

## Comparative Study on the Pro-Inflammatory Activity of Turmeric (*Curcuma longa*) and flaxseed (*Linum usitatissimum*)

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### ABSTRACT

**Background:** A wide spectrum of therapeutic effects of curcumin and flaxseed has been shown by extensive scientific research as an anti-inflammatory. IL6 cytokines are produced in large quantities during inflammation and serve as mediators in chronic inflammatory conditions. Moreover, IL-10 is classified as strong immunosuppression and anti-inflammatory cytokine.

**Objective:** The purpose of our study to assess and compare the anti-inflammatory capacity of turmeric and flaxseed either alone or in combination in a healthy animal model.

**Method:** We evaluate the serum level of IL-6 and IL-10 in healthy male swiss albino mice (n=72) after low dose oral intake of curcumin and flaxseed for six weeks by enzyme-linked immunosorbent assay.

**Results:** Our results demonstrate that dietary turmeric as well as flaxseed had a non-significance decrease in serum IL-6 level and could induce the expression and production of IL-10 level significantly. Moreover, turmeric and flaxseed co-administration appear to be more positive health impacts to boost inhibition of IL 6 and improve IL 10 induction.

**Conclusion:** Turmeric and flaxseed may modulate the level of IL-10. Moreover, their combination having a greater anti-inflammatory potential efficiency than each one separately.

**Key words:** Turmeric, Flaxseed, anti-inflammatory, IL-6, IL-10, cytokines.

### Introduction

Turmeric is obtained from the *Curcuma longa* rhizome and is widely used in our diet. Curcumin is the most researched bioactive nutraceuticals derived from turmeric for its advantage in the treatment of diverse diseases and its capacity to regulate inflammatory response [1]. Moreover, curcumin exhibited therapeutic efficacy in a variety of inflammatory diseases through regulations on various signaling molecules as cytokines, tumor suppressor genes, transcription factors, and microRNAs [2]. Recent studies were carried out on the use of curcumin as a health-improving agent. Therefore, turmeric and its ingredient, curcumin, are becoming increasingly interested [3].

Flaxseed is one of the oldest crops, its Latin name is *Linum usitatissimum*. Flaxseed contains several phytonutrients which promote health and have anti-inflammatory action [4]. Owing to the health benefits related to some of its biologically active elements, flaxseeds have become particularly involved in our diets and disease studies in the last two decades [5].

The initiation and development of various types of serious diseases are usually associated with inflammatory and infection conditions [6]. Inflammation reduction is one of the most effective forms of health-promoting. Fortunately, our diet can regulate the inflammatory process. Some nutrients have strong medicinal properties which can relieve inflammation [7]. Recently, research into novel anti-inflammatory compounds has been based on the natural products curcumin and flaxseed.

Cytokines are proteins produced on the cell surface by various kinds of cells and have a particular impact on cellular signals and communications [8]. IL-6 acts as both a pro-inflammatory cytokine and an anti-inflammatory cytokine [9]. Many studies revealed that the downregulation of IL-6 is associated with curcumin therapeutic effects [10]. Likewise, flaxseed has a remarkable significant inhibition of IL-6 [11]

IL-10, a potent anti-inflammatory mediator, and its levels play a crucial role in inflammation counter-regulation and play a key role in infections, by limiting immune response and preventing host damage [12,13]. The health benefits of curcumin may be linked to its ability to increase the expression of IL-10 [14]. As well as flaxseed can induce IL-10 production [15].

To date, curcumin and flaxseed studies have been carried out with the number of populations with existing health problems. The purpose of our study to assess and compare the level of IL-6 and IL-10 on healthy mice after administration with a safe dose of turmeric and flaxseed either alone or in combination for six weeks.

## Methodology

### Animals

A total number of 72 male healthy swiss albino mice (C57BL/6 strain), aged 6-8 weeks with bodyweight 20-30 gm. The animals were obtained from the European Country Farms in Egypt. Mice were well examined to ensure that they are free from any signs of microbial or parasitic diseases, and they were housed in Theodor Bilharz Research Institute (TBRI) in suitable plastic cages under a temperature of 21 C° and 60% humidity. Moreover, they were kept on a standard diet containing 24% protein, 4% fat, 4-5% fiber, and water ad-libitum. Animals were fed, housed, and handled according to the recommendation of (Guide For Care And use Of Laboratory Animals) and were approved by an institution responsible for animal ethics concerning care for animals and safe disposal of their wastes at TBRI.

### Experimental Design

Briefly, mice were allocated into 6 groups (12 animals/group). The first group was allocated to a control group. The Turmeric group administrated 2 gm turmeric and 20 mg piperine /kg body weight. Flaxseed group that administrated grinded flaxseed with daily consumption dose amount of 45 g/kg body weight. Flaxseed oil group administrated 0.2 ml flaxseed oil /25 gm. Turmeric/Flaxseed group administrated 2 gm turmeric and 20 mg piperine /kg and 45 g/kg grind flaxseed. Turmeric/Flaxseed oil group administrated 2 gm turmeric and 20 mg piperine /kg and 0.2 ml flaxseed oil /25 gm. Turmeric has been demonstrated by poor bioavailability due to poor absorption, fast metabolism, and rapid systemic elimination [16]. The bioavailability of curcumin was improved by the combination of piperin and turmeric [17]. In our study, we increased the bioavailability of turmeric by its combination with piperin.

All administration to the mice by P.O. gavage continued daily for 6 weeks. Four mice of each group were sacrificed after 2 weeks, 4 weeks, and 6 weeks. IL 6 and IL 10 levels were measured for each interval period.

### Assessment of interleukins (IL-6 and IL10) by ELISA:

Blood samples were collected, allowed to clot at room temperature, and centrifuged at 4000 rpm for 15 min (Eppendorf, Germany). Serum samples were collected and stored in -80 °C till use. IL10 & IL6 were assessed using commercial ELISA kit (NOVA Rat interleukin ELISA kit cat #: In Ra0655, China and Cloud Clone Corp. IL6 ELISA kit cat #: E-20303Mo, USA respectively) according to manufacture instruction.

In brief, both tests were performed by sandwich-ELISA method. The micro-ELISA strip plates have been pre-coated with antibodies specific to either IL-10 or IL6. Standard or samples were added to the appropriate micro-ELISA strip plate wells and incubated to allow the combination to the specific antibody. Then Horseradish peroxidase (HRP) as a conjugated antibody was added and incubated. The substrate solution was added to each well, the blue color of (IL 10 or IL6) and HRP-conjugated antibody complexes turned yellow after the addition of stop solution. The optical density was measured spectrophotometrically at a wavelength of 450 nm. The concentrations of either IL10 or IL6 were determined by comparing the optical density (OD) of the samples to the standard curve.

## Statistical Analysis

SPSS statistical package was used for data analysis, version 20 with Excel programming software used for tabulating and showing the data. All results were expressed as mean  $\pm$  SD. The significant difference between groups was noted using a one-way analysis of variance (ANOVA). P- values  $< 0.05$  were considered significant.

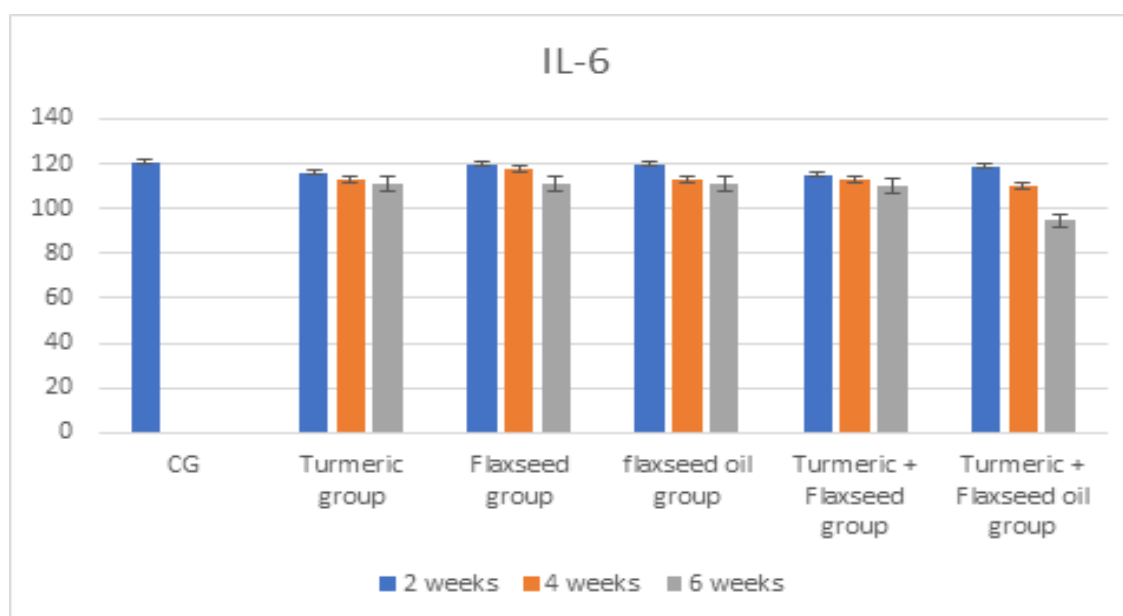
## Results

The current study did not observe significant inhibition of IL-6 release by any oral administrations of turmeric alone, flaxseed (grinded) alone, flaxseed oil alone, and coadministrations of turmeric with flaxseed. The effect of prolonged daily showed a slight decrease in serum IL-6 level. Our findings reported in Table 1 depicted the serum IL-6 level for each group. Decreasing of IL-6 level in each group was observed by increasing the administration period time from 2 to 6 weeks. Co-administration of turmeric and flaxseed oil showed a modest decrease of IL-6 than if they administrated separately even more than combined turmeric and whole grinded flaxseed. Results have been presented as the mean  $\pm$  standard deviation of four samples. The serum IL-6 levels throughout the study time interval are shown in Figure 1. It showed no statistically significant difference between the groups' IL-6 levels.

**Table1:** Changes in IL-6 level throughout the study.

	IL-6		
	2 weeks (Mean $\pm$ SD)	4 weeks (Mean $\pm$ SD)	6 weeks (Mean $\pm$ SD)
<b>Turmeric group</b>	116 $\pm$ 3.3	113 $\pm$ 3.5	111 $\pm$ 2.9
<b>Flaxseed group</b>	120 $\pm$ 2.1	118 $\pm$ 2.4	111 $\pm$ 3.4
<b>Flaxseed oil group</b>	120 $\pm$ 2.9	113 $\pm$ 2.9	111 $\pm$ 3.1
<b>Turmeric + Flaxseed group</b>	115 $\pm$ 4.9	113 $\pm$ 4.5	110 $\pm$ 4.2
<b>Turmeric + Flaxseed oil group</b>	119 $\pm$ 5.1	110 $\pm$ 5.3	94.5 $\pm$ 5.3

Results have been presented as mean  $\pm$  standard deviation of four samples (n = 4).



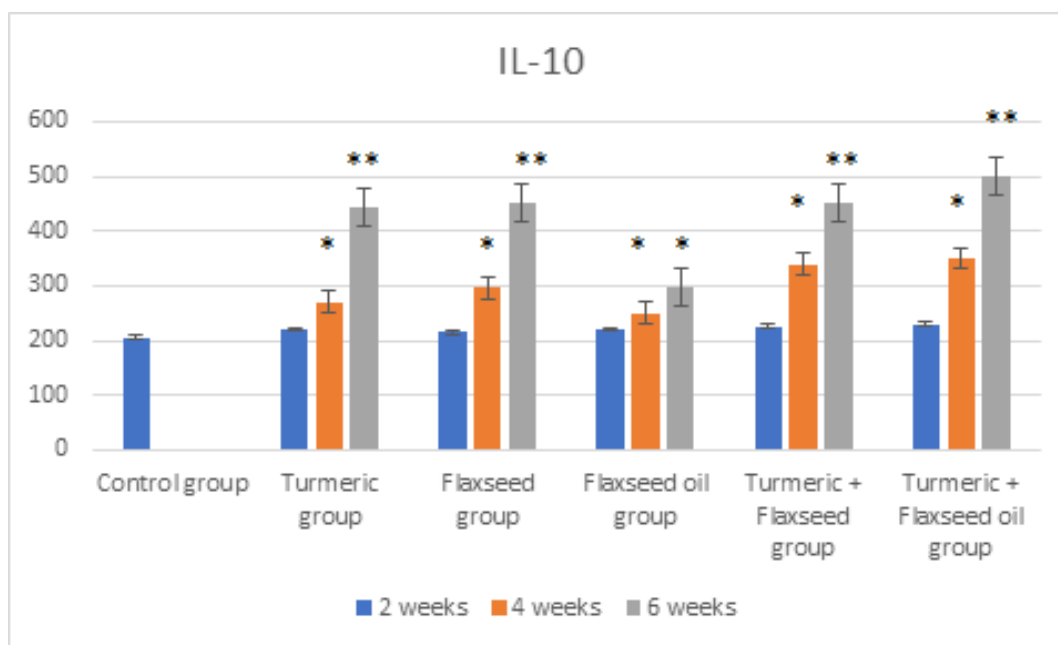
**Figure 1:**Comparative bar diagram showing IL-6 levels in healthy female swiss albino mice with daily oral intake of turmeric, flaxseed grinded, flaxseed oil, combination of turmeric and grinded flaxseed and combination of turmeric and flaxseed oil after 2 weeks, 4 weeks and 6 weeks.

Table 2 showed a significant increase in serum IL-10 relative to the control of the study. On the contrary, all groups induced the expression and production of IL-10. Moreover, changes in IL-10 level were strongly significant through a prolonged experiment administration interval period. We observed a significant increase in IL-10 levels of serum with all the administrated supplements separately or combination after 4 weeks ( $p^* < 0.05$ ). The effect of turmeric, flaxseed grinded, flaxseed oil, and the combined administration of turmeric and grinded flaxseed in addition to the co-administration of turmeric and flaxseed oil on the level of IL-10 after prolonged time 6 weeks ( $p^{**} < 0.001$ ). Figure 2 showed that all potent and efficacious data in promoting IL-10 production.

**Table2:** Changes in IL-10 level throughout the study.

IL-10	2 weeks (Mean ± SD)	4 weeks (Mean ± SD)	6 weeks (Mean ± SD)
<b>Turmeric group</b>	220±7.1	270±6.8*	442.5±9.1**
<b>Flaxseed group</b>	215.5±5.2	295.5±5.2*	450±4.1**
<b>Flaxseed oil group</b>	220±9.3	250±9.3*	296±7.5*
<b>Turmeric + Flaxseed group</b>	225±8.6	340±7.8*	450±9.7**
<b>Turmeric + Flaxseed oil group</b>	230±4.5	350±4.4*	500.5±7.8**

Results have been presented as mean ± standard deviation of four samples (n = 4). Significant effects are indicated by asterisks ( $*P < 0.05$ ,  $**P < 0.001$ )



**Figure 2:**Comparative bar diagram showing IL-10 levels in healthy female swiss albino mice with daily oral intake of turmeric, flaxseed grinded, flaxseed oil, combination of turmeric and grinded flaxseed and combination of turmeric and flaxseed oil after 2 weeks, 4 weeks and 6 weeks. Significant effects are indicated by asterisks ( $*P < 0.05$ ,  $**P < 0.001$ )

## Discussion

Turmeric spice is a therapeutic superfood. It is a relative of ginger and the spice that give a typical yellowish color to curry powder. Most people used to use it daily for flavoring their food in addition to its anti-inflammatory therapeutic action [18]. Turmeric benefits are better obtained by combining curcumin with substances like piperine, which greatly enhance bioavailability [19]. Moreover, turmeric is one of the main studied medical spices against inflammation. It has recognized anti-inflammatory properties for centuries against several diseases. Widespread research in the last two to three decades reported that curcumin is a central ingredient in turmeric's anti-inflammatory effects [20].

Numerous studies on inflammatory diseases demonstrated the effect of turmeric and its active ingredient curcumin on anti-inflammatory cytokines. Many observations correlated its effect through down-regulation of IL-6 level in serum [21,22], in contrast, other studies revealed that curcumin did not significantly reduce the level of IL-6 [23,24]. Moreover, the induction level of IL-10 increased to modulate the immune response on inflammatory conditions [13].

Flaxseed is a rich source of polyunsaturated fatty acid omega-3 fatty acid, alpha-linolenic acid (ALA) that exhibit anti-inflammatory properties [25]. The hardened coat protection of the flaxseed is destroyed by crushing, leading to exposure of alpha-linolenic acid ALA to oxidation and makes it more available and much effective [26]. On the other hand, flaxseed oils have a higher content of alpha-linolenic acid [27]. Their health benefits are various and attract global attention mainly because of their antioxidant and anti-inflammatory pathways.

Recent analysis on flaxseed revealed the ability to reduce IL-6 circulating concentrations in inflammatory diseases [28,29]. Furthermore, other studies showed no significant alteration on IL-10 level [15].

Up to date, curcumin and flaxseed trials have been carried out with populations that having health problems. Our study is carried out on healthy mice to evaluate the pro-inflammatory IL-6 and the anti-inflammatory IL-10 levels with prolonged experiment time extend to 6 weeks with daily oral uptake of turmeric, grinded flaxseed, and flaxseed oil individually. Furthermore, make a comparison analysis for their co-administration effects.

The present study results revealed turmeric, grinded flaxseed, flaxseed oil, turmeric/flaxseed, and turmeric/flaxseed oils oral supplementation in healthy mice did not exert any significant effects on the concentrations of IL-6. Although the administration of turmeric and flaxseed oils individually almost does not affect IL-6 level, their combination showed a relative decrease in IL-6 level. Suggesting the combination of them enhanced the inflammatory responses.

In contrast to their effect on the level of IL-6, the level of IL-10 was induced significantly after 4 weeks ( $P < 0.05$ ) and duplicate its induction after 6 weeks into two folds ( $P < 0.001$ ). Our experimental herbs have anti-inflammatory effects mediated through mild reduction of potent pro-inflammatory cytokines and induction of anti-inflammatory cytokines production.

The main observation of our work is that even with no inflammation induction, turmeric, and flaxseed in addition to their combination can induce IL-10 expression. Our study suggests that the prolonged administration time of turmeric and flaxseed and their combination may have played an indirect role in upregulating the expression of several key genes involved in the expression of IL-10 and modulate its production in higher levels. Therefore, further research into this topic is necessary on the molecular level.

This study demonstrates for the first time that dietary oral administration of turmeric and flaxseed, and their combination in a healthy animal model may have a beneficial effect in suppressing the pro-inflammatory cytokines and expressing the anti-inflammatory cytokines. It seems that a combination of turmeric and flaxseed oil has the best anti-inflammatory effect. Moreover, one of the strong points of our work was the detection of IL-10 level in healthy mice model after daily dietary of turmeric and flaxseed, which responsible power to change in immune system response in the body.

There are some limitations to this study that should be mentioned. The main limitation is other identified inflammatory markers were not assessed. Moreover, a relatively small sample size is considered as other limitations of the study.

## Conclusion

The prolonged administration time of turmeric and flaxseed and their combination may have played an indirect role in upregulating the pro-inflammatory IL-10 and modulate its production in high levels and have no significant effect on IL-6 level.

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