

## ORIGINAL ARTICLE

# Ameliorative Potential of *Cymbopogon citratus* Dried Leaf Powder in Attenuation of Hyperlipidemia

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## ABSTRACT

**Background and Objective:** Cardiovascular disease (CHD) are the primary reason of morbidity and mortality in all around the world so this study was aimed to assess and compare the prospect of ameliorative potential of sun dried *C. citratus* leaf powder against lipid-lowering drugs (statins) in hyperlipidemic non-smoker male patients and also to execute its compositional analysis.

**Materials and Methods:** *C. citratus* powder was firstly subjected to proximate analysis. Then nominated hyperlipidemic, non-smoker males (age 25-50 years) were divided into four groups ( $G_1$ ,  $G_2$ ,  $G_3$  and  $G_4$ ) and each faction included 10 subjects. First three sets were given deliberated amounts of *C. citratus* powder (4, 8 and 12 g, respectively) for 30 days.  $G_4$  set was prescribed the use of statins. Serum lipid profile was analyzed of each volunteer before and after the termination of the trial. The data obtained was subjected to statistical analyses.

**Results:** Most desirable cholesterol reduction was witnessed in  $G_2$  and  $G_3$  ( $235.90 \pm 33.20$  -  $187.30 \pm 47.37$  and  $230.60 \pm 37.43$  -  $182.50 \pm 35.16$  mg dL<sup>-1</sup>, respectively). Levels of TG diminished most significantly in  $G_4$  (statins), which was from  $178.70 \pm 54.40$  -  $131.20 \pm 31.22$ . LDL reduction in both  $G_2$  and  $G_3$  were nearly same ( $G_2$ : 15.1% and  $G_3$ : 15.7%). However,  $G_4$  lowered the HDL level considerably from  $55.30 \pm 10.91$  -  $35.80 \pm 4.66$  mg dL<sup>-1</sup>.

**Conclusion:** So, it was concluded that *C. citratus* attenuated the serum lipid parameters dose dependently; hence, it can be considered as an arsenal for fighting against the health problems that have risen from elevated levels of lipid markers

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## INTRODUCTION

Cardiovascular complications including stroke and coronary heart disease (CHD) are the foremost reason of morbidity and mortality in all around the world<sup>1,2</sup>. According to The Global Burden of Disease (GBD) study estimation, 29.6% of all mortalities globally (>15.6 million deaths) were due to CVD in 2010<sup>2</sup>. Hyperlipidemia donates substantially to the progression of CVD and escalates the risk for various cardiovascular events such as atherosclerosis, ischemic heart disease (IHD), myocardial infarction and stroke<sup>3,4</sup>. Hyperlipidemia can be established as high levels of blood lipids, including triglycerides, cholesterol and lipoproteins that work as vehicle for transportation of cholesterol in blood plasma<sup>4</sup>. Complexes of cholesterol-lipoprotein including very low, low, as well as intermediate densities are termed as pro-atherogenic cholesterol and demonstrate as one of risk factors for CVDs<sup>5</sup>. In contrast, high-density cholesterol-lipoprotein complexes also recognized as HDL is titled as anti-atherogenic cholesterol as it exposes protective effect and shields from cardiovascular disorders<sup>4,6</sup>.

The treatments accessible for hyperlipidemia include dietary modification policies and use of lipid-lowering medicines, mainly statins that are supposed to be very efficient in lowering LDL-cholesterol levels in body<sup>7</sup>. Nonetheless, numerous patients, especially those who initially have greatly elevated LDL cholesterol and those who experience undesirable side effects by the use of high-dose statins, are incapable to reach the recommended target levels of LDL-cholesterol<sup>8</sup>. The use of substitute cures, especially medicinal plants along with their complements, to treat various ailments like cardiovascular diseases, diabetes and hyperlipidemia have amplified over the recent decades in many countries around the world. *Cymbopogon citratus*, commonly acknowledged as lemon grass is well-recognized therapeutic plants among all others and is usually used as a traditional substitute medicine for the cure of numerous diseases because of the presence of numerous bioactive compounds in it<sup>9,10</sup>.

*Cymbopogon citratus* is a tropical herb generally recognized as lemon grass. It is extensively cultivated aromatic plant and is used in traditional medicine as infusion or as decoct<sup>11</sup>. Belonging to *Poaceae* family, lemon grass is native of tropical and semitropical areas of Asia and is also cultivated in South and Central America, Africa and many other tropical countries<sup>12</sup>. It nurtures up to 6 inches and has a bulbous stems and consists of elongated leaf sheaths with rectilinear blade, slender base and acute apex with dimensions of 100 cm length and 2 cm width<sup>6</sup>.

Freshly cut and dried up leaves are widely used in Ayurvedic medicine. They are also utilized as folk remedy for flu, headache, fever, coughs, elephantiasis, hypertension, leprosy, malaria, gingivitis, ophthalmic pneumonia, nervous, gastrointestinal and vascular disorders<sup>13</sup>. The aqueous extracts of dried leaves are used for the treatment of several inflammation-based pathologies<sup>14</sup>. Despite the widespread application of *C. citratus* as folk medicine; to date, little literature is available on the effects of *C. citratus* on hematologic parameters in humans<sup>15-18</sup>. Even in Pakistan, apparently a very few studies have passed the eyes confirming the potential health benefits of *C. citratus*. Hence, the aim of this study was to investigate the biological proficiency of lemongrass leaves on the markers of lipid profile in humans.

## MATERIALS AND METHODS

**Procurement of raw material:** *Cymbopogon citratus* plants were procured from plant market, Faisalabad.

**Preparation of sun dried leaf powder:** Fresh leaves of were acquired from the plant of *C. citratus*. They were washed and dried prior to any experiment. Then the leaves of *C. citratus* were subjected to sun drying. By using an electric blender they were pulverized into fine powder.

**Proximate analysis:** Fresh *C. citratus* leaves were analyzed for moisture, ash, fat, crude fiber, crude protein and NFE according to their respective methods as described in AOAC<sup>16</sup>.

**Moisture content:** The moisture content in *C. citratus* leaves powdered was accessed by drying the 5 g sample in the oven at 105±5 till the weight of the sample became constant.

Moisture content (%) was calculated using following Eq:

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

**Ash content:** Ash estimation was conducted by direct incineration of sample obtained in a crucible. The crucible was heated on the oxidizing flame till it produced no fumes. Then kindled in a muffle kiln at 550°C till grayish white residue was obtained. Ash (%) was calculated according to the Eq:

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

**Crude fat content:** About 5 g of sample was taken in separate thimbles and placed in an extraction tube of soxhlet kit after wrapping in filter paper. The modification of temperature of the heater was so that incessant drops of ethanol fell on the sample in the extraction tube. The remainders were shifted into a dry weighted china dish. Then this china dish was placed into a hot air oven for evaporation for either 4-5 hrs. After that, the dish was taken out and placed in desiccators to cool and then again weighted and percentage was calculated.

**Crude protein:** The percentage of nitrogen in the sample was determined by using kjeldahl method. The sample (2 g) was digested in the digestion tube with the aid of 30 mL conc. H<sub>2</sub>SO<sub>4</sub> in the presence of 5g digestion mixture (CuSO<sub>4</sub>: FeSO<sub>4</sub>: K<sub>2</sub>SO<sub>4</sub> in the ratio of 9:1: 90) for 5-6 hrs. or till the digested material attained light greenish or transparent color. This material was diluted and distillation was done by taking 10 mL of diluted material and 10 mL of 40% NaOH. Ammonia released was collection in 4% boric acid having methyl indicator and the solution was then titrated against 0.1 NH<sub>2</sub>SO<sub>4</sub>. Crude protein was calculated by using the following Eq:

**Nitrogen free extract:** The NFE was computed by using the following Eq:

$$\text{Nitrogen (\%)} = \frac{\text{Vol. of 0.1 NH}_2\text{SO}_4\text{(mL)} \times \text{Vol. of dilution} \times 0.0014}{\text{Wt. of sample (g)} \times \text{Vol. of aliquot sample (mL)}} \times 100$$

**Study setting and experimental design:** Efficacy study was conducted for a period of 30 day on hypercholesterolemic males. There were 4 groups of human volunteers in this efficacy paradigm and each group comprised of 10 male subjects; G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> with the dose of *C. citratus* powder 4, 8 and 12 g day<sup>-1</sup>, respectively. Fourth group (G<sub>4</sub>) was prescribed the use of statins (atorvastatin and simvastatin). Fasting lipid profile tests along with body weight (kg), height (cm) and age of the patient was noted down. These readings were taken before initiation and after termination of the trial.

**Statistical analysis:** Complete randomized design (CRD) was applied to find out the significance level. The statistical analysis of data was performed using two-way ANOVA and Tukey's HSD.

## RESULT

Results along with their interpretations are allocated into two major portions i.e., compositional analysis of *C. citratus* dried powder and efficacy studies.

**Compositional analysis:** *Cymbopogon citratus* dried leaf powder was analyzed for varied quality attributes, such as moisture, ash, fiber, proteins, fat alongside NFE. These six qualities attributed, make up complete components of proximate analysis. According to the results, *C. citratus* dried leaf powder contains  $10\pm 0.2\%$  of moisture content. The Ash contents present in the *C. citratus* dried leaf sample weighed to be  $8.1\pm 0.23$  whereas, protein, fat, fiber and nitrogen free extracts are calculated to be  $2.47\pm 0.15$ ,  $1.28\pm 0.15$ ,  $3.56\pm 0.20$  and  $74.59\pm 0.9$  mg dL<sup>-1</sup>, respectively.

**Table 1:** Effect of *C. citratus* treatment and days on serum lipid profile

Group	Serum lipid profile (mg dL <sup>-1</sup> )			
	CHOL	TG	LDL	HDL
<b>Baseline phase</b>				
G <sub>1</sub> = <i>C. citratus</i> power 4 g	212.30±20.95 <sup>ab</sup>	170.80±40.73 <sup>a</sup>	154.00±28.58 <sup>a</sup>	41.50±5.99 <sup>bc</sup>
G <sub>2</sub> = <i>C. citratus</i> 8 g	235.90±33.2 <sup>a</sup>	198.70±59.88 <sup>a</sup>	189.10±41.08 <sup>a</sup>	43.00±5.89 <sup>bc</sup>
G <sub>3</sub> = <i>C. citratus</i> 12 g	230.60±37.84 <sup>ab</sup>	147.20±30.74 <sup>a</sup>	179.50±50.61 <sup>a</sup>	44.80±7.44 <sup>bc</sup>
G <sub>4</sub> = Statins	220.80±37.43 <sup>ab</sup>	178.70±54.40 <sup>a</sup>	192.90±36.55 <sup>a</sup>	55.30±10.91 <sup>a</sup>
<b>Chronic phase</b>				
G <sub>1</sub> = <i>C. citratus</i> power 4 g	203.60±24.18 <sup>ab</sup>	167.00±49.14 <sup>a</sup>	145.00±29.02 <sup>a</sup>	42.20±349 <sup>bc</sup>
G <sub>2</sub> = <i>C. citratus</i> 8 g	187.30±47.37 <sup>ab</sup>	185.40±71.82 <sup>a</sup>	160.20±31.94 <sup>a</sup>	48.00±5.68 <sup>ab</sup>
G <sub>3</sub> = <i>C. citratus</i> 12 g	182.50±35.16 <sup>b</sup>	132.20±35.82 <sup>a</sup>	151.20±61.74 <sup>a</sup>	55.60±10.67 <sup>a</sup>
G <sub>4</sub> = Statins	193.20±51.83 <sup>ab</sup>	131.20±31.22 <sup>a</sup>	180.00±30.26 <sup>a</sup>	35.80±4.66 <sup>c</sup>

**Efficacy study:** Maximum cholesterol reduction was recorded in G<sub>3</sub> (230.60±37.84 - 182.50±35.16) and then in G<sub>2</sub> (235.90±33.20 - 187.30±47.37) as shown in Table 1. G<sub>1</sub> and G<sub>4</sub> demonstrated lower mean cholesterol reduction; 212.30±20.95 - 203.60±24.18 and 220.80±33.07 - 193.20±51.83, respectively. Results obtained from G<sub>2</sub> and G<sub>3</sub> were approximately similar and both groups decreased cholesterol to a considerable level.

No significant change was observed in drop of TG levels in G<sub>1</sub> (170.80±40.73 - 167.00±49.14). Slightly considerable change was witnessed in both G<sub>2</sub> and G<sub>3</sub>; 198.70±59.88 - 185.4±71.82 and 147.20±30.74 - 132.20±35.82, respectively. On the other hand, maximal reduction in TG contents was recorded in G<sub>4</sub> (statins), which was from 178.70±54.40 - 131.20±31.22. This leads to the conclusion that the G<sub>4</sub> was most successful in attenuating the levels of TG in human subjects.

Minimum LDL drop was documented in G<sub>1</sub> and G<sub>4</sub>. This decrease ranged from 154.00±28.58 - 145.00±29.02 in G<sub>1</sub> and 192.90±36.55 - 180.00±30.26 in G<sub>4</sub>. G<sub>2</sub> diminished LDL numeric from 189.10±41.08 - 160.50±31.94 and in G<sub>3</sub> the values shrunk from 179.50±50.61 - 151.20±61.74.

The HDL contents improved in all three groups (G<sub>1</sub>, G<sub>2</sub> and G<sub>3</sub>). However, this upsurge was minimal in G<sub>1</sub> (1.7%) where the HDL levels increased from 41.50±5.99 - 42.20±3.49. G<sub>2</sub> showed a moderate increase from 43.00±5.89 - 48.00±5.68. In G<sub>4</sub> maximum escalation in HDL was observed from 44.80±7.44 - 55.60±10.67. On the other hand, G<sub>4</sub> showed a highly significant decrease in HDL levels from 55.30±10.91 - 35.80±4.66. This obtained data concluded that the increment of HDL in both groups (G<sub>3</sub> and G<sub>4</sub>) is to a considerable level (Table 1).

## DISCUSSION

The present research findings regarding proximate characterization of dried leaves of *C. citratus* were in nearby conformity with the ranges termed in the literature with minor

differences. Possible reason behind the variations in values could account to environmental factors such as climate and location. Furthermore, variance in genetic makeup could also serve as a contributing factor for differences in values<sup>17,18</sup>.

Analysis of proximate composition between fresh leaves and dried leaf powder of *C. citratus* were conducted in previous study<sup>19</sup>. The objective of this comparison was to determine the significant difference between the nutrient levels of *C. citratus* in both forms.

Current values for moisture, ash, crude protein and crude fat, coincide with the figures evaluated and provided in previous researches<sup>19,20</sup>. The values range from 7.01-10.12 for moisture, 5.01-11.28 for ash, 0.34-11.15 for crude protein and 0.98- 1.45% for crude fat. However, Current values for crude fiber and NFE (3.56 and 74.59%, respectively) reside nearby the numerics provided by Thorat *et al.*<sup>19</sup> which are 3.33% for crude fiber and 65.78% for NFE.

The use of therapeutic plants in folk medicine has depended greatly on prolong clinical experience rather than scientific data on their efficacy or safety. A few toxicology researches have been directed on the secure dosage and use of *C. citratus* essential oil (EO). The findings of this study, to a slight extent agree with the study conducted by Costa *et al.*<sup>11</sup>. This study illustrated that the *C. citratus* EO offers low toxicity and reflected as comparatively safe for use in the long term treatment at the doses up to 100 mg kg<sup>-1</sup> b.wt. Consequently, no deleterious effects were detected on the functions of liver or kidney functions during and after the experimentation at all the doses that were examined. This leads to the authentication of the safety of doses of *C. citratus* leaf powder that were chosen for this present study. The only negative feedback that was given by the volunteers was the difficulty in ingestion of powder as it was dry and had an astringent affect. Otherwise, they experienced no complications<sup>11</sup>.

Researchers were mentioned about the changes that were dose-dependent such as hypolipidemia in the Wistar rats after oral administration of daily single dose of fresh leaf aqueous extract at dose of 125, 250 and 500 mg kg<sup>-1</sup> for duration of 42 days. There was no significant drop in triglycerides values in each group. However, cholesterol, VLDL and LDL decreased most significantly ( $p < 0.001$ ) in 500 mg kg<sup>-1</sup> treatment<sup>6</sup>. In another study model, 22 hypercholesterolemic volunteers ingested *C. citratus* EO at 140 mg of dose in the form of capsules daily, it was witnessed that the bioactive constituents present in *C. citratus* EO efficiently lowered the levels of cholesterol between this specific hypercholesterolemic subgroup<sup>21</sup>.

Cholesterol-lowering potential of *C. citratus* may be attributed to alteration of the cholesterol uptake from intestine alongside the cholesterol transformation to the bile acids and raising their excretion. This indicated the hypocholesterolaemic possibilities of this extract that may well elucidate its usage in enthomedicine for the management of the heart diseases<sup>22</sup>.

## CONCLUSION

On the basis of the results of this research, it can be concluded that hypolipidemic effects of *C. citratus* as compared to statins were significant. It can be used as an arsenal for fighting against the raising levels of serum lipid parameters in individuals nowadays. Its unique ability to raise HDL levels in body makes it ideal as compared to the use of some statins that lower HDL in body. It has also to some extent the ability to reduce appetite and hence result in weight loss.

## SIGNIFICANCE STATEMENT

This study helped us to investigate the biological proficiency of *C. citratus* leaf powder and discovers that its powder attenuates the serum lipid profile of humans, dose dependently and fight against the health complications that arise from elevated levels of lipid markers. This study will provide preliminary data that can use for researchers to further work on the diet based therapies with *C. citratus* for a better human health.

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