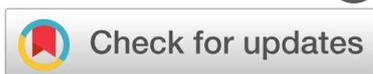


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Research



## Quercetin and Apigenin of *Cymbopogon citratus* mediate inhibition of HCT-116 and PC-3 cell cycle progression and ameliorate Doxorubicin-induced testicular dysfunction in male rats

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## Abstract

**Introduction:** This study aims to elucidate the possible ameliorative effect of *Cymbopogon citratus* (CC), i.e. lemon grass, in a Doxorubicin (Dox)-induced male testicular damage and infertility model. Moreover, the anticancer role of the main active constituents of CC (Apigenin and Quercetin) was evaluated on prostatic and colon cancer (PC-3 and HCT-116 carcinoma cell lines, respectively). **Methods:** In vitro studies of the cell lines were carried out to determine the IC<sub>50</sub> of each active constituent of CC, in order to select the most suitable dose for *in vivo* studies. For the *in vivo* studies, 24 rats were divided into 4 groups: Group 1 received saline treatment (negative control), Group 2 received treatment with CC (300 mg/kg body weight (b.w.)), Group 3 received Dox treatment (25 mg/kg b.w. intraperitoneally (i.p.)) on day 9 of the study, and Group 4 received CC treatment followed by Dox treatment on day 9 of the study. All the treatments were administered orally for 10 consecutive days. **Results:** The data revealed that Apigenin and Quercetin (AQ) had greater potency against PC-3 cells than HCT-116 cells. *In vivo* studies demonstrated that Dox treatment induced testicular damage and increased nitrosative stress, inflammation, and cell death, while decreasing cell energy markers for testes. In contrast, rats treated with CC only, or in parallel with Dox, showed ameliorative responses against Dox; they also showed greater testicular activity than normal, which reflected the ability of CC to significantly improve reproductive health performance. **Conclusion:** In conclusion, CC inhibits the activity and growth of PC-3 and HCT-116 carcinoma cell lines and, moreover, the whole extract of CC ameliorates testicular dysfunction induced by Dox.

## 1. Background

Anticancer drugs, such as Cyclosporine, Allopurinol, Colchicine, Sulfasalazine, Spironolactone, and other drugs (such as marijuana and alcohol), especially if used in large quantities, may slow down and affect sperm production [1]. About two-thirds of male patients with cancer who are treated with anticancer drugs have trouble forming sperm in the testicle, leading to sperm deficiencies and/or loss of function [2]. Chemotherapy is the treatment to eliminate cancer cells. Drugs have been used in the treatment of many diseases; in fact, there are currently more than twenty drug species in use. The chemotherapeutic drug is distributed through the blood throughout the body, which gives a greater chance of eliminating cancerous cells in the body that may have migrated from the primary tumor to another organ in the body [3]. As aforementioned, chemotherapy for cancer may disrupt testicular function, either permanently or temporarily, after a period of administration. It can destroy growing cancerous cells which growing more rapidly than normal cells [4]. As the cancer cells grow, they multiply and divide at a rate greater than that of the rest of the body's normal cells, yet chemotherapy is able to destroy them [5]. Some natural cells in the body grow and multiply quickly too. Thus, chemotherapy cannot differentiate between the normal cells and cancerous cells. Thus, the side effect of chemotherapy, i.e. non-specific killing of normal cells, can be a huge downturn. However, after chemotherapy is ended, the effects on non-cancerous cells will return to normal [6]. Doxorubicin (Dox) is an anticancer drug that is widely used to treat various types of cancers, and can cause side effects in the short term. Following administration into the vein, Doxorubicin penetrates the cells and forms stable complexes with DNA molecules, thereby killing the cancer cells preferentially [7]. In order to multiply, cancer cells need to constantly replicate samples of DNA, which are no longer able to do so when complexed with cisplatin. However, this therapy has major obstacles. Within an hour or even half an hour after injection, some patients have side effects or develop many disorders [8]. Cymbopogon citratus (CC), i.e. lemon grass, is a long-standing perennial herb grown in the tropical regions of Asia and India [9]. It has a number of health and medicinal benefits which include anticancer, antioxidant, antibacterial, antifungal, and antimicrobial properties [10]. The benefits of lemon grass are that it can relieve gastrointestinal and stomach disorders, treat insomnia and fever, contribute to the treatment of rheumatoid arthritis, help maintain blood cholesterol levels, promote the health of the immune system, help in the treatment of obesity, type II diabetes and cancer, and contribute to the removal of toxins from the body [11,12]. The PC-3 cell line is one of the human prostate cancer cell lines used in research. These cells are helpful in examining the biochemical alterations in prostatic cancer and in surveying their reaction to chemotherapeutic agents [13]. The HCT-116 cell line is a human colon cancer cell line that has been used to investigate or induce a rat colon cancer model [14]. The aim of the present study was to investigate the possible ameliorative effects of Dox in combination with CC on the reproductive male function. In addition, the study aimed to evaluate the potential anticancer activity of the main active constituents of CC (Apigenin and Quercetin) on growth inhibition of PC-3 and HCT-116 cell lines.

## 2. Methods

### (a) Plant extracts

Plant materials were identified by the Applied Research Center for Medicinal Plants (ARCMP) in Egypt. The materials were dried in a lyophilizer at -50°C until dried, then grinded. The ethanolic extract was prepared by macerating the CC leaves with ethanol (70%) overnight. The process was repeated three times. Then, the extraction was filtered and evaporated to dryness in a vacuum by lyophilization/freeze-drying. The filtrate (ethanolic extract) was then stored at -20°C until further use [15].

## (b) Animals

Twenty-four male Wister rats (120-150 g) from the animal facility of the National Organization for Drug Control and Research (NODCAR) in Egypt were used in the study. The animals were kept at room temperature ( $22\pm 1^\circ\text{C}$ ), with 12 h light and free access to diet and tap water.

## (c) Measurement of potential cytotoxicity of Apigenin and Quercetin on PC-3 and HCT-116 cell lines by Sulfo-Rhodamine-B (SRB) staining assay

The potential cytotoxic effects of the compounds were tested using the method of Skehan and Storeng [16], which was as follows:

- Cells were plated in a 96-multiwell plate ( $10^4$  cells/ well) for 24 h before treatment with the compounds to allow for attachment of cells to the wall of the plate.
- Different concentrations of the compounds were evaluated (0, 12.5, 25, 50 and 100  $\mu\text{g}/\text{ml}$ ). The different concentrations were added to the cell monolayer in triplicate wells for each dose.
- The monolayer cells were incubated with the compounds for 48 h at  $37^\circ\text{C}$  in 5%  $\text{CO}_2$  atmosphere.
- After 48 h, cells were fixed, washed, and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with acetic acid and the attached stain was recovered with Tris-EDTA buffer.
- The 11-color intensity was measured in an ELISA reader at 450nm.
- The relation between the surviving fraction and drug concentration was plotted to obtain the survival curve of each tumor cell line after treatment with the specified compound.
- The  $\text{IC}_{50}$  value was expressed as the concentration of each extract which inhibited the growth of cells by 50%.

## (d) Determination of the active compounds of Cymbopogon Citratus (CC) by high performance liquid chromatography (HPLC)

Flavonoids of CC leaves were extracted according to the method outlined by Fernandez de Semon *et al.* [17] and Dimitrios *et al.* [18].

## (e) Experimental design

Rats were divided into four groups (n=6 rats per group), as follows:

- Group 1: rats received saline orally every day for 10 days (normal control group).
- Group 2: rats received the ethanolic extract of CC every day for 10 days (300 mg/kg b.w., per os (p.o.)) [19].
- Group 3: rats received Dox (25 mg/kg b.w., i.p.) 24 h before decapitation (positive control group) [20].
- Group 4: rats received CC orally every day for 10 days and then were injected with Dox for 24 h before decapitation.

The testis tissues were kept at  $-80^\circ\text{C}$  until use for measurement of nitrosative stress, arachidonic acid (AA), malondialdehyde (MDA), glutathione (GSH), ATP, phosphatidylcholine, and phosphatidylserine, as well as genomic DNA analysis (on 1% agarose). A part of the testis tissue was fixed in 10% formaldehyde for histopathological examination.

**(f) Determination of tissue nitric oxide (NO) ( $\mu\text{mol/g}$  tissue)**

Nitric oxide (NO) levels were determined with anion exchange column (Hamilton PRP-X100) using the parameters (150\*4.1mm, 10  $\mu\text{m}$ , mobile phase 45:55 of 0.1 M NaCl-methanol); wavelength was adjusted to 230 nm, according to HPLC procedure [21].

**(g) Determination of tissue MDA (nmol/g tissue) by HPLC**

HPLC, sample preparation, and chromatographic conditions were carried out with some modifications of protocols from Algohary *et al.* [22].

**(h) Determination of tissue ATP ( $\mu\text{mol/g}$  tissue) by HPLC**

HPLC, sample preparation, and chromatographic conditions were carried out with some modifications of protocols from Abd-Elrazek *et al.* [23].

**(i) Phospholipid extraction**

Phospholipid extraction and determination were done according to previous protocols from study by Yassin *et al.* [24].

**(j) Determination of arachidonic acid (AA)**

Arachidonic fatty acid was determined using gas chromatography. Standards were prepared in hexane:chloroform (1:1) and combined into a single fatty acid mixture. Extraction of the serum samples was prepared with Folch reagent, chloroform:methanol (2:1), then vortexed for 5 min and centrifuged at 4000 rpm for 10 min. Samples were evaporated at room temperature prior to derivatization. Finally, the supernatant was mixed with 2 ml of 95% methanol:sulfuric acid, then extracted with hexane for injection [25].

**(k) Extraction of genomic DNA**

DNA extraction and detection of apoptosis (DNA fragmentation assay) were done according to the modifications from Hassab El-Nabi *et al.* [26].

**(l) Agarose gel electrophoresis for DNA fragmentation**

Gel electrophoresis was used for separation and analysis of macromolecules and their fragments, based on size and charge. The 1% agarose gel was prepared according to the method of Awwad *et al.* [27].

**(m) Histological examination of testis sections**

After the experimental period, animals were sacrificed and the testis section removed immediately, sliced, and washed in saline. The tissue pieces were preserved in 10% formalin for histopathological studies, then the pieces were processed and embedded in paraffin wax. Sections were taken and stained with hematoxylin and eosin (H&E) and photographed [28].

**(n) Statistical analysis**

The data were demonstrated as mean $\pm$ SEM (standard error of the mean). One-way ANOVA was carried out and statistical comparisons among groups were performed with Duncan's test [29] using a statistical package of social science (SPSS) software (17.5). All analyses were two-tailed, and  $p < 0.05$  was considered as significant for all the statistical analyses in the study.

### 3. Results

#### (a) Anti-proliferative activity or cytotoxicity of Apigenin and Quercetin on human prostate cancer (PC-3) and human colon cancer (HCT-116) cells

The SRB assay was used to assess the potential cytotoxic effects of Apigenin and Quercetin on human PC-3 prostate cancer cells and human HCT-116 colon cancer cells at different concentrations. The anti-proliferative activity on cancer cells was expressed as the IC<sub>50</sub> value. IC<sub>50</sub> is the inhibitory concentration that causes 50% inhibition of the cancer cell population. **Table 1** and **Figure 1** show that the cytotoxicity of Apigenin and Quercetin (AQ) on PC-3 and HCT-29 increases with increasing drug concentration. Thus, AQ had a dose-dependent effect on the cell viability, with cell lysis occurring after 48 h of drug treatment. The IC<sub>50</sub> for A was 10.9 µg/ml and for Q was 11.6 µg/ml on PC-3 cells; the IC<sub>50</sub> for A was 36.0 µg/ml and for Q was 31.8 µg/ml on HCT-116 cells.

**Table 1. IC<sub>50</sub> values for Apigenin- and Quercetin-mediated cytotoxicity against Human Prostate Cancer (PC-3) and Human Colon Cancer (HCT-116) carcinoma cell lines**

Cell Lines	Compounds	
	Apigenin	Quercetin
Prostate cancer (PC-3)	10.9 µg/ml	11.6 µg/ml
Colon cancer (HCT-29)	36.0 µg/ml	31.8 µg/ml

#### (b) Determination of the active compounds of CC leaves (Apigenin and Quercetin) by HPLC

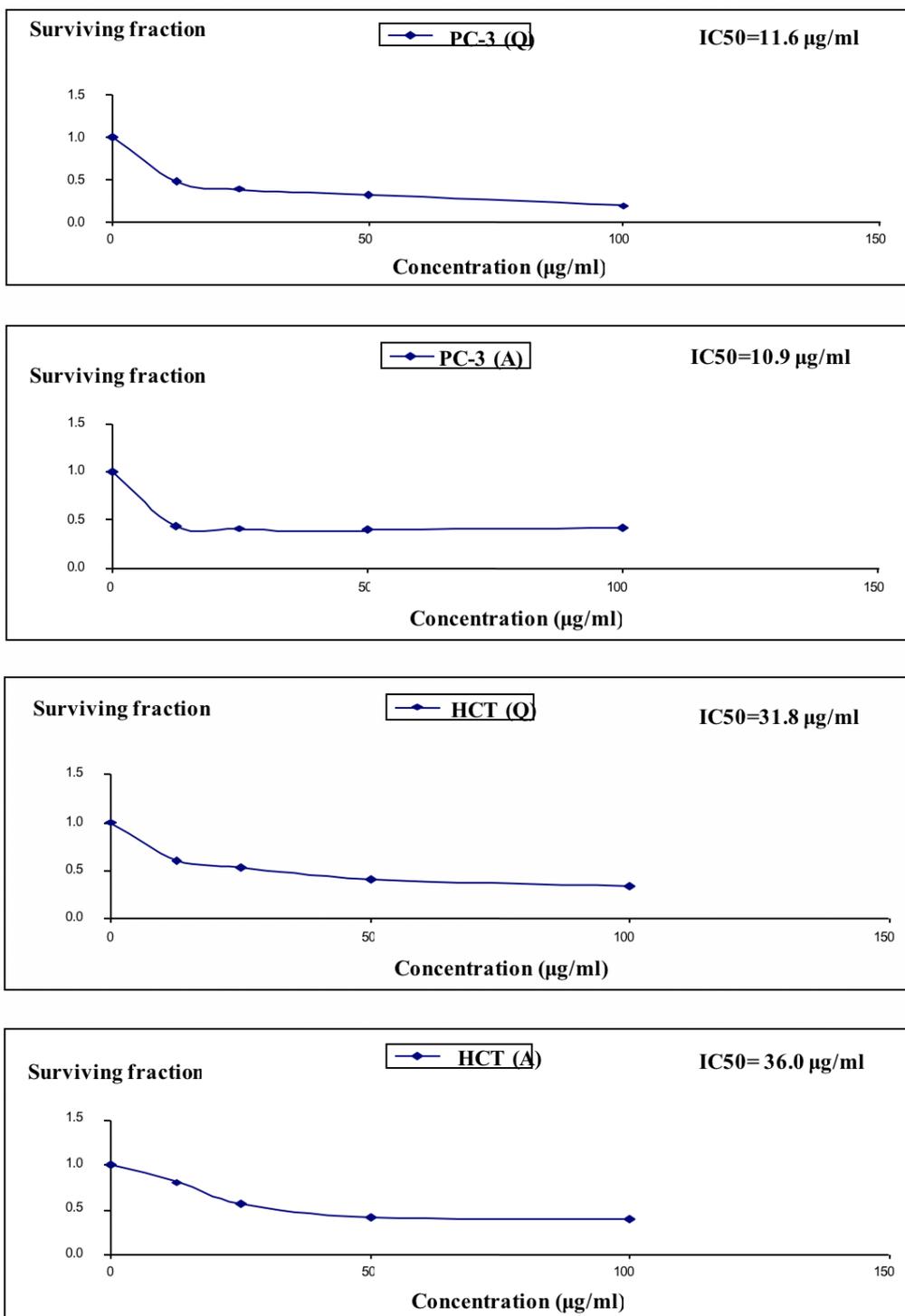
The anticancer activity of flavonoids has been well-documented in the literature. Moreover, flavonoids have been reported for their anticancer activity. **Figure 2** shows that CC leaves contain major components with a high amount of flavonoids: Quercetin (c), followed by Apigenin (b).

As shown in **Table 2**, Dox increases the reductive stress marker MDA, decreases the endogenous antioxidant marker GSH, increases the nitrosative stress marker NO, decreases the precursor of PGE-2 (AA), and increases testicular cell energy (ATP), in comparison with the control group. In contrast, CC treatment alone increases testicular function, and ameliorates the negative effects induced by Dox (when CC+Dox are used in combination).

As shown in **Table 3**, Dox decreases the reductive stress marker MDA, decreases endogenous antioxidant marker GSH, increases the nitrosative stress marker (NO), decreases the precursor of PGE-2 (AA), and increases testicular cell energy (ATP), in comparison with the control group. In contrast, CC alone increases testicular function and/or ameliorates the negative effects induced by Dox in the combination treatment group.

#### (c) Effects of CC ethanolic extracts alone or in combination with Dox-induced male reproductive toxicity, and effects on genomic DNA concentration in rat testis

**Figure 3** demonstrates the identification of DNA of rat testis (by 1% agarose gel electrophoresis) after various treatments. The resulting bands were compared against the DNA Ladder (100-3000 bp). It was observed, from the DNA results, that DNA appeared to be damaged after treatment with Dox (which induces toxicity in rat tests) at 25 mg/kg b.w. i.p. However, pre-administration

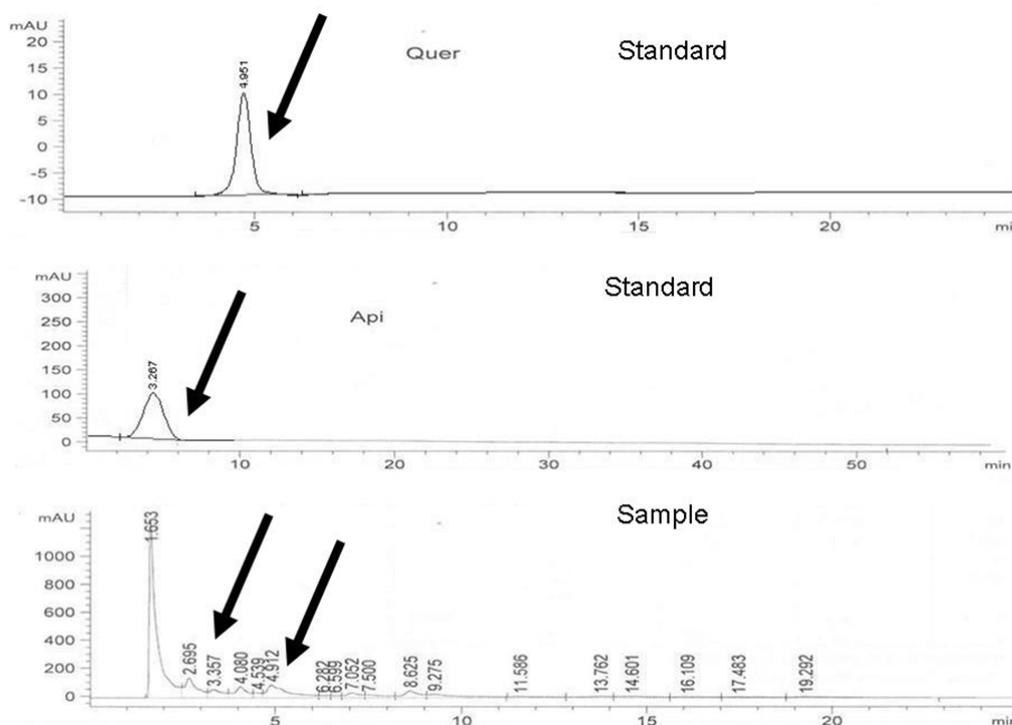


**Figure 1.** Effects of Apigenin (A) and Quercetin (Q) on cancer cell viability of Human Prostate Cancer (PC-3) and Human Colon Cancer(HCT-116) cell line. Cancer cells were incubated for 48 h with various concentrations of each compound (0.0, 12.5, 25.0, 50.0 and 100.0 µg/ml).

**Table 2.** Effect of *Cymbopogon citratus* against Doxorubicin-induced testicular dysfunction in terms of nitrosative stress, arachidonic acid (AA) oxidative stress markers (MDA, GSH), and cell energy (ATP) in male rats

Groups	Parameters				
	MDA nmol/g tissue	GSH μmol/g tissue	NO μmol/g tissue	AA μmol/g tissue	ATP μmol/g tissue
Control	35.02 ± 0.97	12.02 ± 0.32	0.394 ± 0.01	139.4 ± 3.7	28.02 ± 0.79
CC	26.94 ± 0.76a	16.19 ± 0.37a	0.302 ± 0.01a	117.3 ± 3.12a	22.76 ± 0.61a
Dox	108.1 ± 2.80a	7.031 ± 0.20a	1.813 ± 0.05a	66.02 ± 1.7a	14.02 ± 0.38a
Dox+CC	79.02 ± 1.90a	9.701 ± 0.29ab	1.424 ± 0.03ab	85.07 ± 2.3ab	19.03 ± 0.53ab

Data represents the mean ± SD. <sup>a</sup>Significant difference from control group; p<0.05; <sup>b</sup> Significant difference from DR group; p<0.05.



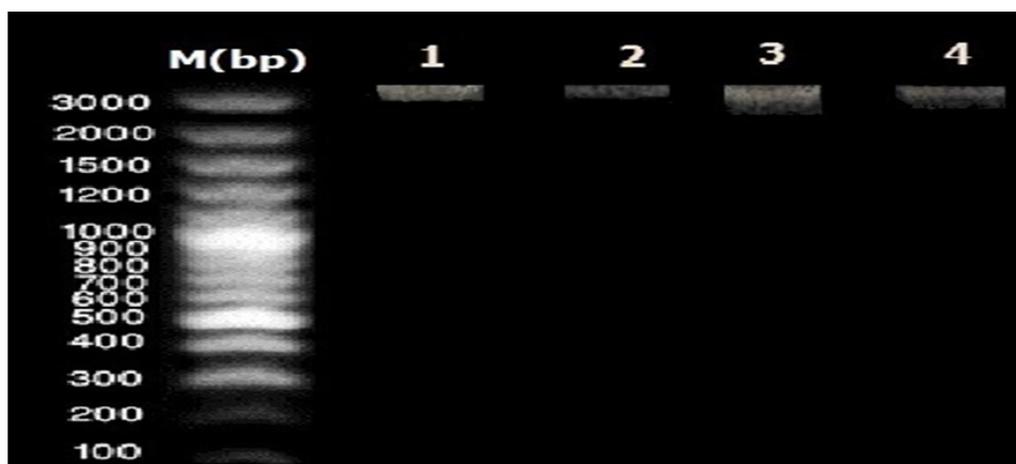
**Figure 2.** HPLC-chromatograms of *Cymbopogon citratus* (i.e. lemon grass; LG) leaves (a), Apigenin (b), and Quercetin (c).

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**Table 3. Effect of *Cymbopogon citratus* against Doxorubicin-induced testicular dysfunction with respect to total lipids, phospholipids, and deferential phospholipids (phosphatidylcholine and phosphatidylserine) in male rats**

Groups	Parameters				
	Total lipid (TL) (mg/g testis)	Phospholipids (PL) (mg/g testis)	PC ( $\mu$ g/g testis)	PS ( $\mu$ g/g testis)	PC/PS
Control	14.02 $\pm$ 0.38	9.521 $\pm$ 0.25	398.2 $\pm$ 11.0	99.01 $\pm$ 2.71	4.052 $\pm$ 0.10
CC	16.70 $\pm$ 0.45	10.21 $\pm$ 0.26	431.6 $\pm$ 11.8a	89.62 $\pm$ 2.32a	4.244 $\pm$ 0.11
Dox	9.412 $\pm$ 0.25a	6.643 $\pm$ 0.20	234.3 $\pm$ 6.61a	135.5 $\pm$ 3.63a	1.783 $\pm$ 0.05a
Dox+CC	10.33 $\pm$ 0.28ab	8.941 $\pm$ 0.25	281.1 $\pm$ 7.40ab	111.2 $\pm$ 2.91ab	2.561 $\pm$ 0.07ab

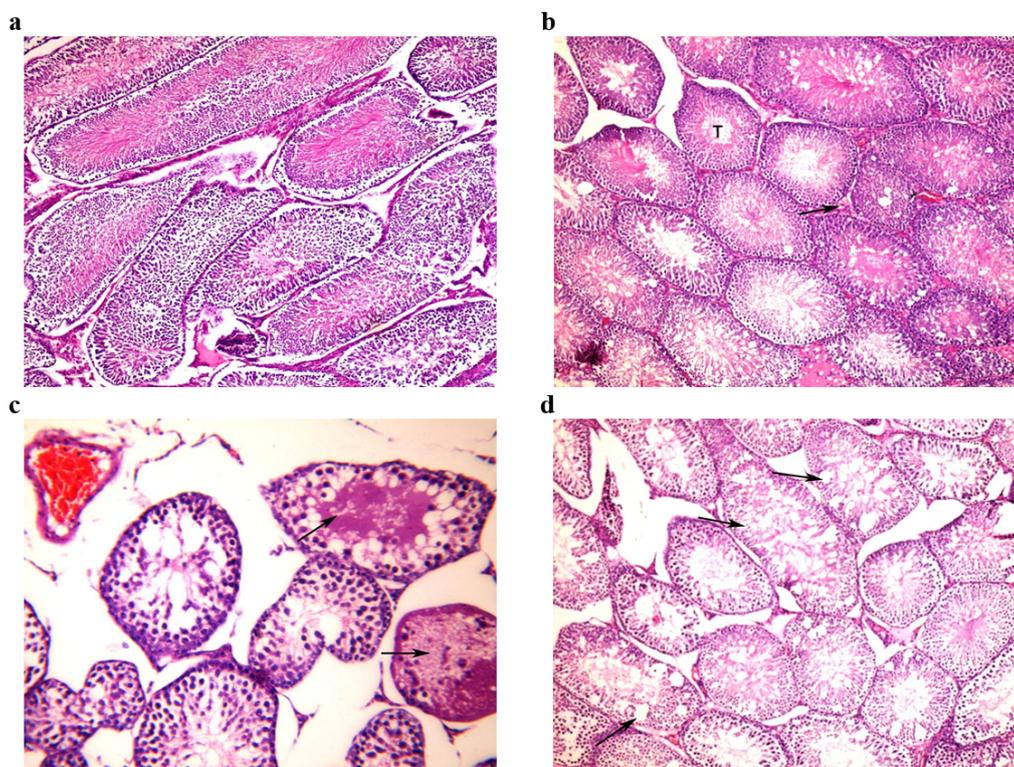
Data represents the mean  $\pm$  SD; <sup>a</sup> Significant difference from control group,  $p < 0.05$ ; <sup>b</sup> Significant difference from DR group,  $p < 0.05$ .



**Figure 3. Genomic DNA fragmentation pattern of testes tissues on 1% agarose gel electrophoresis. Lane M: DNA ladder; Lane 1: Saline(-ve control); Lane 2: CC Treatment; Lane 3: Dox Treatment (+ve control); and Lane 4: CC then Dox treatment 24 h before decapitation**

with CC in combination with Dox, or administration of CC alone, reduced the damage. All DNA bands were observed within the same molecular weight (more than 2000 bp).

Our results demonstrated that in the group with Dox-induced toxicity of rat testis, the DNA damage was clearly observed; this may be due to the oxidative stress and cell membrane damage as a result of the toxic effects of free radicals of lipid peroxidation, which act on DNA, membrane proteins, and lipids. It has been reported that the anti-proliferative effect of some medicinal plants, such as CC, is due to active flavonoid compounds which reduce DNA damage [30]. Thus, our findings are in accordance with previous results. Administration of the CC ethanolic extract in rats produced a significant increase in DNA content in both induced and non-induced toxicity groups (less damage in the latter). This enhancement in DNA concentration following CC treatment was in a higher percentage in the non-induced toxicity group (than the induced group). The effect of CC on DNA appeared to be dose-dependent.



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**Figure 4. Histopathological examination of cymbopogon citratus against doxorubicin-induced testicular dysfunction with respect to total lipids, phospholipids, and deferential phospholipids (phosphatidylcholine and phosphatidylserine) in male rats. a.** Testicular section of the control group (untreated) showed healthy architecture with normal spermatogenesis (H&E,200X). **b.** Testicular section group of rats treated with Cymbopogoncitratrus (CC) showed healthy architecture with normal spermatogenesis (H&E, 200X). **c.** Photomicrograph of testicular tissue of Dox-treated animals showing reduction in spermatogenesis lineage and uniformed seminiferous tubules with vacuolation (H&E; 200X). **d.** Photomicrograph of testicular tissue of Dox+CC treated animals showing improvement in spermatogenesis lineage and seminiferous tubules H&E, 200X).

Histological examination of testis tissues (**Figure 4a**) showed normal cell structure and seminiferous tubules. Meanwhile, the Dox-treated group (**Figure 4b**) resulted in a reduction in spermatogenic lineage (T) and seminiferous tubules with vacuolation. Pre-treatment with CC alone (**Figure 4c**) or in combination with Dox (**Figure 4d**) for 10 days ameliorated the effect of Dox-induced toxicity on testis tissues. The data obtained in the study suggested that the CC extract is conceivably defensive against Dox-induced male reproductive damage. Biochemical investigations were affirmed by histopathological data which showed damage induced by Dox after treatment (**Figure 4b**).

#### 4. Discussion

The testicles deliver the male gametes and male sexual hormones (androgens). Gonadal dysfunction is a common consequence of cytotoxic chemotherapy [31]. *Cymbopogon citratus* (CC) is popularly known as lemongrass. CC has gained great interest because it is among the commercially valuable essential oils and also widely used in food technology as well as in traditional medicine [32]. Nowadays, herbal remedies (such as CC) are a solution for patients with influenza, elephantiasis, pneumonia, and vascular issues. Data obtained from our

study show that, indeed, CC has anticancer and cytoprotective effects against Dox-induced testicular dysfunction. Doxorubicin, an anthracycline antibiotic, is a generally utilized anticancer agent. Dox is integral to intercalation and DNA biosynthesis by inhibiting the progression of topoisomerase II, which decreases the mechanical action for cutting DNA strands and relaxing DNA tangles and supercoils [33]. Dox suppresses the hydrolysis of ATP and topoisomerase Type-I, which change the linking number of circular DNA. Another stress that may be established after Dox induction is the generation of toxic reactive oxygen species (ROS) through disruption of transcription. First and foremost, the Dox-treated group showed a significant increase in MDA, NO, AA, and PS, and a decrease of GSH, ATP, TL, PL, and PC, in comparison with the control group. Compared to results of previous research studies, the results obtained in our study are consistent. Like other studies, our study shows the harmful effect of Dox and acceleration of lipid peroxidation, resulting in maximization of MDA levels and other inflammatory markers. According to pathogenesis features which occur through Dox-induced testicular toxicity, ROS has been implicated. Testicular lipid synthesis and cell membrane formation may be disrupted after Dox treatment, and PL may be degenerated by converting cell wall shape and charge [34]. In normal cases, PC is stable at the outer layer of the cell membrane and PS is embedded inside the membrane. However, reciprocal transformation between PC and PS may occur and serve as a marker for cytotoxic drug use or drug abuse after long term induction. Previous studies have shown that Dox treatment was concomitant with decrease of enzymatic or non-enzymatic antioxidant enzyme levels [35]. It is worth mentioning that the main side effects of Dox treatment are cardiotoxicity; in fact, nitric oxide is one of them. Several trials have been evaluated and have established that a mechanism of Dox-associated toxicity is cardiotoxicity [36]. Currently, the most accepted reason is injury of myocardial cells by free radicals occurring after its metabolism. Nitric oxide oversecretion after Dox induction can lead to stimulation of free radicals which may induce cardiac failure, ischemia/perfusion injury, and cardiomyopathy. Free radicals, especially NO, is an intense vasodilator and huge contributor in myocardial contraction and in the pathogenesis of cardiac diseases. Indeed, an increase of NO in testicular cells disrupts all reproductive functions [37]. Additionally, Dox affects the number of germ cells, induces atrophy of Leydig cells, and decreases the rate of spermatogenesis. Previous reports have confirmed that Dox can induce deterioration of sperm motion, sperm content and sperm morphology, which are responsible for the side effects on male fertility, due to ROS oversecretion [38]. Flavonoids are a class of polyphenolic mixes which show an assortment of pharmacological activities. There has been recent interest in using flavonoid derivatives therapeutically as anticancer drugs. At pharmacological levels, various naturally occurring flavonoids have been shown to be cancer-protective in a variety of animal models [39]. Quercetin and Apigenin are chemical molecules belonging to the flavonoid group, in particular, the flavonol family. Quercetin is one of the important molecules known to supplement vitamin C; it possesses anti-oxidant, anti-inflammatory, and anti-allergic properties [40]. The data obtained data in our study showed an increase in the endogenous antioxidant system (GSH), a decrease in oxido-nitrosative markers (MDA and NO), a decrease in inflammatory markers (e.g. AA), and an increase in energy (ATP) after fifteen days of treatments. Furthermore, the rat group treat with CC showed cell membrane protection, as represented by stabilization of lipids and phospholipids; there was an increase of total lipids, phospholipids, and PC, and a decrease in PS, compared to the Dox-treated group. Quercetin and Apigenin are oxidized by radicals, resulting in more stability, which decreases radical reactivity, especially in ROS (through reacting with the reactive compounds of the radicals). The main effects of Quercetin and Apigenin may reach hydroxyl groups which induce inactive radicals, according to the following equation:

Flavonoid (OH) + R• > flavonoid (O•) + RH; whereby R• is a free radical, and O• is an oxygen free radical.

Stabilizing the lipid and phospholipid profile of testicular cells, and repairing the PC/PS ratio, as a result of scavenging highly reactive oxygen-derived radicals (e.g. peroxyxynitrite, ROS), propagated after Dox treatment [41]. According to the oxidative stress markers, inflammatory

mediators, endogenous antioxidant and testicular cell energy, Quercetin ameliorated the most negative symptoms from Dox induction. Nitric oxide produced by endothelial cells or macrophages, and the higher concentrations produced by inducible nitric-oxide synthase in macrophages, can result in oxidative damage. Flavonoids, thereby, ameliorate NO radicals which produce the mostly irreversible damage to the cell membrane. Our obtained data is in agreement with results by van Acker *et al.* [42], which showed NO as a radical that can be directly scavenged by flavonoids. The authors also demonstrated that Apigenin represses the development of prostate cancer cells by causing cell cycle capture and apoptosis. This is similar to results observed by Shukla *et al.* [32], who showed that Apigenin (at 6 to 64 mg/day) may inhibit the movement of prostate cancer cells in a transgenic adenocarcinoma mouse prostate (TRAMP) model. Furthermore, our data is in agreement with data from a study by Van *et al.* [43], who reported that Apigenin initiates FoxO3a and its DNA restriction can prompt the declaration of downstream target proteins (BIM and p27/Kip1), bringing about cell cycle capture and diminished expansion in prostate tumors. Apigenin could be used as a potential preventive and therapeutic agent in the management of prostate cancer in humans. Indeed, mice treated with low and high dose of Apigenin (20 and 50 mg/day, respectively) with the middle Apigenin dose group (40-50 mg/day), which is the equivalent administration of a dose of 120 mg/d of Apigenin in a grown-up. Taken together, our investigation supports the idea that Apigenin may confer a wide range of natural and chemopreventive effects on cells by influencing various cell cycle progression pathways or apoptotic cascades [44]. Hung *et al.* [45] stated that Quercetin has a synergetic chemopreventive efficacy, when combined with Kaempferol, via the arrest of HCT-116 cells due to inhibition of DNA synthesis. As for the cascade pathway, Quercetin induces loss of HCT-116 viability and subsequently supports the structure/activity of 3'-OH moiety and/or 4'-OH moiety.

## 5. Conclusions

In conclusion, the data obtained in this study show the anticancer activity of Apigenin and Quercetin (constituents of CC) since they inhibited the growth and activity of human PC-3 prostate cancer and human HCT-116 colon cancer cell lines. The whole extract of CC led to an approximate convalescence from the reproductive side effects (induced by Dox treatment); this recovery was due to the flavonoids of the mixture (which were separated by HPLC). Thus, CC is cytotoxic against PC-3 and HCT-116 carcinoma cell lines and, moreover, the whole extract of CC ameliorates testicular dysfunction induced by Dox.

## 6. Open Access

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## 7. List of abbreviations

**AA:** arachidonic acid; **AQ:** Apigenin and Quercetin; **CC:** Cymbopogon citratus; **Dox:** Doxorubicin; **GSH:** glutathione; **H&E:** Hematoxylin and eosin; **HPLC:** High performance liquid chromatography; **MDA:** malondialdehyde; **NO:** Nitric oxide; **NODCAR:** National Organization for Drug Control and Research; **SRB:** Sulfo-Rhodamine-B

## 8. Ethics approval and consent to participate

The protocol for the conducted animal experiments was approved by the Research Ethics Committee of the NODCAR which followed the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

## 9. Competing interests

The authors declare that they have no conflicts of interest.

## 10. Authors' contributions

All authors contributed equally in the study design, interpretation of the data and writing of the final manuscript.

## 11. Acknowledgments

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