Protective Effect of Sage (*Salvia officinalis* L.) on Cyclophosphamide toxicity evaluated By Cytogenetic, Biochemical and Histopathological Assays in Male Albino Mice

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**SUMMARY**

The induction of chromosomal aberration, biochemical and pathological alterations by Cyclophosphamide (CP) and their reduction by two concentrations of Sage (*Salvia officinalis* L.) were studied in male albino mice. Six groups of mice were used included the control group, cyclophosphamide (CP) treated group and the other groups treated with the extract alone or in combination with CP. The results indicated that treatment with CP resulted in significant clastogenetic effects. Also, biochemical results revealed that CP increased Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Glucose levels and decreased testosterone level. Moreover, the results showed different histopathological lesions in kidneys, liver and testes of CP treated mice. However, Salvia did not have toxic effects of its own and the two concentrations of sage extracts exhibited a protection against CP-induced cytogenetic, serum biochemical and pathological changes in mice. The results provide some support for antimutagenic potency of sage *in vivo*.

**Keywords:** Chromosomal aberration, *Salvia officinalis*, Albino mice, Cyclophosphamide, Histopathology.

**INTRODUCTION**

Chemoprevention of mutation-related disease and cancer is an important research field and the dietary use of antimutagens and anticarcinogens has been proposed as the most promising approach to protection human health (Fe-
rguson, 1994 and Vukcevic-Knezevic et al., 2005) Medicinal plant are a valuable natural re-
source and regarded as potentially safe drugs (Khalil et al., 2005).

Many species of salvia have been used as medicinal plants and show several activities. There are four main categories of chemical compounds in plants of genus salvia including monoterpenes, diterpenes, phenolic acids and flavonoids. Regarding phenolic acids, the majority of these compounds in salvia species and possesses a variety of biological activities including antioxidant, antiplatelet, antitumor and antiviral activities (Weiss and Fintembmann 2000; Lu and Foo 2002 and Hosseinzadeh et al., 2009).

Salvia officinalis L. are well known for their anti-oxidative properties (Baricevic et al., 1996). Moreover, the antimutagenic potential of sage extracts was demonstrated on Escherichia coli repair proficient strains (Filipic and Baricevic 1997 and 1998).

On the other hand it should be considered that some of the S. officinalis that have antimutagenic effect in certain concentrations are cytotoxic in higher concentrations (Vujosevic and Blagojevic 2004). Also, Abd El-Aziem et al. (2004) reported that sage have a protective role against acrylamide induced genotoxicity in rats. Therefore, it is of interest to investigate whether sage reveals its ant MUTagenic activity in mammalian cells in vivo. In this study, the mice bone marrow chromosomal aberrations test was applied to evaluate the role of sage in modulating the cytogenetic damage induced by indirect mutagen cyclophosphamide. The activities of ALT, AST as well as levels of Glucose and testosterone were measured. Also, histopathological examination of mice tissues (kidneys, liver and testes) was done.

MATERIALS AND METHODS

1- Cyclophosphamide (CP; CAS No. 6055-19-2) was obtained from Sigma chemical Company (St. Louis, MO, USA).
2- Dried Sage leaves (Salvia officinalis) was supplied by the department of crop production, faculty of Agriculture, Ain Shams University.
3- Kits: Transaminase(ALT and AST) were purchased from Randex Labs(San Francisco, CA,USA).Glucose and testosterone were obtained from Biomerieux Lab of reagents and products (Marcy Letoile, France).

Experimental animals:

Forty eight (six to eight weeks old) Swiss albino male mice (20-
25g), purchased from animal house colony, Giza, Egypt were main-
tained on standard lab diet (Protein: 160, 4; Fat: 36.3; fibre: 41g/kg and
metabolizable energy 12.09 MJ), and housed in a room free from any
source of chemical contamination, artificially illuminated and ther-
mal control, at the Animal House Lab., National Research
Centre, (N.R.C.) Cairo, Egypt. After
an acclimatization period of one
week, the animals were divided into
six groups (8 mice/group) and
housed in filter-top poly carbonate
cages. All animals received humane
care in compliance with the guide-
lines of the Animal Care and Use
Committee of the N.R.C. Cairo,
Egypt.

Experimental design:

Animals within different groups
were orally treated for 2 weeks as fol-
low:
Group 1: served as negative control
Group 2: treated with sage (0.3 g/
kg. b.w.).
Group 3: treated with sage (0.6 g/kg.
b.w.).
Group 4: treated with intraperitoneal
injection with CP (25 mg/kg b.w.).
Group 5: treated with sage (0.3 g/kg,
 b.w.) for two weeks then injected
with CP (25 mg/kg b.w.).
Group 6: treated with sage (0.6 g/kg
 b.w.) for two weeks then injected
with CP (25 mg/kg b.w.).

Cytogenetic analysis: for chromo-
somal analysis both treated and
control animals were sacrificed by
cervical dislocation. Two hours be-
fore sacrifice, mice were injected
with 5mg colchicines/kg b.w. fe-
murs were removed and the bone
marrow cells were aspirated using
saline solution. Metaphase spreads
were prepared using the method of
Preston et al., 1987). Fifty meta-
phase spreads per animals were
analyzed for scoring the different
types of chromosomal aberrations.

Biochemical analysis: At the end
of experimental period, blood
samples were collected from all
animals from the retro-orbital ve-
 nous plexus for biochemical analy-
ses. These analysis included, se-
rum ALT, AST (Young, 1995),
Glucose (Trinder, 1995), and Test-
tosterone (Hall, 1988).

Histopathological examination:

Tissue specimens from kidneys,
 liver and testes were collected from
all experimental groups at the end of
experiment and fixed in neural buff-
ered formalin 10%, dehydrated in
ascending concentration of ethanol,
 cleared in xylene and embedded in
paraffin. Sections 4-5m thick were
prepared and stained with Hema-
toxyl in and Eosin (Bancroft et al,
1996).

Statistical analysis:

The obtained results of Cyto-
genetic examinations or biochemi-
cal analysis were statistically ana-
lyzed by ANOVA (one way) using
Excel 2003 Microsoft Crop
RESULTS

Cytogenetic analysis:
The data on chromosomal aberrations were shown in Table (1). Cytogenetic results showed that the frequencies of total structural chromosome aberrations were low in the two groups of mice which treated with single treatments of (0.3 or 0.6 g/kg b.w.) sage compared to those found of the control group of mice.

The established mutagen CP significantly induced chromosomal aberration in male mice as compared to the control and salvia treatment.

The third and fourth groups which treated with (0.3 or 0.6 of salvia) respectively, resulted in a significant decrease in the total chromosomal aberrations when compared to the control or the positive control (CP). But there in no statistically significant difference between the two concentrations of salvia was found on the total chromosomal aberrations induced by 0.3 or 0.6 g/kg b.w. (Table 1).

The combined treatments of salvia (0.3 g/kg b.w.) or (0.6 g/kg b.w.) resulted in a significant decrease in the frequency of total chromosomal aberrations in bone marrow of male mice induced by cyclophosphamide (25 mg/kg b.w.), and significant increase when compared to control group and salvia alone. Also, when compared slavia (0.6 g/kg b.w. + CP) with slavia (0.3 g/kg b.w. + CP) there was significant decrease in total chromosomal aberration (Table 1).

Biochemical results:
The data of serum biochemical studies on different parameters in all experimental treatments are shown in Table 2. The data show that cyclophosphamide affected the liver enzymes (AST and ALT), Glucose and testosterone. CP caused a statistically significant increase in all measured biochemical parameters of males mice when compared with the control except the plasma testosterone levels it was significantly decreased. However, the (AST and ALT) and Glucose were significantly decreased at (P < 0.05) when the two concentrations of salvia singly was administrated when compared to the control or other treatments.

Meanwhile, a statistically significant increase in testosterone level was observed in combined treatments of (Salvia and CP) when compared with the positive control (CP) alone.
Table (2): Serum levels of AST, ALT, glucose and testosterone in control, Salvia extract and or cyclophosphamide treated mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST U/l</th>
<th>ALT U/l</th>
<th>Glucose mg/dl</th>
<th>Testosterone ng/ml</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>120.20 ± 1.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45.80 ± 1.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.40 ± 1.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.40 ± 0.75&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Salvia (0.3 g/kg)</td>
<td>102.00 ± 1.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.00 ± 2.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.00 ± 2.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salvia (0.6 g/kg)</td>
<td>72.00 ± 1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.00 ± 2.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.80 ± 1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.20 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cyclophosphamide (25 mg/kg)</td>
<td>194.20 ± 1.66&lt;sup&gt;f&lt;/sup&gt;</td>
<td>81.20 ± 4.65</td>
<td>181.80 ± 0.92&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3.00 ± 0.89&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salvia 0.3 + Cyclophosphamide</td>
<td>144.60 ± 1.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.80 ± 1.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>133.80 ± 1.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.40 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salvia 0.6 + Cyclophosphamide</td>
<td>122.60 ± 1.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.60 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.6 ± 3.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.20 ± 0.58&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

The different letters (a,b,c,d,e,f) in the same column are significantly different at level (P < 0.05)

**Histopathological Results:**

Kidneys of mice from control group (group 1) or from mice treated with Salvia either with (0.3g/kg b.w.)or (0.6g/kg b.w.) (groups 2 & 3) revealed normal histological structure of renal parenchyma from cortex and medulla (Figure 1).

Meanwhile, kidneys of mice treated with CP showed congestion of renal blood vessels and glomerular tufts. There were granular and vacuolar degeneration of epithelial lining renal tubules (Figures. 2&3) as well as pyknosis of some nuclei of the epithelial lining and focal interstitial leucocytic cells infiltration (Figure 3). However, kidneys of mice treated with CP plus salvia 0.3 g/kg b.w revealed no pathological changes except congestion of glomerular tufts (Figure 4). Moreover, apparent normal renal parenchyma was noticed in examined sections from mice treated with CP plus salvia 0.6 g/kg b.w (Figure 5).

Regarding liver, examined sections from control mice (group 1), or from mice treated with 0.3 or
0.6 g/kg b.w. of Salvia (groups 2 & 3) revealed no changes as the liver showed the normal histological structure of hepatic lobule from central vein and concentrically arranged hepatocytes. On the other hand, liver of mice treated with CP (group 4) revealed different histopathological changes which summarized as vacuolations of hepatocytes and pyknosis of some nuclei of hepatocytes (Figure 6). Focal hepatic hemorrhages associated with necrosis of hepatocytes were also observed in all examined sections (Figure 7). Additionally, some examined sections from this group (group 4) showed marked dilatation of bile duct as well as cholangitis described by inflammatory cells infiltration in the wall of bile duct (Figure 8). However, examined liver sections from mice treated with CP combined with 0.3 g/kg b.w. of Salvia revealed slight improvement in the histopathological picture as examined sections showed kupffer cells activation (Figure 9), vacuolar degeneration of some hepatocytes and cholangitis (Figure 10). On the other hand, liver of mice treated with CP combined with 0.6 g/kg b.w. of Salvia showed marked improvement in the histopathological picture as the liver showed no histopathological changes except Kupffer cells activation (Figure 11).

Concerning testes, examined sections from control mice or from mice treated with 0.3 or 0.6 g/kg b.w. Salvia revealed the normal histology of semineferous tubules which lined by different spermatogonial cells (Figure 12).

Microscopically, testes of mice treated with CP revealed vacuolations of spermatogonial cells lining the semineferous tubules (Figure 13), interstitial oedema dispersed the semineferous tubules far away from each other, degeneration of spermatogonial cells lining semineferous tubules (Figure 14) with slight thickening of basement membrane. On the other hand, testes of mice treated with CP plus 0.3 g/kg b.w. of Salvia showed improvement in the histopathological picture, examined sections revealed slight degeneration of spermatogonial cells lining some semineferous tubules associated with slight interstitial oedema (Figure 15). Whereas, testes sections of mice treated with CP plus 0.6 g/kg b.w. of Salvia revealed no histopathological alterations except slight degeneration of spermatogonial cells lining of some semineferous tubules associated with incomplete spermatogenesis (Figure 16).
Fig. (1): Kidney of Control, untreated mice showing the normal histological structure of renal parenchyma (H and E x 200).

Fig. (2): Kidney of mice treated with CP showing congestion of glomerular tuft, granular and vacuolar degeneration of epithelial lining renal tubules (arrow) (H and E x 200).

Fig. (3): Kidney of mice treated with CP showing vacuolar degeneration of epithelial lining renal tubules and focal interstitial leucocytic cells infiltration (arrow) (H and E x 200)

Fig. (4): Kidney of mice treated with CP and 0.3 Salvia showing congestion of glomerular tuft (arrows) (H and E x 200)
Fig. (5): Kidney of mice treated with CP and 0.6 Salvia showing apparent normal renal parenchyma (H and E x 200).

Fig. (6): Liver of mice treated with CP showing clearance of the cytoplasm and pyknosis of some nuclei of hepatocytes (arrow) (H and E x 200).

Fig. (7): Liver of mice treated with CP showing focal hepatic hemorrhage (arrow) associated with necrosis of hepatocytes and pyknosis of their nuclei (H and E x 200).

Fig. (8): Liver of mice treated with CP showing marked dilatation of bile duct (small arrow) associated with cholangitis (large arrow) (H and E x 200).
Fig. (9): Liver of mice treated with CP and 0.3 *Salvia* showing kupffer cells activation (arrow) (H and E x 200).

Fig. (10): Liver of mice treated with CP and 0.3 *Salvia* showing cholangitis (H and E x 200).

Fig. (11): Liver of mice treated with CP and 0.6 *Salvia* showing kupffer cells activation (arrow) (H and E x 200).

Fig. (12): Testis of control, untreated mice showing the normal histology of semineferous tubules (arrows) (H and E x 200).
Fig. (13). Testis of mice treated with CP showing degeneration of spermatogonial cells lining seminiferous tubules (arrow) (H and E x 200).

Fig. (14): Testis of mice treated with CP showing vacuolation of spermatogonial cells lining the seminiferous tubules as well as interstitial edema (arrow) (H and E x 200).

Fig. (15): Testis of mice treated with CP and 0.3 *Salvia* showing degeneration of spermatogonial cells lining seminiferous tubules and interstitial edema (arrow) (H and E x 200).

Fig. (16): Testis of mice treated with CP and 0.6 *Salvia* showing slight degeneration of spermatogonial cells (arrow) (H and E x 200).
DISCUSSION

The results of the present study show that *Salvia officinalis* (Sage) has some antimutagenic properties against CP induced chromosomal aberrations and improve the changes in the biochemical parameters (AST, ALT, Glucose and testosterone). From histopathological point of view, the present work indicated that CP was able to induce many of alterations in kidneys as well as in liver and testes tissues. Those alterations could be attributed to the oxidative stress induced by increasing lipid peroxidation production by cyclophosphamide as well as to the ability of the activated metabolites of cyclophosphamide (which are alkylating agents) to cause cross-linking of DNA strands, interfering with normal cell division in all rapidly proliferating tissues. In addition, cyclophosphamide can disrupt the redox balance of tissues suggesting that biochemical and physiological disturbances may result from oxidative stress caused by the generation of free radicals and ROS (Sabik and Abd El-Rahman 2009).

Possible application of plant antimutagens as a dietary prevention of cancer and other mutation related disease makes the study of plant antimutagens an important in research field (Craig, 1999). *Salvia* sp. is an important genus consisting of about 900 species in the family Lamiaceae.

*Salvia officinalis* is an aromatic and medicinal plant of Mediterranean origin and well known for its antioxidant properties due to its composition in phenolic compounds. The antioxidant activity mainly based on results from several subcellular and non cellular in vitro (Baricevic and Barto 2000). Also, Farhat et al., (2001) reported that the antioxidant activity of sage may be due to 1,8-cineole, the main constituent, although other components included ketones such as limonene and alpha, beta-pinene and alcohols such as bornel and linalool. Moreover, sage tea drinking has shown the ability to improve liver antioxidant status in mice and rats (Lima et al., 2005).

Consequently, Amin and Hamza (2005) have shown that the treatment of rats with a water extract of sage protected against azathioprine. In agreement with these findings, we found in the present study that salvia could inhibit the toxic effects of CP in males mice and the high dose (0.6 g/kg b.w.) of saliva more effective to reduce the mutagenic effects and pathological alterations in all examined tissues. The results of another investigation showed clearly the antioxidant effects at cellular level of sage namely preventing cell death, lipid peroxida-
tion and GSH depletion induced by tert-butylhydroperoxide in HePO₂ cells (Lima et al., 2007). Also, Abd - ElAziem et al., (2004) reported that sage supplemented diet decreased acrylamide mutagenicity in rats.

On the other hand, it should be considered that some of the Salvia species (such as S. officinalis) that have antimutagenic effect in certain concentrations, are cytotoxic in higher concentrations (Vujosevic and Blagojevic 2004). The protective effect of sage from CP may be caused by a change in the activity of biotransformation enzymes needs to produce its active mutagenic metabolite phosphoramide mustard (Rompelberg et al., 1995).

The data of the biochemical study in the present work show that oral administration of sage caused a significant decrease in the liver enzyme (AST and ALT) and Glucose in the two concentrations (0.3 and 0.6 g/b.w.). These biochemical data were supported with the histopathological results, as the examined tissues from those groups revealed marked improvement in the histopathological picture. Furthermore, sage has a protective role against CP to reduce these biochemical parameters, increase testosterone levels and improve the histopathological picture. These results were in agreement with Hosseinzadeh and Eghbal (2002) who investigated the possible hepatoprotective effects of the alcoholic leaf extract of Salvia leriifolia in mice. At present, it is known that Salvia has relatively unambiguous antiendoxin effects and can directly neutralize and destroy endoxin. It has been pointed out in some studies that Salvia can protect hepatic cells and maintain liver function through reducing inflammatory mediator levels and improving microcirculation. Salvia is also able to significantly improve the liver function at the early postoperative stage. Salvia is able to reduce endotoxemia, regulate the production and secretion of vasoactive substances, and improve renal blood perfusion, thereby exerting protective effects against damage to kidney function (Ye et al., 2010).

Conversely, the lower doses of the S. Leriifolia extract (50 mg/kg and 100 mg/kg) did not reduce the elevated activities of ALT and AST significantly when administrated 1 hr after established hepatoxins, carbon, tetrachloride and acetaminophen. In addition, treatment of mice with a single oral dose (2 g/kg) of alcoholic seed extract of S. leriifolia caused a significant reduction in blood glucose level (Hosseinzadeh et al., 1998). Moreover the higher content of antioxidant substances in sage and barley led to improvement the serum clinical chemistry in rats treated with acrylamide (Abd El-Aziem, et al., 2004). Also, several
reports indicated that blood cholesterol and lipoprotein concentration can be reduced in human (Braaten et al., 1994) and animal (Kalra and Jood 2001) by aglucan from barley. Moreover, the change of plasma testosterone levels in male mice treated with CP may result from a local inhibition of the fetal testicular function. Elangovan, et al., (2006), also reported that CP treatment causes impairment of sperm and its fertilizing ability in mice.

In conclusion, the present study revealed that CP induced cytogenetic, biochemical and histopathological alterations typically to those reported in the literature. Both two concentrations of sage have a protective role against these deleterious effects possibly due to their contents of antioxidant substances.

REFERENCES


التأثير الوقائي لنبات المرمرية على سمية السيلكوفوسفاميد باستخدام التحليل
الوراثي السيتولوجي والبيوكيميائي والهستوباتولوجي في ذكور الفئران البيضاء

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الملخص العربي

هدفت هذه الدراسة إلى تقييم الدور الوقائي لمستخلص نبات المرمرية على سمية السيلكوفوسفاميد باستخدام الاختبارات الوراثية الخلوية (التغيرات الكروموسومية) والتأثير الكيميائي الحيوي على انزيمات الكبد والجلوكوز وتغيره في مستويات هرمون التستوستيرون وأيضا التغيرات النسيجية في خلايا كل من الكبد والكبد والخصبة في ذكور الفئران البيضاء. قسم الدراسات 5 مجموعات بالإضافة إلى المجموعة المضبوطة كل مجموعة 8 فئران وbast كانت كالتالي: المجموعة الأولى معاملة مفردة بالسيلكوفوسفاميد بتركيز 20 جم لكل كلغ من وزن الجسم والمجموعة الثانية والثالثة معاملات مفردة من المرمرية بتركيز 20 جم لكل كلغ من وزن الجسم و 4 جم لكل كلغ من الوزن السوسي والثاني أما المجموعة الرابعة والخامسة معاملات مشتركة من السيلكوفوسفاميد ومرمرية بتركيزها. وقد أوضح النتائج أن المعالجة بالمولبوفوسفاميد أحدثت تأثير معنوي على مستوى التغيرات الكروموسومية التركيبية وأيضا على انزيمات الكبد ومستوي الجلوكوز في الدم وانخفاض هرمون التستوستيرون كما أظهرت النتائج تغيرات نسيجية في كل من خلايا الكبد والكليه والخصبة في ذكور الفئران، بينما المعاللات المفردة من المرمرية لم تظهر أي تأثير سلبي وراثي على مستوى التركيزات المستخدمة بل كان هناك تأثير معرفي إيجابي في المعاملات المشتركة للسيلكوفوسفاميد والمرمرية أظهر دورها الوقائي والثاني عند التركيز العالي. نستخلص من هذه الدراسات أن مستخلصات نبات المرمرية له قدره على تقليل وحماية من التغيرات الكروموسومية والكيميائية الحيوية وأيضا الهستوباتولوجي نتيجة المعاملة بالسيلكوفوسفاميد.

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