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## Research Article Protective Effect of *Cymbopogon schoenanthus* Extract Against Formalin Hazards in Rats

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### Abstract

**Background and Objective:** Formalin can be credited for raising the number of patients with kidneys failure, which is increasing in incidence in Egypt annually, according to WHO and the Egyptian Ministry of Health. Lemon grass *Cymbopogon schoenanthus* (CS) is highly reputed in Egyptian folk medicine as an effective renal antispasmodic and diuretic agent. This study aimed to use the CS extract to eliminate the formalin hazard in rats. **Materials and Methods:** The experiment was designed and rats divided into six groups are group 1 (untreated) served as control group; group 2 received formalin solution, group 3 and 4 orally treated with CS extract at different two doses (50 and 100 µL). Groups 5 and 6 received formalin solution plus CS extract at mentioned doses. The changes in rat's body weight were noted and the biochemical blood parameters were determined as well as histopathological examination of liver and kidney. **Results:** The results indicated that all tested biochemical parameters were in normal value for the control group and had no significant differences for CS groups. While, the animals treated with formalin at dose of (100 mg kg<sup>-1</sup> b.wt.) showed significant increase in tested biochemical parameters compared with control and CS groups. **Conclusion:** It is worthy to mention that the rats treated with CS plus formalin showed significant changes in all tested parameters compared with the group received formalin alone. The histopathological examination confirmed the blood biochemical analyses and treatment with CS extract improved the liver and kidney tissues.

Key words: Formalin, rats, liver, kidneys, Cymbopogon schoenanthus extract

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**Competing Interest:** The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### **INTRODUCTION**

The National Toxicology Program (NTP)<sup>1</sup> lists formaldehyde as known to be a human carcinogen as well as the International Agency for Research on Cancer (IARC)<sup>2</sup> has concluded that formaldehyde is "Carcinogenic to humans" based on higher risks of nasopharyngeal cancer and leukemia. In addition, the (EPA)<sup>3</sup> has classified formaldehyde as a probable human carcinogen. It has been observed that acute tubular necrosis can develop due to the edematous obstruction or acute renal failure caused by the intravesical formalin used for the treatment of chronic hemorrhagic cystitis<sup>4,5</sup> induced acute renal failure<sup>6</sup>. Formaldehyde is an organic carbon compound frequently used in occupational environments (hospitals, textiles, papers, resins and wood composites) and house indoor environments (insulating materials, fabrics, chipboard and cooking emissions) and is considered as one of the major components responsible for sick building syndrome<sup>7-9</sup>. The NCI<sup>10</sup> considered exposure to formaldehyde may cause leukemia, particularly myeloid leukemia, in humans. Formalin is added to milk in the production of cheese and dairy products as an antimicrobial agent. It is a very reactive compound, reacting with cellular proteins and nucleic acids; thus, safety evaluation of formalin as an additive to milk and dairy products (cheese and yoghurt) must be thoroughly considered<sup>11</sup>. Formalin has been shown to induce oxidative stress in mouse brain, liver and lung<sup>12</sup>.

Antioxidants can prevent or moderate the oxidative damage in the body through free radical scavenger. Lemon grass *Cymbopogon schoenanthus* is used in folk medicine. Its decoction and infusion are taken as diuretic to reduce intestine spasm and to act against food poisoning, anti-rheumatism, anti-anorexia and digestive<sup>13</sup>. Recent study demonstrated that lemon grass extract and essential oil have antioxidant and acetyl cholinesterase inhibitory properties<sup>14,15</sup>. Based on the obtained reports this study aimed to prepare the lemon grass extract has the ability to protect or moderate the formalin toxicity in rats.

#### **MATERIALS AND METHODS**

**Materials:** *Cymbopogon schoenanthus* purchased from the Egyptian herbal Markets, Dokki, Giza. All Kits of Biochemical analyses: AST, ALP, bilirubin, albumin, cholesterol, triglyceride, HDL, LDL, urea, creatinne and urea were purchased from Biomeieux, Laboratory of Reagents and Products (France).

#### Methods

**Preparation of** *Cymbopogon schoenanthus* **extract:** About 200 g of dried powder plants were added to 1000 mL of

distilled water: Ethanol (20:80 v/v) and incubated at room temperature for 24 h. The slurry was filtered through a Whatman No. 1 filter paper and then solvent was fully evaporated with a rotary evaporator at 35°C. The residues were dissolved in 100 mL of distilled water and stored at 4°C.

**Total phenol content:** The total polyphenols content of CS extract was determined calorimetrically using the Folin–Ciocalteau reagent according to the modified method described by Gutfinger<sup>16</sup>.

#### **Antioxidant activity**

**DPPH radical scavenging method:** The antioxidant activity was evaluated by using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) according to a modification method of Bandoniene *et al.*<sup>17</sup>.

**Experimental animals:** Dawley Albino rats of 3 months of age were purchased from the Animal House Colony, National Research Center, Giza, Egypt. All animals were maintained on a standard diet only and housed in a room free from any source of chemical contamination.

**Experimental design:** After an acclimatization period for 1 week, animals were randomly equally divided into six groups and housed in filter top polycarbonate cages. The different groups were treated for consecutive 30 days as following. Group 1 served control group, fed a basal diet. The second group served as a toxins group, which treated orally with formalin (100 mg kg<sup>-1</sup> b.wt.) third and fourth group treated with SC extract at doses 50 and 100  $\mu$ L, respectively, the fifth and sixth groups were treated with formalin plus SC at previously mentioned doses. The animals were observed daily for signs of toxicity and weighted as well. At the end of experimentation period (i.e., day 30), blood samples were collected from all animals from retro-orbital venous plexus for biochemical analyses. Then all animals were killed and samples of the liver and kidneys tissues of each animal were removed and hydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin.

**Histopathological examination:** All histological analyses were performed in routinely processed formalin-fixed, paraffin embedded tissue sections of 5 mm thickness. They were stained with hematoxylin-eosin stain and the slides were examined with light microscope. Randomly selected fields were evaluated for cellular and tubular structures. Degeneration in epithelium and interstitial spaces were also noted.

**Statistical analysis:** All data were expressed as means $\pm$ standard error of mean (SEM). One-way analysis of variance (ANOVA) was performed to test for differences between the groups mean. Significant differences between the means were determined by Duncan's multiple range test and p<0.05 were regarded as significant<sup>18</sup>.

#### **RESULTS AND DISCUSSION**

**Analysis of CS extract:** Table 1 showed to the total polyphenol and antioxidant activity of CS extract and found that the extract contained 3.64 mg mL<sup>-1</sup> and 76.6%, respectively. Polyphenol contribute directly to the antioxidant activity, CS extract possess a high phenolic content and showed an antioxidant activity<sup>15</sup>.

**Biological study:** The effect of formalin and CS extract at different mentioned doses on the experimental animals and their biochemical parameters as well as their liver and kidneys tissues were studied. The results in Table 2 showing the protective effect of CS at two doses against harmful effects of formalin on liver functions in rats treated with 100 mg kg<sup>-1</sup> b.wt., formalin for 30 days. The results clearing the negative effect of formalin on the liver function AST, ALT, ALP (U L<sup>-1</sup>), bilirubin, albumin (mg dL<sup>-1</sup>) and induced significant increase these values comparing with control group which gave the values 29.3, 29, 102.6, 0.58, 4, respectively. It is worthy to report that rats treated orally

Table 1: Polyphenol and antioxidant activity of *Cymbopogon schoenanthus* extract

Total polyphenol (mg mL <sup>-1</sup> )	3.64
Antioxidant activity (%)	76.60

with CS extract at two doses slightly enhanced the liver function. The values were decreased comparing to the control values to be in the range 29-25, 21-23, 91-102, 0.47-0.54 and 3.6-3.5 for AST, ALT, ALP (UL<sup>-1</sup>), bilirubin, albumin (mg dL<sup>-1</sup>), respectively.

Rats treated orally with formalin alone had a negative effect on liver function, while the combination of CS extract at two doses plus formalin restored the biochemical parameters towards the control group. Changes in the liver after absorption of formalin consist of mild or severe grade of cloudy swelling accompanied by vacillation of the protoplasm, changes in the nuclei and leukocyte infiltration. Focal necrosis may result similar changes follow the inhalation of formaldehyde<sup>19</sup>. Furthermore, formalin was found to cause fatty changes through accumulation of lipid droplets in hepatocytes<sup>20</sup>.

Data summarized in Table 3 showed that the control rats and CS groups had normal values of kidney function, while the rats of formalin group showed significant elevate in kidneys functions compared with control and CS groups at two doses. Whilst, the animals of groups treated with formalin plus CS at two doses showed a significant restoration in tested kidney function. Formaldehyde inhalation inflicts various harmful effects on many organs, such as testis<sup>21</sup>, brain<sup>22</sup>, kidneys<sup>23</sup> and liver<sup>24</sup>.

The lipid profile cholesterol (mg dL<sup>-1</sup>), triglycerides (mg dL<sup>-1</sup>), HDL (mg dL<sup>-1</sup>) and LDL (mg dL<sup>-1</sup>) of experimental animals were studied and the results tabulated in Table 4 showed that almost all lipid profile values are in normal range but the formalin induced some decrease in lipid profile to record the values 78, 106, 25, 30 for cholesterol (mg dL<sup>-1</sup>),

Table 2: Effect of CS at two doses as a protective agent against formalin risk on liver functions of rats

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Groups	$AST(UL^{-1})$	ALT (U L <sup>-1</sup> )	ALP (U L <sup>-1</sup> )	Bilirubin (mg dL <sup>-1</sup> )	Albumin (mg dL <sup>-1</sup> )
Control	29.30±0.8ª	29.00±5.3ª	102.67±3.71ª	0.58±0.06ª	4.00±0.06ª
CS extract (50 µL)	29.67±3.6ª	21.33±3.2 <sup>b</sup>	91.00±6.66ª	$0.47 \pm 0.07^{b}$	$3.63 \pm 0.2^{b}$
CS extract (100 μL)	25.67±1.33ª	23.00±2.52 <sup>b</sup>	102.33±9.68ª	0.54±0.06ª	3.50±0.23 <sup>b</sup>
Formalin	36.33±2.40 <sup>b</sup>	33.00±3.18°	131.00±6.66 <sup>b</sup>	$0.46 \pm 0.04^{b}$	3.67±0.20 <sup>b</sup>
Formalin+CS extract (50 µL)	29.00±2.00ª	26.33±2.2ª	112.33±11.35°	$0.60 \pm 0.07^{a}$	3.70±0.21 <sup>b</sup>
Formalin+CS extract (100 μL)	28.33±2.91ª	27.53±2.03ª	102.00±5.57ª	0.57±0.03ª	$3.67 \pm 0.18^{b}$

Each column, means superscripted with different letters are significantly different (p<0.05), SC: Cymbopogon schoenanthus extract

Table 3: Effect of CS extract at two doses as a protective agent against formalin risk on kidney function in rats

Groups	Urea (mg dL <sup>-1</sup> )	Creatinine (mg dL <sup>-1</sup> )	Uric acid (mg dL <sup>-1</sup> )
Control	41.30±2.03ª	0.52±0.03ª	2.80±0.12ª
CS extract (50 μL)	41.00±0.58ª	0.50±0.05ª	2.50±0.17ª
CS extract (100 μL)	42.67±2.03ª	0.45±0.03ª	2.40±0.06ª
Formalin	51.00±2.08 <sup>b</sup>	0.38±0.03 <sup>b</sup>	3.10±0.12 <sup>b</sup>
Formalin+CS extract (50 µL)	46.33±2.90ª	$0.50 \pm 0.60^{a}$	2.50±0.31ª
Formalin+CS extract (100 µL)	43.33±2.03ª	0.44±0.30ª	2.50±0.26ª

Each column, means superscripted with different letters are significantly different (p<0.05), SC: Cymbopogon schoenanthus extract



Fig. 1(a-f): Effects of Cymbopogon schoenanthus on kidney of rats treated with formalin (H and E X200)

Table 4: Effects of CS extract at two doses as a	protective agent on lipid r	profile of rats treated with	100 mg kg <sup>-1</sup> b.wt., formalin

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Groups	Cholesterol (mg dL <sup>-1</sup> )	TG (mg dL <sup>-1</sup> )	HDL (mg dL <sup><math>-1</math></sup> )	LDL (mg dL <sup>-1</sup> )
Control	96.67±4.41ª	150.00±15.2ª	30.33±1.45ª	36.33±4.33ª
CS extract (50 μL)	94.00±18.9ª	125.40±7.2 <sup>b</sup>	28.33±2.33ª	41.04±17.35 <sup>b</sup>
CS extract (100 μL)	95.70±8.6ª	135.00±10.41 <sup>b</sup>	29.33±1.76ª	51.67±11.26°
Formalin	78.33±7.2 <sup>b</sup>	106.00±10.93°	25.33±3.18 <sup>b</sup>	30.67±3.71ª
Formalin+CS extract (50 µL)	100.00±2.89ª	133.00±4.41 <sup>ab</sup>	29.2±3.61ª	51.33±3.18°
Formalin+CS extract (100 μL)	$100.00 \pm 8.66^{a}$	146.33±7.26ª	31.67±1.45°	46.67±8.37 <sup>b</sup>

Each column, means superscripted with different letters are significantly different (p<0.05), SC: Cymbopogon schoenanthus extract

triglycerides (mg dL<sup>-1</sup>), HDL (mg dL<sup>-1</sup>) and LDL (mg dL<sup>-1</sup>), respectively, while lipid profile values for control are 96, 150, 30 and 36, respectively. Combination of formalin plus CS at two doses 50 and 100  $\mu$ L induced increase the lipid profile values and were in range between 100-100, 133-146, 29-31 and 51-46, respectively. Formalin was found to cause fatty changes through accumulation of lipid droplets in hepatocytes<sup>25</sup>.

**Histopathological study:** The biochemical parameters were confirmed by the histopathological examination for both liver and kidney tissues. Figure 1a shows the control untreated kidney rats showing normal renal glomeruli and renal tubules, while at the contrary the kidney rats treated with formalin showed congested interstitial blood vessel with thickened muscle wall (arrow) and hemorrhages caused by formalin treatment alone (Fig. 1b) but the kidney rats treated with CS at two doses showed normal renal glomeruli and renal tubules (Fig. 1b, c).

Kidney of rats treated with formalin alone showed congested interstitial blood vessel with thickened muscle wall (arrow) and hemorrhages (arrowhead).

Also, the results showed that kidney of rats treated with formalin+CS at the two doses showed slightly congestion in the interstitial blood vessel (arrow). Approximately similar microscopic manifestations in the liver include alterations in centrilobular vacuolization and focal cellular necrosis was noticed previously after exposing to formalin<sup>26</sup>. Inflammatory mononuclear cells were also found in the liver of formaldehyde exposed rats<sup>27</sup>.

Liver of rats in control group showed normal hepatocytes, blood sinusoids and central vein (Fig. 2a) also liver of rats treated with CS at two tested doses had no any different of liver of rats of control group, they showed normal liver cells and normal hepatic vein (Fig. 2b, c). Conversely, the liver of rats of formalin group showed dilated blood sinusoids (blue arrow) and atrophied hepatocytes (black arrow) (Fig. 2d). Otherwise, the liver of rats in group 5 which treated with the CS at (50  $\mu$ L kg<sup>-1</sup> b.wt.) plus formalin showed some vacuolated hepatocytes (arrow) (Fig. 2e). While liver of rats in groups 6 which treated with CS at (100  $\mu$ L kg<sup>-1</sup> b.wt.) plus formalin showed restoration to normal hepatic tissues (Fig. 2e). From the histological examination cleared that the safety of CS extract at two mentioned doses on liver and



Fig. 2(a-f): Effects of *Cymbopogon schoenanthus* on liver of rats treated with formalin (H and E X200)

kidney tissues and CS extract moderate the negative effect of formalin on liver and kidney.

#### CONCLUSION

From the obtained results it could be concluded that the safety of CS extract at two studied doses on the blood biochemical parameters and lipid profile as well as liver and kidney tissues and could be used to moderate or eliminate the formalin hazard in rats.

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