

Lemongrass (Cymbopogon Citratus), and Coenzyme Q10, ameliorates sodium valproate induced reproductive toxicity in rat model

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Abstract

This study was conducted to analyze the impact of lemongrass (*Cymbopogon Citratus*) powder (CP) and aqueous extract (CA) with and without coenzyme Q10 supplementation on reproductive toxicity induced by sodium valproate (SVA) in rat model. Forty-eight male albino rats (210 ± 5g) were divided randomly into 6 groups (n=8 rats). Experimental design; group (1) fed on basal diet as normal control (-ve). **Groups(2 - 6)** received sodium valproate (SVA) orally at dose 500mg/kg/BW daily on the final week of the experimental duration (45 days) to induce reproductive toxicity. Group(2) fed on basal diet with orally SVA at dose 500mg/kg/BW daily (on the final week) as reproductive toxicity control (+ ve), group (3) treated with coenzyme Q10 at dose (150 mg/kg/BW) + (SVA), group (4) treated with lemongrass (*Cymbopogon Citratus*) powder (CP) at dose (150 gm/kg diet) + (SVA), group (5) treated with lemongrass (*Cymbopogon Citratus*) aqueous extract (CA) at dose (150 mg/kg/BW)+ (SVA) and group (6) treated with mixture of (coenzyme Q10+CP+CA) at (50 mg/kg/BW+ 50 gm/kg diet + 50mg/kg/BW, respectively)+(SVA). The antioxidant and phenolic compounds of lemongrass (*Cymbopogon Citratus*) leaves and its aqueous extract were estimated. Lemongrass (*Cymbopogon Citratus*) leaves and its aqueous extract contained remarkable amounts of antioxidant compounds. The results showed the groups treated with lemongrass (*Cymbopogon Citratus*) leaves, aqueous extract and coenzyme Q10 affected the relative weight of (testis and seminal vesicles) positively. Moreover, the parameters determined in this study showed improvement in these levels when compared with reproductive toxicity control. The best results for parameters that were determined in this study, as serum lipid profiles and liver enzymes, testicular antioxidant and lipid peroxidation biomarkers, serum testosterone, (FSH) and (LH) levels in addition, sperm count were shown in groups were treated with (coenzyme Q10+CP+CA) then CP followed by coenzyme Q10 and CA respectively, but most results showed non-significant difference between these groups. The study showed that, lemongrass (*Cymbopogon Citratus*) alone or combined with coenzyme Q10 has a powerful protection benefits versus SVA-induced reproductive toxicity in rat model, without causing severe oxidative injury to the reproductive organs and hormonal levels **that could** be attributable to its antioxidants, phenolics and flavonoid contents that related mainly to ameliorate reproductive toxicity induced by sodium valproate in rat model.

Keywords: Lemongrass (*Cymbopogon Citratus*)- rat model - sodium valproate - coenzyme Q10- antioxidants - reproductive toxicity.

Introduction

One of the most fragrant herb that is frequently grown for its excellent aroma is lemongrass (*Cymbopogon Citratus*), Botanically, this herb belongs to the grass family of *Poaceae*. Fresh lemongrass (*Cymbopogon Citratus*) leaves take place in traditional salads in many countries as an example, Mideast and Southwest Asia, India, and Brazil [**Rahim et al., 2013**].

Lemongrass(*Cymbopogon Citratus*), whether fresh or dried, is a rich source of minerals like potassium, zinc, calcium, iron, manganese, copper, and magnesium. Its leaves are very good in folate, also rich in many essential vitamins such as pantothenic acid (vitamin B5), pyridoxine (vitamin B-6) and thiamin (vitamin B-1) [**Chaisripipatet al., 2015 and Tabassu,2020**]. Lemongrass(*Cymbopogon Citratus*)also contains L-carnitine which is a conditionally required amino acid in the mitochondrial system, which plays a crucial role as cofactor in the production of cellular energy [**Nhu-Trang et al., 2006**].

As herbal remedy, lemongrass(*Cymbopogon Citratus*) is often used in some countries, as a tranquilizer, treat fevers and to alleviate some gastrointestinal disorders [**Rauber et al., 2005**]. Many scientific researches revealed the efficacies of lemongrass(*Cymbopogon Citratus*)that has various medicinal actions specifically, antibacterial, antidiarrheal and antifungal[**Cheel et al., 2005**].

Coenzyme Q10 also recognized as ubiquinone-10 which have significant functions in cell membranes and mitochondria as powerful bioactive natural antioxidant against free radical lipid peroxidation processes. Ubiquinone-10 supplementation to rat model and humans resulted in enhancing mitochondrial activity by synthesizing of energy-carrying molecule adenosine triphosphate (ATP) which is found in all the cells of living things, with remarkable enhances of the free coenzymes pool, therefore poses the body immune defense system [**El-khadragy et al., 2020**].

Some widely applied usage of sodium valproate (SVA) is to manage pain, chronic neurological disorders migraine and chronic headache as co-enhancer for immune system in chemotherapy. Sodium valproate (SVA) toxicity is infrequent but conceivably fatal resulting severe idiosyncratic liver injury and testicular injury. Additionally, SVA causes oxidative stress and reproductive toxicity in experimental rat model. [**Rossi, 2013; Shelbaya, 2016 and Oztopuz et al., 2020**].

The current study was designed to evaluate the effects of *Cymbopogon Citratus* versus reproductive toxicity in rat model induced by sodium valproate(SVA).

Materials and Methods

Materials and chemicals

Lemongrass (*Cymbopogon Citratus*) was obtained from agriculture research center, Riyadh, Saudi Arabia.

Sodium valproate (SVA) was obtained from a local pharmacy in Riyadh city, Saudi Arabia, name Depakine.

Coenzyme Q10 capsules were obtained from a local pharmacy in Riyadh city, Saudi Arabia.

Other chemicals were obtained from Sigma Aldrich, USA.

Preparation of dried lemongrass (*Cymbopogon Citratus*) and aqueous extract:

Lemongrass (*Cymbopogon Citratus*) was dried at shade after being washed and minced, then dried in dessicators to remove moisture to up level. The water extract was prepared daily as tea by putting 5 gm of lemongrass (*Cymbopogon Citratus*) leaves into the teapot, added 100ml of distilled water, boiled for fifteen minutes and then filtered to insure better extraction according to **Guleria and Sehgal, [2020]**. The lemongrass (*Cymbopogon Citratus*) dried powder was used at dose (150 gm/ kg diet) and lemongrass (*Cymbopogon Citratus*) aqueous extract was given to rats orally at dose (150 mg/kg BW).

Estimation of antioxidant compounds of lemongrass (*Cymbopogon Citratus*) leaves:

The antioxidant compounds of the lemongrass (*Cymbopogon Citratus*) leaves were evaluated according to **Hertog et al. [1992] and Hakkinen et al., [1998]**.

Determination of phenolic compounds of lemongrass (*Cymbopogon Citratus*) aqueous extract:

Identification of phenolic compounds for lemongrass (*Cymbopogon Citratus*) aqueous extract according to **Chang et al. [2002]** by comparison with the reference standard retention rate (RT/min), mass spectrometry detection was carried out using an Agilent Mass Selective Detector system to identify and confirm the components in chromatographic peaks. Analysis of phenolic compounds for lemongrass (*Cymbopogon Citratus*) extract was held by HPLC method according to **Shelbaya, [2016]**.

Animals

Forty-eight healthy adult male albino rats initially weighed around $210 \pm 5g$, at age of 10-12 weeks were obtained from Laboratory of Animal Colony, College of Pharmacy, King Saud University. Rat model were housed in stainless cages in Applied Medical Sciences, KSU. The experimental protocol was conducted in ethical number (CAMS-001-3940), also animal care procedures were in agreement with the National Institutes of Health (NIH).

Preparation of basal diet

Basal diet was prepared according to, **Reeves et al., [1993]**. It consists of 20% protein (as casein), sucrose 10%, corn oil 4.7%, choline chloride 2%, vitamin mixture 1%, salt mixture 3.5%, fibers 5%, and corn starch up to 100%.

Methods

Experimental design.

Experimental rats were fed on the basal diet for adaptation in a precisely calibrated setting for 7 days before beginning the trial (25 ± 2 °C temperatures, 55–60% humidity and a 12 h light/dark cycle). Rats were divided randomly into 6 groups (n=8 rats), group 1 (normal control) animals were fed on basal diet only. In accordance with **Hamza and Amin, [2007]**, rats of groups (2-6)(n= 40 rats) were fed on basal diet, and were given orally sodium valproate (SVA) at dose (500mg/kg/BW daily on the final week) of the experimental duration (45 days) to induce reproductive toxicity.

Group (1): Fed on basal diet without treatment and assigned as normal control (-ve).

Group (2): Fed on basal diet with orally sodium valproate (SVA) (500 mg/kg/BW daily) on the final week as reproductive toxicity control (+ ve)

Group (3): Fed on basal diet with were given orally coenzyme Q10 (150mg/kg/BW) for the period of experiment (45 days) + (SVA) (500 mg/kg/BW daily on the final week).

Maha M. Essam El-Din

Group (4): Fed on basal diet contains lemongrass (*Cymbopogon Citratus*) powder(150 gm/kg diet)for the period of experiment (45 days)+ (SVA) (500 mg/kg/BW daily on the final week).

Group (5):Fed on basal diet were givenorally lemongrass (*Cymbopogon Citratus*) aqueous extract(150 mg/kg/BW) for the period of experiment (45 days) + (SVA) (500mg/kg/BW daily on the final week).

Group (6):Fed on basal diet with mixture of(coenzyme Q10+CP+CA) (50 mg/kg/BW + 50 gm/kg diet + 50 mg/kg/BW, respectively) for the period of experiment (45 days)+ (SVA) (500 mg/kg/BW daily on the final week).

Feed intake was recorded daily and the body weight gain assigned weekly, feed efficiency ratio (FER) was estimated by equation FER = weight gain (g)/ feed intake (g)

Calculation of sexual organs relative weight were calculated by equation:

Organs relative weight = (organ weight / animal final weight) x 100

Biochemical analysis

Determination of serum lipid profiles:

Serum total cholesterol (TC) was performed according to *Henry et al., [1974]*. According to *Fossati and Precnie, [1982]*, Serum triglycerides (TG) was determined. Serum high density lipoproteins cholesterol (HDL-c) was assayed according to *Burstein, [1970]*. Serum low density lipoproteins cholesterol (LDL-c) and very low density lipoproteins cholesterol (VLDL-c) were calculated according to *Friedewald et al., [1972]* as following:

LDL- c (mg/dl) = total cholesterol –[HDL cholesterol - (TG/5)].

VLDL-c (mg/dl) = triglycerides (TG) / 5

Determination of serum liver enzymes:

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentration were determined according to the method described by *Young [1990], and Sherwin [1984]*, respectively.

Determination of antioxidant and lipid peroxidation biomarkers in testicular tissues of rats:

As illustrated by *Sun et al., [1988];Ohkawa et al., [1979] and Moron et al., [1979]* determined the activity of antioxidant and lipid peroxidation biomarkers in testicular tissues such as total antioxidants capacity (TAC), superoxide dismutase activity (SOD), glutathione (GSH) and malondialdehyde (MDA) were determined after weighing the genitalia, each testis was homogenized as described by *Koracevic et al., [2001] and Prathima et al., [2017]* for previous biochemical analysis.

Determination of serum hormonal levels and sperm count:

According to the methods described by *Maruyama, [1987]* concentrations of serum testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were estimated. The sperm count determined according to *Zambrano et al., [2005]* by hemocytometer after sperm dilution, were counted with a light microscope at 400x.

Statistical analysis:

Data were expressed as means ± standard deviation. In order to compare the groups, Analysis of Variance (ANOVA) test was used.Values at P<0.05 were considered to be statistically significant according to *SAS,[2006]*.

Results

Antioxidant compounds of lemongrass (*Cymbopogon Citratus*)leaves:

Data presented in Table (1) recorded that, lemongrass "*Cymbopogon Citratus*" leaves contains some important bioactive components expressed as total antioxidant activity (94.46%), total phenols (12.89%) and flavonoids (3.88%), also contains tannins (39.16mg/100g) and chlorophyll (2.69mg/100g).

Table (1):
Antioxidants compounds Contents in lemongrass (*Cymbopogon Citratus*)leaves.

Samples	Total antioxidant activity (%)	Total phenols (%)	Flavonoids (%)	Tannins (mg/100g)	Chorophyll (mg/100g)
Lemongrass(<i>Cymbopogon Citratus</i>) leaves	94.46	12.89	3.88	39.16	2.69

Phenolic compounds in lemongrass (*Cymbopogon Citratus*)aqueousextract:

Many active compounds were detected in lemongrass (*Cymbopogon Citratus*)extract as shown in Table (2). The extract of lemongrass (*Cymbopogon Citratus*) contain of high amount as concentration from caffeic acid, syringic acid, quercetin, catechin, gallic acid, epicatechin andvanillic acid as it recorded 15.35, 12.31, 11.68, 7.85, 6.88, 6.54, 6.01mg/100 ml, respectively while the lowest amount was dihydroxybenzoic acid at 2.15 mg/100 ml.

Table (2):
Phenolic Compounds in lemongrass (*Cymbopogon Citratus*)aqueous extract at Standard Retention Rate(RT/min)and Concentration (mg/100 mL).

Compounds	Standard Retention Rate (RT/min)	Concentration (mg/100 mL)
Syringic acid	4.01	12.31
Dihydroxybenzoic acid	4.32	2.15
Gallic acid	5.2	6.88
Terpenes and terpenoids	5.85	5.23
Epicatechin	6.41	6.54
Catechin	8.05	7.85
Caffeic acid	8.99	15.35
Vanillic acid	9.32	6.01
3,7-dimethyl-2,6-Octadienal	20.35	4.61
Quercetin	29.33	11.68

Maha M. Essam El-Din

Effects of lemongrass (*Cymbopogon Citratus*) on feed intake, body weight gain and FER for reproductive toxicity rats.

As presented data in Table (3), reproductive toxicity control group showed a significant $P < 0.05$ decline in feed intake; body weight gain and feed efficiency ratio compared to normal control group. Contrarily, the all treated groups showed significant $P < 0.05$ elevation in these parameters compared to the positive control.

Table (3):

Effects of lemongrass (*Cymbopogon Citratus*) on feed intake, body weight gain and FER for reproductive toxicity rats.

Groups	Parameter	Feed intake (g/d)	Body weight gain (g/45 days)	FER*
Normal control		25.24 ± 3.10 ^a	94.73 ± 2.41 ^a	0.085 ± 0.004 ^a
Reproductive toxicity control		11.30 ± 1.02 ^b	40.42 ± 1.32 ^c	0.078 ± 0.005 ^b
SVA+ Coenzyme Q10 (150 mg/kg BW)		19.53 ± 4.11 ^a	81.14 ± 2.23 ^b	0.093 ± 0.003 ^a
SVA+ CP (150 gm /kg diet)		21.46 ± 2.14 ^a	85.27 ± 4.33 ^b	0.088 ± 0.002 ^a
SVA+ CA (150 mg/kg BW)		20.38 ± 3.13 ^a	77.09 ± 3.22 ^b	0.085 ± 0.003 ^a
SVA+ (Coenzyme Q10+CP+CA)		23.94 ± 1.12 ^a	87.24 ± 1.14 ^{ab}	0.081 ± 0.001 ^a

* FER= feed efficiency ratio

** Values are expressed as mean ± SD. n= 8 rats/group.

** Letters indicates the significant differences at $P < 0.05$ (DMRT)

Effects of lemongrass (*Cymbopogon Citratus*) on sexual organs relative weight for reproductive toxicity rats.

Data expressed in Table (4) showed the impacts of lemongrass (*Cymbopogon Citratus*) treatments on relative weight of testis, seminal vesicles, and prostate organs on reproductive toxicity control group. The results showed reproductive toxicity control group were reduction significantly ($P < 0.05$) in the testis and seminal vesicles relative weight but not significant change in prostate relative weight when compared with normal control group. Notwithstanding, the groups treated with coenzyme Q10, CP, CA and combination of (coenzyme Q10 + CP + CA) group were shown significant $P < 0.05$ relative weight improvement of some sexual organs (testis and seminal vesicles) when compared with reproductive toxicity control group.

Table (4):

Effects of lemongrass (*Cymbopogon Citratus*) on sexual organs relative weight for reproductive toxicity rats.

Groups	Parameter	Testis	Seminal vesicles	Prostate
	Relative weight of organs (%)			
Normal control		3.545 ± 0.20 ^a	0.879 ± 0.09 ^a	0.419 ± 4.02 ^a
Reproductive toxicity control		1.990 ± 0.21 ^d	0.521 ± 0.03 ^c	0.295 ± 3.11 ^{ab}
SVA+ Coenzyme Q10 (150 mg/kg BW)		2.917 ± 0.22 ^c	0.758 ± 0.05 ^a	0.381 ± 3.09 ^{ab}
SVA+CP (150 gm /kg diet)		2.994 ± 0.28 ^{bc}	0.781 ± 0.17 ^a	0.388 ± 4.01 ^{ab}
SVA+CA (150 mg/kg BW)		2.784 ± 0.13 ^b	0.727 ± 0.06 ^a	0.379 ± 4.02 ^{ab}
SVA+ (Coenzyme Q10+CP+CA)		3.082 ± 0.16 ^a	0.818 ± 0.11 ^a	0.395 ± 4.02 ^{ab}

** Values are expressed as mean ± SD. n= 8 rats/group.

** Letters indicate the significant difference at P < 0.05 (DMRT)

Effects of lemongrass (*Cymbopogon Citratus*) on serum lipid profiles for reproductive toxicity rats.

The impacts of lemongrass (*Cymbopogon Citratus*) powder and aqueous extract on the serum lipid profiles versus reproductive toxicity in rat model presented in Table (5). Rats received sodium valproate (SVA) were shown significant P<0.05 increase in total cholesterol, triglycerides, LDL-C and VLDL-C vice versa, for HDL-C, that decreased. Results showed the tested groups treated with coenzyme Q10, CP and CA and group treated with combination of (coenzyme Q10+CP+ CA), were shown significant enhance in lipid profile levels; that were elevated when rats received SVA.

Table (5):

Effects of lemongrass (*Cymbopogon Citratus*) on serum lipid profiles for reproductive toxicity rats.

Groups	Parameters	TC	TG	LDL-c	VLDL-c	HDL-c
	mg/dl					
Normal control		120.22 ± 4.13 ^c	105.13 ± 2.88 ^d	83.09 ± 3.43 ^c	21.09 ± 2.62 ^c	58.26 ± 3.12 ^a
Reproductive toxicity control		183.19 ± 2.15 ^a	169.23 ± 1.03 ^a	192.99 ± 3.43 ^a	33.98 ± 4.82 ^a	24.28 ± 2.54 ^c
SVA+Coenzyme Q10(150 mg/kg BW)		140.16 ± 3.36 ^{bc}	128.25 ± 1.95 ^c	123.25 ± 1.37 ^{bc}	26.88 ± 3.02 ^b	42.27 ± 2.66 ^b
SVA+CP (150 gm /kg diet)		137.24 ± 3.44 ^{bc}	120.17 ± 3.80 ^c	111.59 ± 2.36 ^{bc}	25.07 ± 2.34 ^{bc}	48.20 ± 1.93 ^b
SVA+CA (150 mg/kg BW)		146.42 ± 3.18 ^b	139.24 ± 4.04 ^b	131.94 ± 1.76 ^b	27.95 ± 1.08 ^b	43.22 ± 2.76 ^b
SVA+ (Coenzyme Q10+CP+CA)		125.25 ± 4.01 ^c	110.15 ± 2.77 ^{cd}	96.86 ± 0.37 ^c	21.69 ± 1.45 ^c	51.27 ± 2.75 ^{ab}

** Values are expressed as mean ± SD. n= 8 rats/group.

** Letters indicates the significant differences at P < 0.05 (DMRT)

Maha M. Essam El-Din

Effects of lemongrass (*Cymbopogon Citratus*) on serum liver enzymes (AST and ALT) for reproductive toxicity rats.

The effects of lemongrass (*Cymbopogon Citratus*) powder and aqueous extract on serum liver enzymes (AST and ALT) on reproductive toxicity rats presented in Table (6). Rats received sodium valproate (SVA) were shown significant ($P < 0.05$) increased in AST and ALT. While, the groups treated with coenzyme Q10, CP and CA and group treated with combination of (coenzyme Q10 + CP + CA), were shown significant improvement on liver enzymes levels when compared with reproductive toxicity group.

Table (6):

Effects of lemongrass (*Cymbopogon Citratus*) on liver enzymes (AST and ALT) for reproductive toxicity rats.

Groups	Parameter	AST (IU/L)	ALT (IU/L)
Normal control		64.87 ± 1.29 ^c	40.10 ± 1.95 ^d
Reproductive toxicity control		95.50 ± 1.67 ^a	82.67 ± 3.54 ^a
SVA+ Coenzyme Q10 (150 mg/kg BW)		74.87 ± 2.24 ^b	49.52 ± 1.26 ^b
SVA+CP (150 gm /kg diet)		71.99 ± 2.82 ^{bc}	47.38 ± 2.18 ^b
SVA+CA (150 mg/kg BW)		76.44 ± 1.57 ^b	50.42 ± 0.08 ^b
SVA+ (Coenzyme Q10+CP+CA)		69.05 ± 1.89 ^c	45.25 ± 0.15 ^{cd}

** Values are expressed as mean ± SD. n= 8 rats/group.

**Letters indicates the significant differences at $P < 0.05$ (DMRT)

Effects of lemongrass (*Cymbopogon Citratus*) on testicular antioxidant and lipid peroxidation biomarkers for reproductive toxicity rats.

The effects of treatment with lemongrass (*Cymbopogon Citratus*) on testicular antioxidant and lipid peroxidation parameters presented in Table (7). Results showed the reproductive toxicity control group were shown significant ($P < 0.05$) decrease on testicular levels of total antioxidants capacity, superoxide dismutase (SOD) and GSH, while significant increase in testicular malondialdehyde (MDA) level when compared to normal control group. All groups treated with lemongrass (*Cymbopogon Citratus*), coenzyme Q10 and combination of (coenzyme Q10+CP+CA), were shown significant ($P < 0.05$) increase in total antioxidants capacity, superoxide dismutase (SOD) and GSH as compared to reproductive toxicity control group, while significant decrease in testicular malondialdehyde (MDA) level when compared with reproductive toxicity control group. The best findings were recorded in group treated with combined (coenzyme Q10+CP+CA) followed by groups treated with lemongrass (*Cymbopogon Citratus*) powder, coenzyme Q10 and lemongrass (*Cymbopogon Citratus*) aqueous extract, respectively when compared to reproductive toxicity and normal control groups.

Table (7):

Effects of lemongrass (*Cymbopogon Citratus*) on testicular antioxidant and lipid peroxidation biomarkers for reproductive toxicity rats.

Groups	Parameters	Total Antioxidants capacity (Units/mg protein)	SOD (Units/mg protein)	GSH (μmol /mg protein)	MDA (μmol/mg protein)
Normal control		4.18 ± 0.35 ^a	192.33 ± 8.77 ^a	122.23 ± 1.06 ^a	7.63 ± 1.34 ^e
Reproductive toxicity control		1.33 ± 0.46 ^c	97.22 ± 3.22 ^e	59.36 ± 0.76 ^d	15.32 ± 2.67 ^a
SVA+ Coenzyme Q10 (150 mg/kg BW)		3.28 ± 0.33 ^b	166.17 ± 7.74 ^c	94.32 ± 0.40 ^b	9.66 ± 1.06 ^{bc}
SVA+CP (150 gm /kg diet)		3.58 ± 0.42 ^{ab}	172.16 ± 9.03 ^b	98.24 ± 0.36 ^b	8.39 ± 1.11 ^c
SVA+CA (150 mg/kg BW)		3.11 ± 0.29 ^b	137.17 ± 7.53 ^d	83.33 ± 0.45 ^c	10.77 ± 1.14 ^b
SVA+ (Coenzyme Q10+CP+CA)		3.78 ± 0.28 ^{ab}	181.13 ± 9.52 ^{ab}	113.28 ± 0.38 ^{ab}	7.98 ± 1.31 ^e

** Values are expressed as mean ± SD. n= 8 rats/group.

**Letters indicates the significant differences at P < 0.05 (DMRT)

Effects of lemongrass (*Cymbopogon Citratus*) on serum sexual hormones and sperm count for reproductive toxicity rats.

Table (8) demonstrated the reproductive toxicity control group showed significant (P<0.05) reduction in levels of testosterone, LH and FSH when compared to normal control group. While, significant (P<0.05) improvement in these parameters occurred with treatments by combination (coenzyme Q10+CP+CA) group; lemongrass (*Cymbopogon Citratus*) powder; coenzyme Q10 and lemongrass (*Cymbopogon Citratus*) aqueous extract respectively, when compared with reproductive toxicity control group. Moreover, male rats received SVA caused a significant decrease of sperm count when compared to normal control group, while all treated groups showed significant increase of the sperm count and minimized toxic effects of SVA when compared to reproductive toxicity control group.

Table (8):

Effects of lemongrass (*Cymbopogon Citratus*) on serum sexual hormones and sperm count for reproductive toxicity rats.

Parameter Groups	Testosterone	Follicle- Stimulating Hormone (FSH)	Luteinizing hormone (LH) mg/mL	sperm count (10 ⁶ /mL)
Normal control	35.33 ± 2.9 ^a	193.38 ± 2.87 ^a	6.25 ± 1.82 ^a	79.98 ± 2.80 ^a
Reproductive toxicity control	18.15 ± 1.99 ^d	110.18 ± 1.88 ^d	2.96 ± 2.81 ^d	15.20 ± 3.75 ^d
SVA+ Coenzyme Q10 (150 mg/kg BW)	28.01 ± 2.99 ^b	180.71 ± 1.33 ^b	4.99 ± 2.99 ^c	65.88 ± 1.33 ^b
SVA+CP (150 gm /kg diet)	30.18 ± 2.81 ^b	182.76 ± 2.90 ^{bc}	5.01 ± 2.20 ^b	68.05 ± 3.22 ^b
SVA+CA (150 mg/kg BW)	25.12 ± 3.01 ^c	178.54 ± 3.52 ^c	4.80 ± 1.25 ^c	55.75 ± 4.22 ^c
SVA+ (Coenzyme Q10+CP+CA)	31.89 ± 2.47 ^{ab}	186.98 ± 2.75 ^b	5.85 ± 2.07 ^b	75.53 ± 2.56 ^a

** Values are expressed as mean ± SD. n= 8 rats/group.

**Letters indicates the significant differences at P < 0.05 (DMRT)

Discussion

In this work, rat model received orally sodium valproate (SVA) resulted in reproductive toxicity, the potential prevention role of both powder (dried) and aqueous extract of lemongrass (*Cymbopogon Citratus*) with or without coenzyme Q10 and combination of (coenzyme Q10, CP and CA), were examined. The results showed the groups treated with lemongrass (*Cymbopogon Citratus*) with or without coenzyme Q10 were shown insignificant increase in feed intake, body weight gain when compared with reproductive toxicity group. These agreed with **Shimaa et al., [2019]** that reported the rats fed on lemongrass (*Cymbopogon Citratus*) showed significant higher in feed intake (FI), body weight gain (BWG), body weight %.

Organs relative weight of testis and seminal vesicles were significantly reduced in rats which received orally sodium valproate (SVA) versus normal control and may be attributed to testicular toxicity induced by SVA. These effects were similar to those reported by **Sveberg et al., [2001]** they reported a highly significant decrease relative weight of testis when treated by high dose of SVA. Results showed the groups treated with powder (dried), aqueous extract of lemongrass (*Cymbopogon Citratus*) with or without coenzyme Q10, presented a significant improvement of some sexual organs (testis and seminal vesicles) relative weight, but non-significant differences in relative weight of prostate compared with reproductive toxicity control group, these results agree with the results obtained by **Brandsch et al., (2002)** who reported that, treatment of coenzyme Q10 for rats received SVA to induce reproductive toxicity does not produce any significant change in the relative weight of prostate when compared with the negative and positive controls.

In our study, the results showed significant increase in serum total cholesterol, triglycerides, LDL-C, VLDL-C, ALT and AST levels and a significant decrease in, HDL-C levels in reproductive

toxicity of rats when compared to normal control. In the same line, the results showed significant decrease on testicular levels of total antioxidants capacity, superoxide dismutase (SOD) and GSH, while significant increase in testicular malondialdehyde (MDA) level for reproductive toxicity control group when compared to normal control group. Moreover, the serum levels of sexual hormones and sperm count were significantly decreased for reproductive toxicity control group. These results agreed with **Ergün et al., [2007]** who found, increase the serum VLDL and total triglyceride levels were statistically associated with low sperm motility and increased deleterious effect on the spermatogenesis. Also, **Hagiuda et al., [2014]**, reported that the sperm morphology has associated with the level of serum triglyceride positively. Similarly, **Liu et al., [2017]** reported that, when the elevate serum levels of lipid profiles and total cholesterol the sperm morphology changes were detected by showing a low percentage of spermatozoa with smaller sperm perimeter and head area and intact acrosomes. In addition, the reproductive toxicity control group showed significant reduction in levels of testosterone, LH, FSH levels and decreased sperm count when compared to normal control group. These agreed with **Laxminarayana et al., [2010]** reported sloughing of rat's testis epithelial cells induced by SVA has been observed and decreased levels of testosterone, FSH and LH, also reduced sperm count and motility in male rats.

On another hand, when the reproductive toxicity groups were treated with lemongrass (*Cymbopogon Citratus*) alone or combined with coenzyme Q10 showed significant decrease in serum total cholesterol, triglycerides, LDL-C, VLDL-C, ALT and AST levels and a significant increase in, HDL-C levels when compared with reproductive toxicity control. These results agreed with **Nekohashi et al., [2014]** and **Morgado et al., [2015]**, they reported that flavonoids and tannins in lemongrass (*Cymbopogon Citratus*) are the main responsible for the hypolipidemic effect and showed a correlation between the consumption of polyphenols with a decreased risk of atherosclerosis due to its antioxidant effect. Also, the potential role of flavonoids in lemongrass (*Cymbopogon Citratus*) inhibiting intestinal cholesterol absorption and transport reported by **[Nekohashi et al., 2014 and Ressurreição, 2022]**. Moreover, quercetin inhibiting NPC1L1 so that reduced high blood cholesterol level and catechin, gallic acid, and epicatechin also have hypercholesterolemic activity through their binding to the bile acids so that reduced the cholesterol solubility **[Ngamukote et al., 2011 and Nekohashi et al., 2014]**.

In the same line, the results as shown, the groups were treated with lemongrass (*Cymbopogon Citratus*), coenzyme Q10 and combination of (coenzyme Q10 + CP + CA), were shown significantly increased in testicular total antioxidants capacity, SOD and GSH levels, while the levels of MDA were significantly decreased in treated groups when compared with reproductive toxicity rats. In addition, the serum levels of sexual hormones and sperm count were significantly increased. These results were confirmed with **Hesham and Shaeru, [2002]** they reported free radical scavenging compounds content are high in lemongrass (*Cymbopogon Citratus*) leaves and could be considered as a potential source of antioxidants. MDA and SOD activities as indicators for lipid peroxidation and presence of free radical anions were restored near normal levels when treatment with lemongrass (*Cymbopogon Citratus*) **[Nakamura et al., 2003]**. Also, the results reported by **Cheel et al., [2005]** revealed that the presence of phenolic compounds in lemongrass (*Cymbopogon Citratus*) are responsible for antioxidant properties of the leaves. The potential effect of lemongrass (*Cymbopogon Citratus*) related to high probability of tannins and flavonoids **[Morgado et al., 2015]**. Studies showed lemongrass (*Cymbopogon Citratus*) extracts containing antioxidants such as phenolic compounds and tannins that reduce the levels of lipid peroxide and prevent from the destructive effects, radical scavenging activity, regulatory action on suppressing oxidative stress and stability,

Maha M. Essam El-Din

permeability of cellular [Saenthaweesuk et al., 2017]. In the same line, the action of lemongrass (*Cymbopogon Citratus*) related to its content from phenolic compounds as well as to the fractions of phenolic acids, flavonoids, terpenoids and tannins [Avosehet al., 2015 and Ressurreição, 2022]. While, Nezhad et al., [2021] confirmed the coenzyme Q10 decreased the number of ROS.

The beneficial effects of lemongrass (*Cymbopogon Citratus*) on the rat reproductive system, described herein, was similar to that disclosed by Hanaa [2013], which concluded that lemongrass (*Cymbopogon Citratus*) contains a qualitative phytochemical component that indicates the existence of flavonoids which may be linked to its cytoprotective and antioxidant functions. Also, this may be attributed to the presence of caffeic which have bioactive superoxide anion scavengers, that may inhibit lipid peroxidation process [Cheel et al., 2005 and Ahda et al., 2022]. Lemongrass (*Cymbopogon Citratus*) have anticancer effect related to its content of phenolic compounds component [Puatanachokchai et al., 2002].

Many phytochemical studies were shown, the lemongrass (*Cymbopogon Citratus*) extract has anti-mutagenic properties, this because of phenolic compounds and antioxidant capacity such as caffeic acid, quercetin and catechin the main phenolic compounds extracted from lemongrass (*Cymbopogon Citratus*), mediated apoptosis death in several hematopoietic cancer cell lines [Dudai et al., 2005 and Coelho et al., 2016].

Conclusion

Lemongrass (*Cymbopogon Citratus*) powder and extract alone or combined with coenzyme Q10 has a powerful protection benefits and antioxidant activity on rats against sodium valproate (SVA) induced reproductive toxicity. The present study further justifies the use of the lemongrass (*Cymbopogon Citratus*) to protect against testicular toxicity related to its source of antioxidant capacity. Thus, the lemongrass (*Cymbopogon Citratus*) may be an alternative to treat male infertility. Furthermore, the consumption of lemongrass (*Cymbopogon Citratus*) as functional food in the diet and scientifically supports usage in pharmaceutical industry.

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عشبة الليمون (*Cymbopogon Citratus*) وكوانزيم كيو10، تعمل على تحسين السمية الإنجابية التي يسببها فالبروات الصوديوم في نماذج الفئران

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الملخص العربي

تم إجراء هذا البحث لتقدير مدى تأثير مسحوق بودرة عشبة الليمون (*Cymbopogon Citratus*) ومستخلصها المائي مع وبدون مكملات كوانزيم كيو10 على السمية الإنجابية المحدثة بواسطة فالبروات الصوديوم في نماذج الفئران. تم تقسيم ثمانية وأربعين ذكور من فئران ألبينو (210 ± 5 جم) بشكل عشوائي إلى 6 مجموعات (ن = 8 فئران لكل مجموعة)، حيث تم تقسيمهم كالتالي: المجموعة (1) تم تغذيتها على الغذاء الاعتيادي (الضابطة السالبة)، المجموعات (2-5) تم اعطائهم فالبروات الصوديوم عن طريق الفم بجرعه 500 ملجم/كجم/من وزن الجسم يومياً وذلك في آخر أسبوع بفترة التجربة (45 يوماً) لأحداث السمية الإنجابية. المجموعة (2) تم تغذيتها على الغذاء الاعتيادي مع إعطائها فالبروات الصوديوم عن طريق الفم بجرعه 500 ملجم/كجم/منوزنالجسم يومياً باخر أسبوع من التجربة لأحداث السمية الإنجابية (الضابطة الموجبة)، المجموعة (3) تم معالجتها باستخدام كوانزيم كيو10 بجرعه (150 ملجم / كجم / وزن الجسم) مع اعطائهم فالبروات الصوديوم ، المجموعة (4) تم المعالجة بمسحوق عشبة الليمون (CP) في النظام الغذائي بجرعه (150 جم / كجم من الغذاء) واعطائهم فالبروات الصوديوم ، المجموعة (5) تم المعالجة بالمستخلص المائي لعشبة الليمون (CA) بجرعه (150 ملجم / كجم / وزن الجسم) واعطائهم فالبروات الصوديوم ، المجموعة (6) تم المعالجة بخليط من كوانزيم كيو10 مع مسحوق بودرة عشبة الليمون ومستخلصها المائي (50 ملجم / كجم / وزن الجسم + 50 جم / كجم من الغذاء + 50 ملجم / كجم / وزن الجسم ، على التوالي) مع اعطائهم فالبروات الصوديوم. وقد تم تقدير مضادات الأكسدة والمركبات الفينولية لأوراق عشبة الليمون ومستخلصها المائي. وقد وجد ان أوراق عشبة الليمون ومستخلصها يحتوي على كميات ملحوظة من مضادات الأكسدة. كما أظهرت النتائج ان تناول عشبة الليمون وكذلك كوانزيم كيو 10 كان لهما تأثير إيجابيا على وزن كلا من (الخصية، الحويصلات المنوية). علاوة على ذلك، فقد أظهرت النتائج تحسن مستوى القياسات التي تم تقديرها بالدراسة مقارنة بالمجموعة المحدث لها السمية الإنجابية. وقد ظهرت افضل النتائج للقياسات التي تم تقديرها في هذه الدراسة كمستوى الدهون وانزيمات الكبد بالدم وكذلك مستوى بيروكسيد الدهون والعلامات الحيوية المضادة للأكسدة بالخصية ومستوى التستوستيرون ومستوي كلا من هرمون (FSH) و (LH) بالدم وكذلك عدد الحيوانات المنوية بالمجموعات التي تم معالجتها بخليط مسحوق بودرة عشبة الليمون ومستخلصها المائي مع كوانزيم كيو 10 مجتمعة ثم المجموعة التي تم معالجتها على مسحوق بودرة عشبة الليمون يليها المجموعة التي تم معالجتها بالكوانزيم كيو10 ثم المجموعة التي تم معالجتها على المستخلص المائي لعشبة الليمون، على التوالي ولكن الاختلاف بمعظم النتائج بين تلك المجموعات ليس ذات دلالة إحصائية .

وقد أكدت الدراسة أن عشبة الليمون بمفرده أو مع كوانزيم كيو 10 له فوائد حماية قوية مقابل السمية الإنجابية المحدثة بواسطة فالبروات الصوديوم في نماذج الفئران، دون التسبب في إحداث ضرر للأعضاء التناسلية والمستويات الهرمونية والتي يمكن أن يرجع ذلك لاحتوائها على مضادات الأكسدة والفينولات والفلافونويد والتي تعمل على تحسين السمية الإنجابية المحدثة بواسطة فالبروات الصوديوم في نماذج الفئران.

الكلمات المفتاحية:

عشبة الليمون (*Cymbopogon Citratus*) - نماذج الفئران - فالبروات الصوديوم - كوانزيم كيو 10 - مضادات الأكسدة - السمية الإنجابية.