Effect of *Salvia aegyptiaca* Aqueous Extract on Some Neurohormonal Disorders Induced by Carbon Tetrachloride in Adult Male Albino Rats

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ABSTRACT

Daily intraperitoneal injection of carbon tetrachloride (CCl₄) at a dose level of 1.0 ml/kg body weight caused a gradual and significant decrease in tyrosine amino acid. As a result of this injection, dopamine (DA) and norepinephrine (NE) monoamines were decreased in different brain areas under investigation (cerebellum,pons & medulla oblongata, striatum, cerebral cortex, hypothalamus, midbrain, and hippocampus) where, CCl₄ injection decreased serum luteinizing hormone (LH) and testosterone. Animals that received a daily oral administration of *Salvia aegyptiaca* aqueous extract at a dose equivalent to 2.0g/kg body weight, slightly increased brain contents of tyrosine, dopamine and norepinephrine. Moreover a significant increase was observed in LH and testosterone from the first day till the end of the experiential period. However, animals that were pretreated with a daily oral administration of *Salvia aegyptiaca* extract at a dose level equivalent to 2.0 g/kg body weight one hour before being intraperitoneally injected with CCl₄ showed a significant increase in DA, NE, and tyrosine as compared to those treated with CCl₄ alone; and thus *Salvia* extract may be attenuate CCl₄ effect through experimental period.

Key Words: CCl₄ / *Salvia aegyptiaca* / Tyrosine / Dopamine / Norepinephrine / Luteinizing hormone / Testosterone / Brain areas / Rats.

INTRODUCTION

Herbal plants have been utilized as medicinal treatments since the beginning of civilization. The wide spread use of herbs and medical plants has been tract to the occurrence of natural products with medicinal properties (1). *Salvia aegyptiaca* L. (family: Labiatae) is a green dwarf shrub that grows in the mediterranean area (2). It is commonly used in local folk medicinal practices and cosmetics. The extract of the different species of *Salvia* are used as anti-inflammatory (3), hypoglycemic (4) and used in the treatment of alcoholism (5). The extract is also used as a neuroprotective agent against anoxic damage in hippocampal neurons (6).

Carbon tetrachloride is a potent, lipid-soluble hepatotoxic agent that, when bound to lipid and protein, produces peroxidative degeneration of many tissues (7). CCl₄ can cause a generation of reactive oxygen species (ROS) in tissues other than liver, such as kidneys, heart, lung, testis, blood and brain (8). The oxidative stress resulting from increasing the free radical produced after CCl₄-induced toxicity may play an important role in degenerative processes in the tissues.

In order to study the neuronal and hormonal protective effect of plants extract it is necessary to induce neurohormonal disorders in experimental animal models. A large number of chemicals are known which on administration to animals like rats will produce a neuronal disorder and will also affect the hormonal system. Thus this investigation aims to study the protective properties of *Salvia aegyptiaca* aqueous extract against xenobiotics using CCl₄ as a model of substances that causes neurohormonal disorders.
MATERIALS AND METHODS

Chemicals

*Salvia aegyptiaca* was obtained from open markets and the whole plant was air-dried and extracted according to the method described by Al-Yousuf *et al.* (9).

Carbon tetrachloride (CCl₄) was supplied from Al-Gomhoria Company for chemicals. The animals were daily intraperitoneal injected 1ml/kg according to Hsu *et al.* (10).

The percentage difference was calculated \[
\frac{\text{Mean of treated} - \text{Mean of control}}{\text{Mean of control}} \times 100
\]

Animals

Adult male Wister albino rats weighing from 100 to 120g were obtained from the National Research Centre, Dokki, Giza. The animals were allowed to acclimatize to surroundings for few days before carrying out the experiments. The animals were supplied with food and water *ad libitum* under standard conditions of light, humidity and temperature and were divided into three main groups each of 60 rats which were then divided into two subgroups, a treated one and its corresponding control.

**First group (G1)** Each group, half of these animals were administered *Salvia* extract equivalent to 2.0 g/kg body weight (Al-Yousuf *et al.* (9)) daily and orally for 15 days and the other half of the group were administered distilled water orally and daily for 15 days and served as a control.

**Second group (G2)** 30 animals of this group were intraperitoneally injected with 1 ml/kg CCl₄ daily for 15 day and the other 30 animals were injected intraperitoneal with saline solution for 15 days and served as its corresponding control group.

**Third group (G3)** Animals of this group received an oral administration of *Salvia* extract at a dose level equivalent to 2.0 g/kg body weight one hour later they receive an intraperitoneal injection (1 ml/kg) CCl₄ body weight. Where its corresponding control group was administered distilled water orally and one hour later animals were injected with the saline solution interaperitoneally.

Animals of all groups and their corresponding control group were sacrificed by sudden decapitation after 1, 3, 7, 10 and 15 days.

Biochemical analyses

Brain tissues were rapidly and carefully removed and then divided into two halves. Each half was dissected on a dry ice glass plate to seven areas (cerebellum, pons & medulla oblongata, striatum, cerebral cortex, hypothalamus, midbrain, and hippocampus) according to the method of Glowinski and Iversen (11). Brain areas were wiped with filter paper, weighed, wrapped in plastic film and then in aluminum foil and quickly frozen in dry ice for further analysis.

In the first half of brain tissue, dopamine (DA) and norepinephrine (NE) were extracted and estimated according to Chang (12) as modified by Ciarlone (13).

In the second half of brain tissue, tyrosine content was estimated by high performance liquid chromatography HPLC derivatization technique as described by Heinriksen and Meredith (14).

Trunk blood was collected in tubes, centrifuged at 5000 rpm for ten min. and serum samples were separated and kept frozen at -20° C. Serum LH level was assessed according to Santer *et al.* (15) by a specific double antibody radioimmunoassay technique. Serum testosterone was determined as described by Holand (16).

Statistical analysis:
The obtained data were represented as mean ± standard error. The statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by Duncan’s test (17).

RESULTS

As shown in table (1), comparing to control, *Salvia aegyptiaca* aqueous extract at a dose level equivalent to 2.0 g/kg body weight slightly increase tyrosine contents showing a significant increase on 7th day in all brain areas (cerebellum, pons & medulla oblongata, striatum, cerebral cortex, hypothalamus, midbrain and hippocampus). The maximum effect of Salvia extract was observed on midbrain and hippocampus recording 15.15% and 15.38% as a percentage change respectively, on the 15th day. In contrary, CCl₄ (1 ml/kg body weight) resulted in a gradual and significant decrease in tyrosine contents in all brain areas under investigation starting from first day and continued till the end of the experimental time. The maximum effect was observed in all brain areas on day 15. In group 3, intraperitoneal injection of CCl₄ one hour after being orally administered Salvia extract resulted in a non significant change in tyrosine contents in most brain areas throughout the experimental period. However, a significant change was observed between group 3 and group 2 starting on the 3rd day in all brain areas.

The data illustrated in table (2) represented the effect of daily oral administration of *Salvia* aqueous extract, intraperitoneal injection of CCl₄ and a combined treatment (*salvia*+CCl₄) on dopamine contents in different brain areas. In comparison to control, administration of *Salvia* extract resulted in a significant increase in DA contents in most brain areas starting from the 3rd day (pons & medulla oblongata, hypothalamus, midbrain and hippocampus). A significant increase was observed in all brain areas on the 7th and 10th day. The maximum effect was observed on day 15. However, CCl₄ caused a significant decrease in DA contents starting from 1st day in all brain areas except in the cerebellum; the significant decrease was observed on the 3rd day and continued in all brain areas till day 15. The maximum decrease was observed in cerebellum recording -27.12% on day 15. Whereas, animals received combined treatment (*Salvia* extract & CCl₄) resulted in a non significant decrease in DA contents in brain areas under investigation except pons & medulla oblongata, midbrain and hippocampus on day 15. Comparing group 3 with group 2 revealed a significant change in all brain areas except cerebellum on 1st and 3rd day.

The data reported in table (3) showed that *Salvia* aqueous extract gradually increase NE contents in different brain areas. The significant increase in NE contents was noticed in all brain areas under investigation started on the 3rd day. The maximum effect of Salvia extract was observed in striatum on the 15th day recording 17.07% as a percentage change. CCl₄ at a dose level 1ml/kg significantly decreased NE contents on the 1st day in all brain areas under investigation; the significant decrease was continued till the end of the experiment. The maximum decrease in NE contents was noticed in cerebellum on the 15th day recording -38.46% as a percentage change. NE contents in brain areas of animals that received 1 ml/kg body weight CCl₄ one hour after being treated with *Salvia* extract showed a non-significant change through the first ten days of the experiment. The significant decrease in NE contents was observed in pons & medulla oblongata, cerebral cortex, midbrain, and hippocampus on the 15th day comparing to its corresponding control. In comparing group 3 with group2 values, a significant difference was observed all over the experimental period in all brain areas under investigation started from the first day except in striatum and hypothalamus the significant difference started on the 3rd day.

As shown in figure (1), comparing with control values, *Salvia aegyptiaca* aqueous extract at a dose equivalent to 2.0 g/kg body weight significantly increased serum luteinizing hormone (LH) one day after oral administration of the extract; LH gradually and significantly increase till the end of the experimental period. Where, CCl₄ at a dose level 1 ml/kg significantly decreased LH throughout the experimental time. Although animals were treated with CCl₄ after being treated with *Salvia* extract LH showed a significant increase all over the experimental time. The maximum effect in all tested groups was noticed on day 15. Comparing group3 with group 2, LH values were significantly increased throughout the experimental period.
Table (1): Effect of daily oral administration (2.0 g/kg body weight) of *Salvia frutescens* aqueous extract and/or intraperitoneal injection (1.0 ml/kg) of CCl₄ on Tyrosine content (μg/g tissue) in different brain areas of adult male albino rats at different time intervals.

<table>
<thead>
<tr>
<th></th>
<th>1st day</th>
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<th>7th day</th>
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<tr>
<td>Treated</td>
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- The data are represented as mean ± S.E. Where (G1) salvia group; (G2) CCL group; (G3) Salvia + CCL group.
- Statistical analysis were performed between control (n=5) and treated (n=6) animals by one way ANOVA.
- *a* Significant at P < 0.5 compared to its corresponding control whereas (b) significant compared to CCL values.

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Table (2): Effect of daily oral administration (2.0 g/kg body weight) of *Salvia argyrophylla* aqueous extract and/or intraperitoneal injection (1.0 ml/kg) of CCl₄ on Dopamine content (µg/g tissue) in different brain areas of adult male albino rats at different time intervals.

<table>
<thead>
<tr>
<th></th>
<th>1st day</th>
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<th>10th day</th>
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<td>O₃</td>
<td>O₁</td>
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<tr>
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<tr>
<td>Pre + Treated</td>
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</table>

- The data are represented as mean ± S.E. Where (G₁) *Salvia* group; (G₂) CCl₄ group; (G₃) *Salvia + CCl₄* group.
- Statistical analysis were performed between control (n=6) and treated (n=6) animals by using one way ANOVA.
- (a) Significant at P < 0.05 compared to its corresponding control where (b) significant compared to CCl₄ group.
Table (3): Effect of daily oral administration (2.0 g/kg body weight) of *Salvia sclarea* aqueous extract and/or intraperitoneal injection (1.0 ml/kg) of CCl₄ on Norepinephrine content (ng/g tissue) in different brain areas of adult male albino rats at different time intervals.

<table>
<thead>
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<th>Brain Area</th>
<th>1st day</th>
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<th>7th day</th>
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<td><strong>1st day</strong></td>
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<td>Control</td>
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</table>

- The data are represented as mean ± S.E. Where (G1) salvia group; (G2) CCl₄ group; (G3)Salba- CCl₄ group.
- Statistical analysis was performed between control (n=6) and treated (n=6) animals by using one way ANOVA.
- Significant at P < 0.5 compared to its corresponding control where (S) significant compared to CCl₄ group.

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Fig (1): Effect of daily oral administration (2.0 g/kg body weight) of *Salvia aegyptiaca* aqueous extract and/or intraperitoneal injection (1.0ml/kg) of CCl₄ on serum luteinizing hormone (mlU/ml) of adult male albino rats at different time intervals.

Similar to LH; daily oral administrations of *Salvia* aqueous extract at a dose level 2.0 g/kg significantly increased testosterone hormone on the 1st day (fig.2); the significant increase was continued till the end of the experiment. In contrary, intraperitoneal injection of CCl₄ (1 ml/kg body weight) significantly decreased testosterone all over experimental period. In spite of being treated with CCl₄ one hour after *Salvia*, testosterone hormone was significantly increased all over the experimental time comparing to control and CCl₄ values.

Fig (2): Effect of daily oral administration (2.0 g/kg body weight) of *Salvia aegyptiaca* aqueous extract and/or intraperitoneal injection (1.0 ml/kg) of CCl₄ on serum testosterone hormone (ng/ml) of adult male albino rats at different time intervals.
DISCUSSION

In the present study, the i.p. injection of CCl₄ (1 ml/kg b. wt.) caused a significant decrease in tyrosine, dopamine, and norepinephrine contents during most of the experimental period and in LH and testosterone hormones overall the experimental period.

This result was in agreement with those obtained by Abdel-Rahman and El-Nahary (18) who recorded a decrease in monoamines content and in LH and testosterone hormones after i.p. injection of CCl₄ (1 mg/kg b. wt.).

CCl₄ has been used extensively as a model compound for free radical damage in different tissues (19). It is reductively bioactivated by cytochrome P450 to trichloromethyl radical (CCl₃⁺) which, in the presence of oxygen, is subsequently converted to peroxy radical (OOCCl₂⁻) (20). The brain is extremely sensitive to these free radicals that directly interfere with the polyunsaturated fatty acids in the membrane, resulting in changes in the membrane structures associated with neurotransmitter uptake disorders (21).

Free radicals are generally very reactive molecules possessing an unpaired electron. They have been implicated in multiple central nervous system (CNS) disorders (22). This understandable since this tissue is highly sensitive to oxidative stress due to its high oxygen consumption, its high iron and lipid contents, especially polyunsaturated fatty acids and the low activity of antioxidant defenses (23).

Oxidative damage to normal human tissue was induced following exposure to hydroxyl (OH⁻) or superoxide (O₂⁻) free radicals. Both enzymatic and cytoskeleton proteins showed substantial oxidative damage. Excess of free radicals may in turn lead to peroxidative impairment of membrane lipids and consequently disrupt neural functions and result in cell death (24).

The toxicity produced by CCl₄ is thought to be due to the reaction of free radicals (CCl₃⁺ or OOCCl₂⁻) with lipid or proteins. The free radicals caused the peroxidation of the polyenoic lipids of endoplasmic reticulum and the generation of secondary free radicals derived from these lipid peroxidation leads to breakdown of membrane structure and function and an increase in the intracellular cytoplasmic Ca²⁺ resulting in cell death (25-26).

From the present results it is clear that the daily oral administration of Salvia aegyptiaca extract caused a significant increase in tyrosine, DA and NE contents in all brain areas at almost all tested times.

There are a few reports on the pharmacological properties of Salvia aegyptiaca and no toxicological studies have been conducted on its effect on animals (9). Phytochemically, the whole plant contains flavonoids, tannins, sterols (triterpenes), diterpene quinines and coumarins (27). Some related Salvia species have been shown to have CNS depressant effect (28).

Rutherford et al. (29) reported that the isolation of two compounds from Salvia officinalis, the diterpenes and carnosol, inhibit [³⁵S] tertiary-butylcyclophosphorothionate ([³⁵S] TBPS) binding to rat brain membrane in vitro. This ligand is considered to bind to the chloride channel of the GABA/benzodiazepine receptor complex. Ten diterpene quinines, which inhibited the binding of [³H] flunitrazepam to central benzodiazepine receptors, were isolated from the extract of Salvia miltiorrhiza (30). Huiyum and Rong (31) reported that, Salvia miltiorrhiza caused a decrease in the spontaneous activity with increasing dose in rats. The extract also decreased exo cerebral fluid (ECF) of DA, NE and 5-HT during cerebral ischemia (32). Hosseinazadeh and Lary (33) found that 500 mg/kg of Salvia leriifolia was as effective as a dose of 5.0 mg/kg of diazepam.

Al-Yousuf et al. (9) cited that, the extract of Salvia aegyptiaca possesses sedative, antipyretic and anti-inflammatory properties. The sedative action may be a result of CNS depression and the anti-inflammatory action may be related to its effect on the cyclooxygenase or the lipooxygenase.
pathways. So it is possible that, the extract contains several chemical constituents that exert more than one action via different mechanisms.

In view of previous studies, it could be concluded that the increment in tyrosine content in the present study may be due to an increase in the peripheral availability of tyrosine to brain for the synthesis of DA and NE. *Salvia aegyptiaca* extract may also act as diazepam, which enhances the action of the inhibitory neurotransmitter, GABA. When GABA binds to its receptors, the influx of Cl⁻ into the cell is increased leading to membrane hyperpolarization and decreases the cell excitability. This leads to limiting the Ca²⁺ current entry to the presynaptic and inhibit the release of the neurotransmitters (34) and as a result, the content of catecholamine is increased.

It should be mentioned that, secretion of pituitary hormones are controlled by hypothalamic hormones which are synthesized by neurosecreting cell whose activity is modulated by different neurotransmitters as dopamine, norepinephrine and serotonin. Centrally acting drugs, interfering with the activity of the neurotransmitters, may influence the secretion of hypothalamic and pituitary hormones (35). Dopamine and serotonin are most directly involved in sexual activity. DA plays a stimulatory role while 5-HT has an inhibitory role. The two monoaminergic systems modulate the secretion of many hormones (GnRH, LH, testosterone and prolactin) involved in the sexual functional capacity (36).

The stimulatory role that DA plays in male sexual behavior is well known. DA agents increase the number of rats that mate with receptive females; this effect is completely blocked by the administration of dopaminergic receptor blocking agents such as haloperidol and pimozide (37,38). Evidence of an increase in sexual activity is noted when patients undergoing therapy for Parkinson’s disease with administration of L-DOPA and in those were treated for acromegaly by administration of bromo-criptine (39-40). However, when dopamine-D₁ receptors were stimulated with fenoldopam, the gonadotropin releasing hormone (LHRH) was increased, and induced the releasing of LH (41).

Ghosh et al. (42-43) studied the effect of alpha-2 U globulin, a sex-dependent male urinary protein on pituitary-gonadal functions and hypothalamic monoamines content in rats and mice. The results indicated that the administration of drug significantly increased plasma LH, FSH and testosterone levels. There was also a decrease in prolactin level. In the medial basal hypothalamus (MBH) of the treated mice, there was a significant increase in DA and NE contents and a decrease in DA turnover in the anterior hypothalamus. Administration of methoxychlor (25 mg/kg/day) produced a decrease in DA content in median eminence leading to decrease in plasma level of LH and testosterone in adult male rats (44).

In the present results it is clear that the extract of *Salvia aegyptiaca* caused a significant increase in the plasma level of LH and testosterone at almost all the tested times. From previous studies it is clear that DA in the hypothalamus plays a stimulatory role in the secretion of LHRH and an inhibitory role in the secretion of prolactin. Thus it is clear from the present data that the increment in DA content in the hypothalamus led to the increase in the secretion of LHRH, which stimulated the release of LH from the anterior pituitary gland and increased its plasma level. LH acted on testicular Leydig cell and stimulated the synthesis of testosterone, so the plasma level of testosterone is increased.

From the present results and previous studies, it could be concluded that *Salvia aegyptiaca* extract caused a significant increase in the content of catecholamine in the brain areas of albino rats which may be due, in part, to the increase in the synthesis of catecholamine by increasing the peripheral availability of tyrosine or the decrease in the release of catecholamine by enhancing GABA/benzodiazepine receptors. The influx of Cl⁻ into the cell is increased leading to membrane hyperpolarization and decreased the Ca²⁺ current entry to the presynaptic. This led to inhibition of the release of catecholamine.
Furthermore, *Salvia aegyptiaca* may also act on hypothalamus-pituitary axis. The increment in DA content led to increasing the release of LHRH release from the anterior pituitary gland. As a result, there was an increase in LH and testosterone levels in serum of treated rats.

The current study showed that, tyrosine, DA and NE in different brain areas of animals that received the combined treatment (*Salvia* and CCl₄) were more or less near to their corresponding control levels.

This may be explained by the reduction effect of the free radicals produced due to CCl₄ metabolism as mentioned before. The plants of the genus *Salvia* are synthesizing several types of secondary products (metabolites or natural products) with antioxidant, antimicrobial and radical scavenging activities. Diterpenoids such as rosmarinic acid and carnosol and polyphenols showed considerable antioxidant activity as well as antimicrobial properties. Moreover, the aqueous extract of the plant which acts as benzodiazepine compound may lead to blocking the release of monoamines and increase the synthesis of peripheral availability of tyrosine amino acid. The results obtained from the current study came in accordance with the findings of other authors worked on different species of *Salvia*. They reported that, *Salvia* is effective therapy for neurodegenerative diseases in utmost importance for aging population and useful against disorders such as Alzheimer and Parkinson’s diseases. Moreover it has a neuroprotective potential.

From the data obtained in the present investigation, it could be elaborated that, *Salvia* extract has a capability to ameliorate the neurohormonal disorders induced by CCl₄.

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