Histological and Immunohistochemical Study to Evaluate the Effects of Chamomile versus Green Tea Extracts on the Salivary Glands of Methotrexate Treated Male Albino Rats

Thesis

Submitted for Partial Fulfillment of MD Degree in Histology

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2019

Summary

Methotrexate is a chemotherapeutic drug that causes major toxic effects on salivary glands. This study aimed to investigate the prophylactic and therapeutic effect of chamomile versus green tea on a methotrexate induced injury on the major salivary glands of adult male albino rats.

Forty eight male albino rats were included in this study. They were divided into six groups of 8 rats each and each group was further subdivided into 2 subgroups 4 rats each according to timing of drugs administration as follows:

● **Group I (control group)**: This group was equally subdivided into**:**

- **Subgroup Ia:** received 1.6 ml single intraperitoneal injection of physiological saline (0.9% NaCl) after 1 week (day 8) of the experiment and distilled water orally from (day 1) to (day 14).
- **Subgroup Ib: received** 1.6 ml single intraperitoneal injection of physiological saline (0.9% NaCl) in the first day (day 1) of the experiment and distilled water orally from (day 8) to (day 14).

● **Group II (MTX treated group)**: This group was equally subdivided into**:**

- **Subgroup II a**: 4 rats received single intraperitoneal injection of 80 mg/kg of MTX dissolved in physiological saline (0.9% NaCl) after 1 week (day 8) of the experiment and distilled water from (day 1) to (day 14).

- **Subgroup II b**: 4 rats received single intraperitoneal injection of 80 mg/kg of MTX dissolved in physiological saline (0.9% NaCl) in the first day (day 1) of the experiment and distilled water from (day 8) to (day 14).

● **Group III (GT treated group)**: all the animals received extract of green tea administered orally at a dose of (40 mg/kg/day) dissolved in distilled water by intragastric gavage tube and equally subdivided into 2 subgroups:

- **Subgroup III a:** received 1.6 ml single intraperitoneal injection of physiological saline (0.9% NaCl) at (day 8) of the experiment and green tea administration started from (day 1) to day 14.
- **Subgroup III b:** received 1.6 ml single intraperitoneal injection of physiological saline (0.9% NaCl) at (day 1) of the experiment and green tea administration started from (day 8) to day 14.

● **Group IV (chamomile extract treated group)**: all the animals received chamomile extract administered orally at a dose of (100 mg/kg/day) dissolved in distilled water by intragastric gavage tube. Each rat received 20 mg chamomile extract (1ml). This group is equally subdivided into 2 subgroups:

- **Subgroup IV a:** received 1.6 ml single intraperitoneal injection of physiological saline (0.9% NaCl) at (day 8) of the experiment and chamomile administration started from (day 1) to day 14.
- **Subgroup IV b:** received 1.6 ml single intraperitoneal injection of physiological saline (0.9% NaCl) at (day 1) of the experiment and chamomile administration started from (day 8) to day 14.

● **Group V (MTX and GT treated group)**: all the animals received extract of GT administered orally at a dose of (40 mg/kg/day) dissolved in distilled water by intragastric gavage tube and this group is equally subdivided into 2 subgroups:

- **Subgroup V a (prophylactic):** received 1.6 ml single intraperitoneal injection of (80 mg/kg) of MTX dissolved in physiological saline (0.9% NaCl) at (day 8) of the experiment and GT administration started from (day 1) to day 14.
- **Subgroup V b (therapeutic):** received 1.6 ml single intraperitoneal injection of (80 mg/kg) of MTX dissolved in physiological saline (0.9% NaCl) at (day 1) of the experiment and GT administration started from (day 8) to day 14.

● **Group VI (MTX and chamomile treated group)**: all the animals received extract of chamomile administered orally at a dose of (40 mg/kg/day) dissolved in distilled water by intragastric gavage tube and this group is equally subdivided into 2 subgroups:

- **Subgroup VI a (prophylactic):** received 1.6 ml single intraperitoneal injection of (80 mg/kg) of MTX dissolved in physiological saline (0.9% NaCl) at (day 8) of the experiment and chamomile administration started from (day 1) to day 14.
- **Subgroup VI b (therapeutic):** received 1.6 ml single intraperitoneal injection of (80 mg/kg) of MTX dissolved in physiological saline (0.9% NaCl) at (day 1) of the experiment and chamomile administration started from (day 8) to day 14.

All rats of all groups were sacrificed after 14days and the major salivary glands (SMG, PG and SLG) were obtained, from which sections were prepared and subjected to the following:

1) Haematoxylin & eosin stain.
2) Mallory trichrome stain.
3) Immunohistochemical staining technique for KI 67 & Caspase-3.

Morphometric study and statistical analysis were performed for area % of **collagen** & **Caspase-3** immunoreactivity and counting of positive **KI 67** immunostained nuclei.

Group I (control group): Both subgroups showed that **SMG** was tightly related to the corresponding SL gland and enclosed together in a common connective tissue capsule and formed of serous acini that separated with areas of connective tissue containing large blood vessels and duct system. Duct system was numerous and consisted of ID, SD, GD and excretory ducts. Immunoreactivity of KI 67 was detected in the nuclei of few acinar and ductal cells and Caspase-3 immunoreactivity was negative.

PG showed serous acini separated with many ducts and very scarce connective tissue containing blood capillaries. Duct system was numerous and consisted of ID, SD and excretory ducts. Immunoreactivity of KI 67 and Caspase-3 immunoreactivity were negative.

SLG showed mucous acini. Many striated ducts**,** interlobar ducts were distributed between acini and fine CT trabeculae. Intercalated ducts were

not frequently observed. Immunoreactivity of KI 67 was detected in the nuclei of few cells and Caspase-3 immunoreactivity was detected in the cytoplasm of few cells.

Group II (MTX group), subgroup IIa (one week after MTX injection), cytotoxic effect appeared in all glands (SMG, PG and SLG). Salivary glands showed loss of normal architecture with degeneration in acini and ducts. Cytoplasmic vacuolations in acinar and ductal cells with darkly stained nuclei. Duct dilatation with stagnant secretion and dilatation and congestion of bl.vs were noticed. In **Subgroup II b**, 2 weeks after MTX injection, all previous features became obvious with appearance of cellular infiltrations and apoptotic bodies. Both **subgroups II a & II b** showed an obvious increase in the thickness of collagen in trabeculae and around ducts, there was statistically significant increase in area % of collagen compared to the control group and statistically significant increase in **subgroup IIb** compared to **subgroup IIa** in SMG & PG. The KI 67 reaction was detected in few nuclei of acinar and ductal cells in both **subgroups II a & II b.** The reaction was statistically no significant difference compared to the control group. Caspase-3 reaction**,** in **group II,** both subgroups revealed a statistically highly significant increase compared to control group in all glands. The increase of apoptosis in **subgroup IIb** was statistically highly significant compared to **subgroup II a** in all glands.

Sections of **group III (green tea) and group IV (chamomile)** showed picture close to normal control sections in all glands. With few dilated bl.vs

in chamomile group. Both **groups III & IV** showed statistically no significant difference in area % of collagen compared to control group. The KI 67 reaction was detected in few nuclei of acinar and ductal cells in all glands of group III and negative reaction in all glands of group IV. The reaction was statistically no significant difference compared to the control group.Caspase-3 reaction**,** was faint in all glands of group III and negative reaction in group IV. The reaction was statistically no significant difference compared to the control group.

In **group V** (mixed MTX and GT group) **subgroup V a (prophylactic)** showed improvement compared to MTX group in the form of regular architecture of acini and ducts. However, few acinar and ductal cells were still affected. Also, in **therapeutic group (subgroup V b),** all glands showed clear improvement compared to MTX group. Collagen fibers were apparently decreased in **subgroup Va** and **subgroup Vb** in all glands comparison with MTX group. This decrease was statistically significant compared to MTX group while no statistically significant difference in PG gland compared to control groups. There was increase in the number of positive Ki 67 immunostained nuclei in **subgroup Va (prophylactic).** This increase was statistically significant in SMG compared to **subgroups IIb & Vb** and **group IV.** While **therapeutic group (subgroup V b),** showed few proliferating cells in all glands which were no statistically significant compared to control. While in PG & SMG were statistically significant decrease of ki67 immunostained nuclei compared to prophylactic group (subgroup V a). Caspase-3 immunoreactivity in **prophylactic (subgroup V a)** showed decrease

in reaction in all glands that was statistically significant compared to **IIa & IIb** in SMG & PG while SLG showed statistically significant decrease with **subgroup IIa** only. Also, **therapeutic group, (subgroup V b)** showed decrease in anti-Caspase 3 area % which were statistically significant compared to **subgroup II a & subgroup II b** in SMG while in PG & SLG were statistically significant compared to **subgroup II b.** Reactions in all glands of both subgroups were statistically significant increased compared to **control, III (green tea) and IV (chamomile)** groups. Reaction in SMG & PG of therapeutic group was statistically significant increased compared to prophylactic group.

In **group VI** (mixed MTX & chamomile)**, subgroup VI a (prophylactic)** showed obvious improvement with minimal bl.vs congestion, few degenerated acini and few apoptotic bodies in SMG & PG & SLG compared to MTX group. Also, in therapeutic group **((subgroup VI b),** all glands showed improvement with minimal cytoplasmic vacuolations and bl.vs congestion compared to MTX group.

In prophylactic, **(subgroup VI a),** SMG & PG & SLG showed decrease in collagen area % in Mallory trichrome stained sections. This decrease was statistically significant in SMG & SLG compared to MTX group. While in PG, collagen area % was statistically significant decreased compared to **subgroup II b** and statistically non-significant difference compared to **subgroup II a.** Also, **subgroup VI b (therapeutic),** all glands showed statistically significant decrease in collagen area % in SMG compared to

subgroup II b, and statistically highly significant decrease in PG & SLG compared to both **subgroups II a & b.** SMG glands in prophylactic group and SLG in prophylactic and therapeutic groups showed statistically significant increase compared to **control, III (GT)** and **IV (chamomile)** groups. KI 67 immunohistochemical stained sections revealed increase in proliferation mainly in duct cells of SMG & PG & SLG in both **subgroups VI a & b.** This increase was statistically highly significant in PG of prophylactic group compared to **control, MTX (II a & b), GT (III), chamomile (IV) & subgroup Vb** and to **control, IV groups, subgroup II a** and **both subgroups V a& b** in therapeutic group. Caspase-3 immunostainined sections in **both subgroup VI a&b** showed decrease in apoptosis in all glands compared to MTX group. This decrease was statistically highly significant in SMG & PG of both **subgroups VI a & b** compared to **subgroups II a&b.** while in SLG gland of **subgroups VI a&b** the decrease was statistically significant compared to **subgroup IIb.**