# Modulatory effects of centrophenoxine on salivary glands in D-galactose induced aged female mice

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## Summary

Three month old female mice were ovariectomised and after 4 days the mice were separated into six groups, I) ovariectomized, II) ovariectomized + D-galactose treated, III) ovariectomized + D-galactose treated + Tween 80, IV) ovariectomized + D-galactose & centrophenoxine treated, V) ovariectomized D-galactose treated + olive oil injected, and VI) ovariectomized + D-galactose treated + estrogen injected. After 15 days the mice were sacrificed. The protein concentration, amylase activity, lipid peroxidation and fluorescence product were measured in various salivary glands, *viz*., submandibular, sublingual, and parotid. In ovariectomized (group II) and ovariectomized + D-galactose treated (group III) mice the protein concentration and amylase activity of various salivary glands decreased, while lipid peroxidation and fluorescence product increased. In ovariectomized + D-galactose + centrophenoxine treated (group IV) and ovariectomized + D-galactose treated + estrogen injected (group VI) mice protein concentration and amylase activity in the salivary glands increased, while lipid peroxidation and fluorescence product decreased. These results reveal that centrophenoxine, and therefore aging, has modulatory effects on salivary glands in D-galactose induced aged female mice.

***Key words:*** Centrophenoxine, ovariectomy, salivary glands.

## Introduction

According to the free radical theory of aging organism's age because cells accumulate free radical damage with outer shell. For most biological structures free radical damage is closely associated with oxidation damage. The free radicals of interest are often referred as reactive oxygen species (ROS). Fifty years ago Harman (1957) postulated free radical theory of aging indicating the imbalance between formation of free radicals and the reduction of anti-oxidant defence system that increase the oxidative damage to the macromolecules, ultimately leading to the cellular deterioration. The anti-oxidant theory implies that anti-oxidants prevent free radicals from oxidizing sensitive biological molecules or reduce the formation of free radicals, and slow aging process and prevent disease (Harman, 1957)

Both enzymatic and non enzymatic anti-oxidants exist in the intracellular and extracellular environments to quench ROS. The enzymatic anti-oxidants include superoxide dismutase (SOD) (Beauchamp and Fridovich, 1971), catalase (CAT) (Luck, 1974) and glutathione peroxidases (GPx) (Beers and Sizer, 1952), while the non-enzymatic anti-oxidants include glutathione, tocopherol (vitamin E), carotenoides, centrophenoxine and ascorbic acid, which react with most of the oxidants. Centrophenoxine (DMAE) is one of the anti-aging, neuro-energizing drugs (Nagy and Zs-Nagy, 1984).

Centrophenoxine enhances neuronal glucose and oxygen uptake, while increasing carbon dioxide production. In addition, centrophenoxine raises neuronal RNA (derived from DNA in the nucleus), which enables neurons to form proteins which help to encode memory as well as repair cell damage. Centrophenoxine reverses the age-related drop in RNA and protein production (Zs-Nagy and Semsei, 1984]

In the females there is protection against free radical due to the presence of estrogen (Lawler, 1991). But after the reproductive period the estrogen level in the blood decreases, which leads to increase in free radical formation. If in order to overcome this effect estrogen is administered the increasing levels of estrogen in blood may become carcinogenic. Therefore, to overcome this problem, we selected the centrophenoxine to find whether centrophenoxine can take the place of estrogen. Thus, the objective of the present investigation is to find whether

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centrophenoxine could offer protection to the females after reproductive period or changes in sex cycle.

**Material and Methods**

Female albino mice (*Mus musculus)* were used in the present investigation. The mice were bred and reared in the departmental animal house (CPCSEA 233) in separate cages under proper conditions of light, temperature and humidity. The animals were supplied with Amrut mice feed (Pranav Agro Industries) and water *ad libitum*. Females mice, 12 to 14 weeks old (35-40 grams), were considered as adults. Adult females were grouped according to the stages of their estrous cycle. The stages of the estrous cycle were confirmed by observation of the vaginal smear. Ovariectomy was performed on 30 proestrous females under ether anesthesia in sterile conditions. The ovariectomised mice were maintained under proper care in the animal house. After four days the ovariectomised mice were separated into following groups.

***Group I****:* Five ovariectomized mice were kept as it is 15 days without any other treatment.

***Group II:*** 5% D-galactose (0.5ml/day) was injected subcutaneously to five ovariectomized mice for 15 days.

***Group III***: Ovariectomized + D-galactose treated (subcutaneous injection, 0.5ml/day) + tween 80 (oral) treated mice from the fifth day after ovariectomy (treatment for 15 days).

***Group IV***: Ovariectomized + D-galactose treated + centrophenoxine (100mg/kg body weight in tween 80, oral) administered from fifth day after ovariectomy (treatment for 15 days).

***Group V***: Ovariectomized + D-galactose treated (for 15 days) + olive oil administered on $3^{rd}$, $6^{th}$, $9^{th}$, $12^{th}$ day along with the D-galactose.

***Group VI***: Ovariectomized + D-galactose treated + estradiol-17ß administered (0.4mg/100g body wt) on $3^{rd}$, $6^{th}$, $9^{th}$, $12^{th}$ day along with the D-galactose.

Then all the animals were sacrificed, dissected and the submandibular gland, sublingual gland and parotid gland were used for the following analysis.

***Estimation of protein:*** Tissue homogenate was centrifuged for 15 min at 3000 rpm; the supernatant was taken for estimation of protein using Bovine Serum Albumin as the standard (Lowry *et al.,* 1951).

***Estimation of amylase activity:*** Amylase activity was determined using starch solution that yields maltose, which was estimated using dinitrosalicylic acid (Doane, 1969).

***Determination of total lipid peroxidation*:** Tissue homogenate was prepared in a chilled mortar using potassium phosphate buffer pH-7.04 containing 1 mM ascorbic acid and $FeCl_3$. MDA was estimated as thiobarbituric acid reacting substance (Wills, 1966).

***Measurement of fluorescence product:*** The lipofuscin granules were extracted using chloroform: methanol mixture (2:1 v/v). The fluresencence was measured in photoflurometer calibrated with quinine sulphate (Dillard and Tappel, 1971).

***Statistical analysis*:** The data were subjected to ANOVA followed by Tukey's post- hoc test.

**Results**

In ovariectomised mice (group I) protein concentration and amylase activity in various salivary glands decreased significantly ($P< 0.01$). In ovariectomized + D- galactose treated mice it was further decreased, and the decrease was significant ($P<0.01$). In ovariectomized + D-galactose treated mice also receiving tween 80 (group III), there was no significant change. In ovariectomized, D-galactose treated + centrophenoxine administered mice (group IV), protein concentration and amylase activity increased significantly as compared to group III ( $P<0.01$) ( Tables 1, 3). In ovariectomized, D-galactose treated + olive oil injected mice there was no significant change as compared to group II. In ovariectomized, D- galactose treated + estrogen administered mice (group VI) protein concentration and amylase activity increased significantly as compared to group III ($P<0.01$)( Tables 2, 4).

The lipid peroxidation and fluorescence product in the various salivary glands increased significantly in group I as compared to control ($P < 0.01$) and also increased in group II ($P < 0.01$) as compared to group I, while there was decrease in group IV and group VI as compared to group III and group V, respectively ($P < 0.01$) (Tables 5-8).

**Discussion**

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**Table 1:** Centrophenoxine effects on protein content of salivary glands of D-galactose treated aged female mice (protein mg/g tissue). Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Group | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 80.12 ±1.29 | | 87.00 ±1.83 | | 101.50 ± 1.29 | |
| 2 | Group I | Ovariectomized (5) | 76.40 ± 2.31 | P< 0.01 | 80.50 ± 1.20 | P< 0.01 | 87.50 ± 1.29 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 71.30 ± 1.99 | P<0.01 | 52.25 ± 1.71 | P<0.01 | 44.50 ± 1.29 | P<0.01 |
| 4 | Group III | Ovariectomized+ D-galactose treated + tween 80 (5) | 72.40 ± 1.90 | P>0.01 | 53.50 ± 1.62 | P>0.01 | 43.60 ± 1.19 | P>0.01 |
| 5 | Group IV | Ovariectomized + D-galactose treated + centrophenoxine (5) | 79.68 ± 1.67 | P< 0.01 | 79.00 ± 2.58 | P< 0.01 | 87.50 ± 1.29 | P< 0.01 |

**Table 2:** Estrogen effect on protein content in salivary glands of D-galactose treated aged female mice (protein mg/g tissue). Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Group | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 80.12 ±1.29 | | 87.00 ±1.83 | | 101.50 ± 1.29 | |
| 2 | Group I | Ovariectomized (5) | 76.40 ± 2.32 | P< 0.01 | 80.50 ± 1.20 | P< 0.01 | 87.50 ± 1.29 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 71.30 ± 1.99 | P<0.01 | 52.25 ± 1.71 | P<0.01 | 44.50 ± 1.29 | P<0.01 |
| 4 | Group V | Ovariectomized + D-galactose treated + olive oil (5) | 70.40 ± 1.57 | P>0.01 | 51.25 ± 1.65 | P>0.01 | 45.60 ± 1.12 | P>0.01 |
| 5 | Group VI | Ovariectomized + D-galactose treated + estrogen(5) | 75.66 ± 1.01 | P< 0.01 | 58.50 ± 2.08 | P< 0.01 | 80.75 ± 0.96 | P< 0.01 |

Protein content of salivary glands decreased in ovariectomized mice and significantly increased in ovariectomized centrophenoxine treated mice and ovariectomized estrogen treated mice. In galactose treated animals the change corresponded to that in aged animals, *i.e.*, increase in lipid peroxidation (Song *et al*., 1999). In the present investigation lipid peroxidation in salivary glands increased in ovariectomized and ovariectomized D-galactose treated mice. This may be due to decrease in the level of estrogen due to ovariectomy (Donato and Sohal, 1981) and treatment of D-galactose (Brunk, 1992).

The decrease in amylase activity in salivary glands, particularly parotid and submandibular glands, can be explained from several perspectives, but mainly there may be change in fidelity of transcription and translation, which are changes in response to intrinsic as well as extrinsic factors.

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**Table 3:** Centrophenoxine effects on amylase activity in salivary glands of D-galactose induced aged female mice. (Amylase activity/mg protein). Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Groups | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 2.47 ± 0.19 | | 0.94 ± 0.07 | | 30.03 ± 0.93 | |
| 2 | Group I | Ovariectomized (5) | 1.68 ± 0.24 | P< 0.01 | 0.73 ± 0.01 | P< 0.01 | 21.13 ± 0.63 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 1.37 ± 0.20 | P<0.01 | 0.63 ± 0.13 | P<0.01 | 18.24 ± 0.33 | P<0.01 |
| 4 | Group III | Ovariectomized + D-galactose treated + tween 80 (5) | 1.27 ± 0.20 | P>0.01 | 0.61 ± 0.11 | P>0.01 | 19.13 ± 0.38 | P>0.01 |
| 5 | Group IV | Ovariectomized + D-galactose treated + centrophenoxine (5) | 2.22 ± 0.39 | P> 0.01 | 0.82 ± 0.14 | P> 0.01 | 28.14 ± 0.69 | P> 0.01 |

**Table 4:** Estrogen effects on amylase activity in salivary glands of D-galactose induced aged female mice. (Amylase activity/mg protein) Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Groups | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 2.47 ± 0.19 | | 0.93 ± 0.07 | | 30.03 ± 0.93 | |
| 2 | Group I | Ovariectomized (5) | 1.68 ± 0.238 | P< 0.01 | 0.73 ± 0.01 | P< 0.01 | 21.13± 0.63 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 1.37± 0.20 | P<0.01 | 0.63 ± 0.13 | P<0.01 | 18.24 ± 0.33 | P<0.01 |
| 4 | Group V | Ovariectomized + D-galactose treated + olive oil (5) | 1.33 ± 0.20 | P>0.01 | 0.56 ± 0.12 | P>0.01 | 17.21 ± 0.32 | P>0.01 |
| 5 | Group VI | Ovariectomized + D-galactose treated + estrogen (5) | 1.88 ± 0.31 | P> 0.01 | 0.64 ± 0.02 | P> 0.01 | 20.14 ± 0.14 | P> 0.01 |

**Table 5**: Centrophenoxine effects on lipid peroxidation in salivary glands of D-galactose induced aged female mice. (Lipid peroxidation, MDA/mg wet tissue). Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Groups | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 11.53 ± 1.69 | | 10.75 ± 1.04 | | 20.39 ± 0.40 | |
| 2 | Group I | Ovariectomized (5) | 18.53 ± 2.69 | P< 0.01 | 32.80 ± 1.61 | P< 0.01 | 34.45 ± 0.17 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 28.84± 1.06 | P< 0.01 | 38.34 ± 1.08 | P< 0.01 | 35.69 ± 0.08 | P< 0.01 |
| 4 | Group III | Ovariectomized + D-galactose treated+ tween 80 (5) | 26.62 ± 0.91 | P>0.01 | 36.31 ± 1.06 | P>0.01 | 34.29 ± 0.08 | P>0.01 |
| 5 | Group IV | Ovariectomized + D-galactose treated + centrophenoxine (5) | 21.89 ± 1.28 | P< 0.01 | 17.23 ± 0.59 | P< 0.01 | 28.85 ± 0.41 | P< 0.01 |

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**Table 6**: Estrogen effects on lipid peroxidation in salivary glands of D-galactose induced aged female mice. (Lipid peroxidation, MDA/mg wet tissue). Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Groups | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 11.53 ± 1.69 | | 10.75 ± 1.04 | | 20.39 ± 0.40 | |
| 2 | Group I | Ovariectomized (5) | 18.53 ± 2.69 | P< 0.01 | 32.80 ± 1.61 | P< 0.01 | 34.45± 0.17 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 28.84± 1.06 | P< 0.01 | 38.34 ± 1.08 | P< 0.01 | 35.69 ± 0.08 | P< 0.01 |
| 4 | Group V | Ovariectomized+ D-galactose treated + olive oil (5) | 26.60 ± 0.90 | P>0.01 | 38.11 ± 1.06 | P>0.01 | 33.33 ± 0.06 | P>0.01 |
| 5 | Group VI | Ovariectomized + D-galactose treated + estrogen (5) | 24.61 ± 1.99 | P< 0.01 | 33.40 ± 1.14 | P< 0.01 | 38.82 ± 0.03 | P< 0.01 |

**Table7**: Centrophenoxine effects on fluorescence product in salivary glands of D-galactose induced aged female mice. Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Groups | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 0.020 ± 0.010 | | 0.008 ± 0.001 | | 0.008 ± 0.001 | |
| 2 | Group I | Ovariectomized (5) | 0.021 ± 0.001 | P< 0.01 | 0.018 ± 0.001 | P< 0.01 | 0.055 ± 0.001 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 0.031 ± 0.001 | P< 0.01 | 0.022 ± 0.001 | P< 0.01 | 0.057 ± 0.001 | P< 0.01 |
| 4 | Group III | Ovariectomized + D-galactose treated + tween 80 (5) | 0.023 ± 0.001 | P>0.01 | 0.021 ± 0.001 | P>0.01 | 0.049 ± 0.001 | P>0.01 |
| 5 | Group IV | Ovariectomized + D-galactose treated + centrophenoxine (5) | 0.018 ± 0.002 | P> 0.01 | 0.018± 0.001 | P> 0.01 | 0.026± 0.001 | P> 0.01 |

**Table 8**: Estrogen effects on fluorescence product in salivary glands of D-galactose induced aged female mice. Values are mean ± SD. Number in parenthesis denotes number of animals.

| Sr. No | Groups | Treatment | Submandibular gland | Statistical significance | Sublingual gland | Statistical significance | Parotid gland | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| 1 | | Control (5) | 0.015 ± 0.008 | | 0.008 ± 0.001 | | 0.008 ± 0.001 | |
| 2 | Group I | Ovariectomized (5) | 0.019 ± 0.006 | P< 0.01 | 0.018 ± 0.001 | P< 0.01 | 0.055 ± 0.001 | P< 0.01 |
| 3 | Group II | Ovariectomized + D-galactose treated (5) | 0.028 ± 0.001 | P< 0.01 | 0.022 ± 0.001 | P< 0.01 | 0.057 ± 0.001 | P< 0.01 |
| 4 | Group V | Ovariectomized+ D-galactose treated + olive oil (5) | 0.022 ± 0.001 | P>0.01 | 0.021 ± 0.001 | P>0.01 | 0.052 ± 0.001 | P>0.01 |
| 5 | Group VI | Ovariectomized+ D-galactose treated + estrogen (5) | 0.023 ± 0.002 | P> 0.01 | 0.021 ± 0.001 | P> 0.01 | 0.056 ± 0.001 | P> 0.01 |

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Centrophenoxine is one of the potent antioxidants that reduce the peroxides formed in the tissues during aging (Nandy, 1978). Thus, it is possible that the antioxidant centrophenoxine is efficient in reducing peroxidation in salivary glands.

Decrease in the proteins and amylase activity in salivary glands can be related to the alteration in DNA transcript and translation due to the alteration in DNA structure. This is because of free radical damage, which may lead to reduction in antioxidant enzymes that are involved in quenching of free radicals (Ship and Baum, 1990)

Decrease in enzyme activity in salivary glands of ovariectomized mice may be related to hormonal level. Mouse salivary gland, especially submandibular gland, is a target organ for estrogen. In the present investigation administration of centrophenoxine to ovariectomized mice produced decrease in the peroxidation and fluorescence level in tissues. Nevertheless, the integrity of genome is not compromised as a very efficient repair system that protects the DNA from damage exists. Thus, it is concluded that centrophenoxine can take the place of estrogen after the reproductive period or change in reproductive cycle.

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