma in the control, 5% turmeric and 5% autoclaved turmeric treatment

of the cells;<sup>12</sup> thus, the autoclaved 5% turmeric may contain a non-protein compound that may improve insulin release and have insulin-like properties that could mobilise GLUT 1 and GLUT 3 proteins for glucose transportation

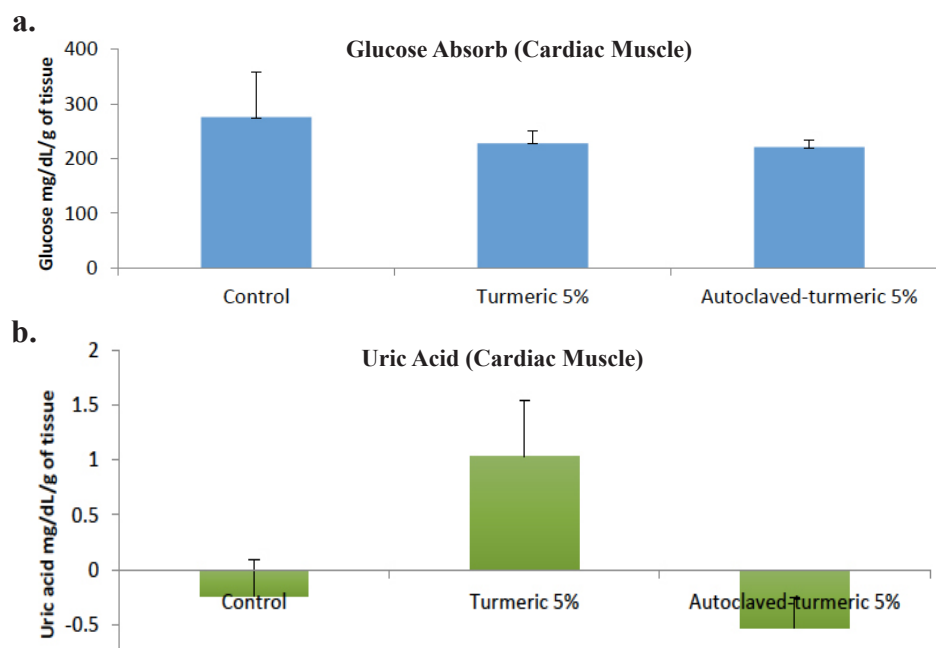
The first glucose transporter to be cloned was GLUT1, one of the most thoroughly studied membrane transport proteins. With 98 percent identity between human and rat GLUT1 and 97 percent identity between human and mouse, rabbit, or pig sequences, the GLUT1 amino acid sequence is highly conserved.<sup>13</sup> The remarkable degree of sequence conservation of this 492-residue protein shows that each of its domains is essential for functional functions. A significant affinity exists between GLUT1 and glucose. These characteristics imply that constitutive glucose absorption is most likely controlled by GLUT1.<sup>13</sup>

The bulk of GLUT1 is located in multiple organs in the endothelial cells of blood-tissue

barriers. As a result, one of the specialized roles of GLUT1 is the passive diffusion of glucose between the blood and organs that have limited access to minute solutes.<sup>13</sup> GLUT3 was first identified in the skeletal muscle of a human fetus.<sup>13</sup> While human GLUT1 and GLUT2 share 64 and 52 percent, respectively, of their amino acid sequences with mouse GLUT3, human GLUT3 shares 83 percent of its amino acid sequence with mouse GLUT3.

As a result, GLUT3's sequence is less conserved across species than GLUT1's is, much like GLUT2's. Although GLUT3 messenger RNA (mRNA) is present in all human tissues in different levels, the brain, kidney, and placenta have the largest concentrations.<sup>13</sup> This transporter, which is assumed to be the main neuronal glucose transporter and is present in both dendrites and axons, exhibits different levels of expression depending on the regional cerebral glucose uptake in the brain. Effective glucose absorption is ensured





**Figure 2. Glucose Absorption and Uric Acid in Cardiac Muscle**

a) The results for cardiac muscle glucose levels after treatment of mice for four weeks in the control, 5% turmeric and 5% autoclaved turmeric groups. b) The uric acid levels in cardiac muscle between the control, 5% turmeric and 5% autoclaved turmeric groups

by GLUT3, which has the largest estimated turnover number of all the GLUT isoforms and the highest affinity for glucose ( $K_m$  1.5 mM). Given GLUT3's extensive distribution in human tissues, it's possible that GLUT1 and GLUT3 are both involved in baseline glucose transport.<sup>13</sup>

#### *Glucose absorption and uric acid in cardiac muscle*

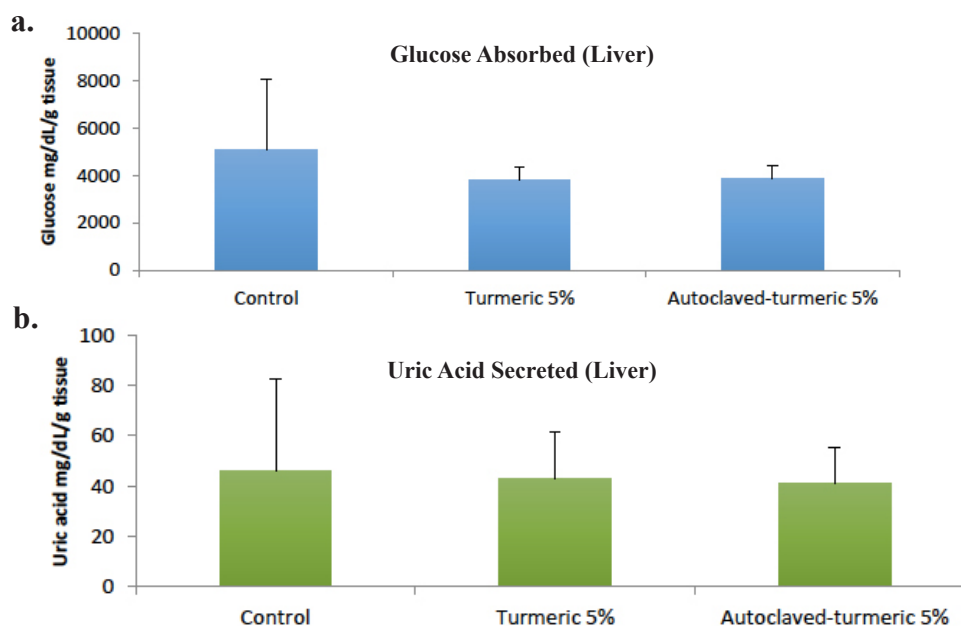
Glucose was absorbed by cardiac muscles as indicated by a decrease in glucose concentration in the medium. Glucose concentrations in cardiac muscles were compared, control group was (275.48 mg/dL/g tissue), 5% turmeric group was (227.66 mg/dL/g tissue) and 5% autoclaved-turmeric group was (220.91 mg/dL/g tissue) (Figure 2a).

However, medium incubated with cardiac muscles showed a change in uric acid concentration between the different treated

groups. The control (-0.23 mg/dL/g tissue) and 5% autoclaved treatment group (-0.53 mg/dL/g tissue) showed little secretion of uric acid when compared to the negative control. The 5% turmeric group (1.03 mg/dL/g tissue) showed absorption of uric acid by cardiac muscle.

The 5% turmeric-treated cardiac muscle tissue had significant ( $p < 0.05$ ) absorption of uric acid when compared to the control and 5% autoclaved turmeric groups, which had higher uric acid levels. There was no significant difference between the control and autoclaved turmeric groups (Figure 2b). There was no significant difference in glucose concentration between groups.

In the heart muscle, similar results were observed. There was glucose absorption in the heart muscle in both the autoclaved and non-autoclaved turmeric treatments. The GLUT 1 and GLUT 4 proteins aid glucose



**Figure 3. Glucose Absorption and Uric Acid in the Liver**

a) The results for liver glucose levels after treatment of mice for four weeks in the control, 5% turmeric and 5% autoclaved turmeric treatment groups. b) The uric acid levels in the liver between the control, 5% turmeric and 5% autoclaved turmeric treatment groups

absorption in the heart muscle.<sup>14</sup> As a result, the turmeric treatment with 5% turmeric has a hypoglycaemic impact.

#### *Glucose absorption and uric acid in the liver*

Glucose was absorbed by the liver tissue. When compared, there was no significant difference in glucose absorption between the control group (5064.71 mg/dL/g tissue), the 5% turmeric (3811.04 mg/dL/g tissue), and the 5% autoclaved turmeric (3855.80 mg/dL/g tissue) treatment groups (Figure 3a). Uric acid was absorbed by the liver tissues; analysis did not show a significant difference between the different groups (Figure 3b).

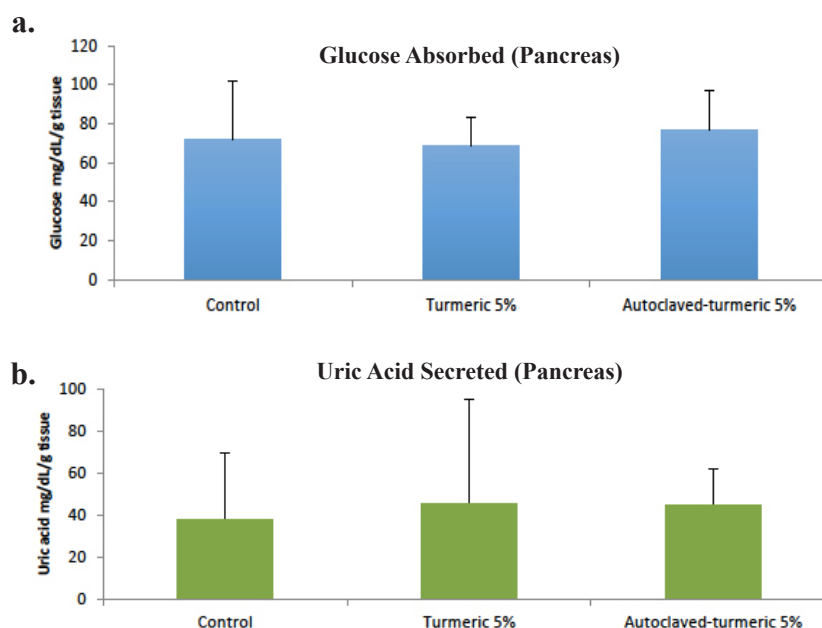
Ingested glucose may cause the liver to respond by dumping the excess glucose.<sup>15</sup> Insulin mobilizes GLUT 2 proteins, which are located in the liver and help with glucose transport. Glucose absorption was seen in both the autoclaved and non-autoclaved turmeric treatments in this investigation. As a result, the 5% turmeric therapy contains a chemical or

protein that works in a similar way to insulin to achieve hypoglycemia.

#### *Glucose absorption and uric acid in the pancreas*

The glucose results in the pancreas showed absorption and secretion in the different treatment groups. The 5% autoclaved turmeric treatment group (76.82 mg/dL/g tissue) was comparable to the control group (71.55 mg/dL/g tissue), which indicated glucose secretion, while the 5% turmeric treatment group (68.66 mg/dL/g tissue) showed glucose absorption (Figure 4a). Uric acid was secreted by the pancreas tissue. When the control (38.65 mg/dL/g tissue), 5% turmeric treatment group (45.93 mg/dL/g tissue) and 5% autoclaved turmeric treatment group (45.37 mg/dL/g tissue) were compared, there was a slight difference in uric acid secretion (Figure 4b).

The glucose sensor is located in the pancreas' Langerhans islets. The glucose transporter GLUT 2 is expressed in pancreatic beta



**Figure 4. Glucose Absorption and Uric Acid in the Pancreas**

a) The pancreas tissue glucose levels after treatment of mice for four weeks in the control, 5% turmeric and 5% autoclaved turmeric treatment groups. b) The uric acid levels in the control, 5% turmeric and 5% autoclaved turmeric treatment groups

cells.<sup>16</sup> Only the autoclaved 5% turmeric therapy demonstrated glucose absorption in mice, indicating that it contains an insulin-like component. Glucose secretion was observed in the non-autoclaved 5% turmeric therapy. This result was unexpected, and it's possible that it was caused by human error.

#### *Glucose and uric acid in skeletal muscle*

Glucose was absorbed by the skeletal muscle. This was shown in the control group of the skeletal muscle (467.59 mg/dL/g tissue) and the turmeric treatment groups: non-autoclaved (174.09 mg/dL/g tissue) and autoclaved (200.27 mg/dL/g tissue), which showed significant reduction (Figure 5a).

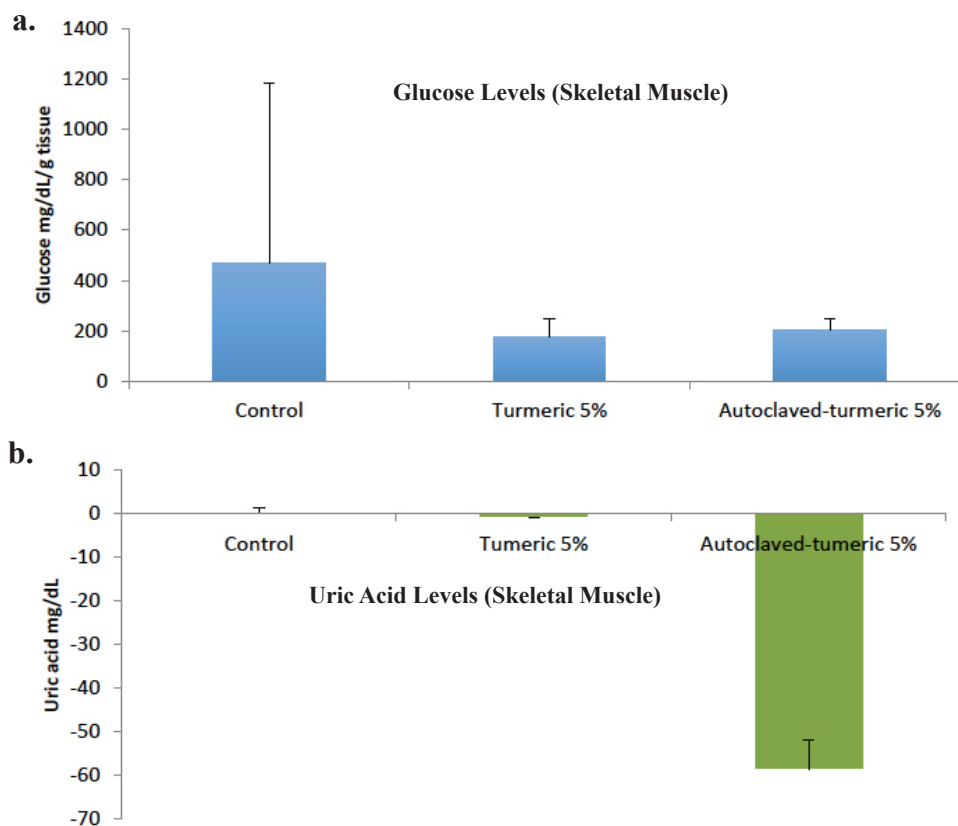
Uric acid was secreted by skeletal muscle tissue in the control and treatment groups. The control had very low or negligible levels of uric acid when compared to the negative control (-0.48 mg/dL/g tissue). Uric acid levels in the 5% turmeric-treated skeletal muscle (1.04 mg/dL/g tissue) and the 5%

autoclaved turmeric-treated skeletal muscle tissues were high (58.52 mg/dL/g tissue). The difference between the control and autoclaved turmeric group was significant ( $p < 0.05$ ), and the difference in the uric acid levels in the turmeric and autoclaved turmeric treatment groups were also significant when compared ( $p < 0.05$ ) (Figure 5b).

In general, oral anti-diabetics and insulin can keep blood glucose levels within a normal physiological range; however, because they don't stimulate the pancreas to generate more insulin, long-term diabetes patients are more likely to develop major complications.<sup>1</sup> This emphasizes the need for more effective and safer treatments.

The anti-diabetic benefits of turmeric aqueous extracts are thought to be mediated by the insulin secretion and action pathway.<sup>17-20</sup> Turmeric may include a single or numerous secondary metabolites that operate directly or indirectly to maintain glucose homeostasis





**Figure 5. Glucose and Uric Acid in Skeletal Muscle**

a) The results for skeletal muscle glucose levels after treatment of mice for four weeks in the control, 5% turmeric and 5% autoclaved turmeric treatment groups. b) The results for skeletal muscle uric acid levels after treatment of mice for four weeks in the control, 5% turmeric and 5% autoclaved turmeric treatment groups

insulin secretion and action pathways. Turmeric has been found to have a stimulatory effect on insulin secretion as well as an insulin-like effect on glucose absorption in additional in vitro experiments.<sup>17-20</sup>

By lowering hepatic glucose production, suppressing the inflammatory state brought on by hyperglycemia, increasing the expression of the GLUT4, GLUT2, and GLUT3 genes, activating AMP kinase, promoting PPAR ligand-binding activity, increasing insulin secretion from pancreatic tissues, enhancing pancreatic cell function, and lowering insulin resistance, curcumin can lower blood sugar levels.<sup>21</sup> Insulin mobilizes skeletal muscle at rest. GLUT Glucose diffusion is aided by four proteins.<sup>22-24</sup>

It's been proposed that glucose control happens at the transcriptional or translational level. Alternatively, cellular absorption of the active substance could necessitate facilitative transport and be sensitive to the kinetics of such a process.<sup>25</sup>

There was glucose absorption in the skeletal muscle in response to both the autoclaved and non-autoclaved turmeric administrations. As a result, the turmeric therapy could contain an insulin-like molecule that activates GLUT 4 for glucose transport. Based on these findings, aqueous preparations of turmeric may be a viable option for people with type 2 diabetes who want to maintain glucose homeostasis while avoiding adverse effects.

The small number of animals used in this study is one of its major limitations. Therefore, careful interpretation is required when using our findings. On a broader scale, more research is necessary.

### Conclusion

*Curcuma longa* Linn, a medicinal herb, was found to have hypoglycaemic properties in this study. In vitro, the probable molecular pathways contributing to *Curcuma longa*'s potentially anti-diabetic action on insulin secretion in the pancreas and glucose regulation by plasma membrane, cardiac muscle, liver, pancreas, and skeletal muscles have been demonstrated. However, more research is needed to isolate and identify the active metabolites responsible for hypoglycaemic activity's effects, as well as the exact mechanisms involved in reducing blood glucose levels.

### Acknowledgement

None

### Funding

None

### Conflict of Interest

None declared

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