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

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Abstract: Background: Methotrexate is a chemotherapeutic drug that causes major toxic effects on salivary glands. Green tea (GT) and chamomile are used to treat these toxic effects due to their anti-oxidant effect. **Aim:** This study aimed to demonstrate the prophylactic and therapeutic effects of chamomile versus green tea on a methotrexate induced injury on the major salivary glands of adult male albino rats. **Materials & Methods:** 48 adult male albino rats were included in the present study. They were equally divided into 6 groups (8 rats each): control, MTX, GT, chamomile, mixed MTX & GT and mixed MTX & chamomile. Mixed groups were further subdivided into prophylactic & therapeutic subgroups. All animals were sacrificed after 14 days. Submandibular, parotid and sublingual glands were obtained and stained with H & E stain, Mallory trichrome stain and immunohistochemical staining for Ki-67 & Caspase-3. The mean area % for collagen and Caspase-3 immunoreaction and the mean number of Ki-67 reactive nuclei were measured using the image analyzer and the results were statistically analyzed. **Results:** MTX group revealed acinar and ductal degeneration, cellular infiltrations and increase area % of collagen significantly compared to control group. However, mixed groups demonstrated improvement and significant decrease area % of collagen versus MTX group. Ki-67 was significantly increased & Caspase 3 was significantly decreased in mixed groups compared to MTX group. GT & chamomile groups showed histological structure and statistically not different from the control group. **Conclusions:** GT & chamomile had a prophylactic and therapeutic effect on MTX-induced salivary glands degeneration through their antioxidant and anti-inflammatory effects. Therapeutic and regenerative effect of chamomile is similar or slightly more effective than GT.
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Key Words: Methotrexate, Green tea, Chamomile, Salivary glands, parotid, submandibular, sublingual,` Ki 67, Caspase 3.

1. Introduction

Humans have three paired major salivary glands: parotid gland (PG), submandibular gland (SMG) and sublingual gland (SLG) as well as hundreds of minor salivary glands ***(Edgar et al., 2012).*** Saliva helps swallowing, protects the mouth from bacteria, acts as a lubricant and helps to digest foods**.** It may be used as a diagnostic tool of multiple systemic disorders ***(Fábián et al., 2008).*** Salivary glands parenchyma is subjected to many local or systemic conditions that may change its histological characteristics ***(Garcia & Bussoloti, 2013).***

Chemotherapy used in treatment of cancer has cytotoxic effects which are not selective for cancer cells but also extend to affect the normal tissues. The degree of damage differs according to the type, dosage and duration of treatment ***(Al-Moula et al., 2012).***

Methotrexate **(MTX)** is an antimetabolite drug. It is widely used in the treatment of many malignant conditions in addition to some other diseases such as rheumatoid arthritis and psoriasis ***(Dadhania et al., 2010)***, ectopic pregnancy, Crohn's disease and ulcerative colitis ***(Herfarth, 2016)*** but its efficacy is limited due to its side effects ***(Klareskog et al., 2004)***.

A major mechanism of MTX cellular toxicity is by reducing effectiveness of antioxidant enzyme defense system, making cells vulnerable to reactive oxygen species (ROS). So, variant antioxidants may protect against oxidative stress induced damage to cells by MTX ***(Sener et al., 2006).***

Chamomile is a well-known medicinal plant species from the Asteraceae family. Its multi therapeutic, cosmetic and nutritional values have been established through years of traditional and scientific use and research ***(Ghasemi et al., 2013***). Chamomile is

proved to have antioxidant ***(Hernández*-Ceruelos *et al., 2010)*** and anti-inflammatory properties ***(Bulgari, et al., 2012).***

Green tea (Epigallocatechingallate, EGCG) is natural product derived from the plant Camellia Sinensis. It was proved as a natural antioxidant as it is very rich in antioxidant polyphenolic flavonoids ***(Chandra et al., 2011).***

2. Materials and methods

Animals:

Forty eight male albino rats weighing 180-220 gm., 10-12 weeks old, were used in this study. The animals were locally bred at the animal house of Kasr Al Aini, Cairo University in hygienic stainless steel cages and in clean well ventilated room. Standard laboratory chow and tap water were available as needed. These measures were approved by the guidelines of animal ethics committee. Following international ethics and regulations for animal research in laboratory applications [Gluck et al., 2002].

Materials & drugs:

1. Methotrexate (MTX): each vial contains 50 mg of methotrexate (Shanxi PUDE pharmaceutical Co., Ltd. Imported by: Techno Pharmafor Investment and Development). Each vial dissolved in 5 ml saline.

2. Green tea extract (GTE): each tablet contains 300 mg of green tea extract (MEPACO-MEDIFOOD. Reg. No. 728/07). Each tablet was crushed and dissolved in 30 ml distilled water.

3. Matricariachamomilla: The fresh leaves of the plant *Matricaria chamomile* (Chamomile) were obtained from the local herbal market from Fayoum, Egypt. From these leaves, extract was prepared. Each 0.1g of extract dissolved in 5ml water.

♦♦ **Preparation of the extract:** Chamomile extract was prepared at biochemistry department, Faculty of medicine, Cairo University as previously described by **Sebai et al., (2014).** Briefly, the plant material was later dried in an incubator at 50°C for 72 hours and powdered in an electric blender. About 10 gm of plant leaves of chamomile was taken in glass jar with double distilled water (1/5; w/v) at 100°C during five minutes under magnetic agitation and the homogenate was filtered through a colander (0.5 mm mesh size). Finally, the obtained extract was stored at –80°C until used.

Experimental design:

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 as shown in table (1):

Table (1): experimental groups

Group	Sub group	drug administration and timing	
Group I (Control)	Ia	Saline (day 8)	DW (days 1-14)
	Ib	Saline (day 1)	DW (days 8-14)
Group II (MTX)	IIa	MTX (day 8)	DW (days 1-14)
	IIb	MTX (day 1)	DW (days 8-14)
Group III (Green tea)	IIIa	Saline (day 8)	Green tea (days 1-14)
	IIIb	Saline (day 1)	Green tea (days 8-14)
Group IV (Chamomile)	IVa	Saline (day 8)	Chamomile (days 1-14)
	IVb	Saline (day 1)	Chamomile (days 8-14)
Group V (MTX & Green tea)	Va	MTX (day 8)	Green tea (days 1-14)
	Vb	MTX (day 1)	Green tea (days 8-14)
Group VI (MTX & Chamomile)	VIa	MTX (day 8)	Chamomile (days 1-14)
	VIb	MTX (day 1)	Chamomile (days 8-14)

Subgroups (a) are considered as prophylactic subgroups

Subgroups (b) are considered as therapeutic subgroups.

Saline: 1.6 ml single intraperitoneal injection.

MTX: 80 mg/kg MTX dissolved in 1.6 ml saline administered as single intraperitoneal injection. At day 8 in (a) prophylactic subgroups and at day 1 in (b) therapeutic subgroups.

DW: 1 ml distilled water orally by intragastric gavage tube.

Green tea: 40 mg/kg/day Green tea dissolved in 1 ml distilled water administered orally by intragastric gavage tube. (days 1-14) in prophylactic subgroups and in (days 8-14) of the experiment in therapeutic subgroups.

Chamomile: 100 mg/kg/day Chamomile dissolved in 1 ml distilled water administered orally by intragastric gavage tube (days 1-14) in prophylactic

subgroups and in (days 8-14) of the experiment in therapeutic subgroups.

After 14 days from starting experiment; rats from all groups were anesthetized using intraperitoneal injection of phenobarbitone at 60 mg/kg and then sacrificed and the major salivary glands (SMG, PG and SLG) were obtained, from which paraffin sections were prepared. Serial sections of 7 µm thickness were cut and subjected to the following stains:

1) Haematoxylin & eosin stain to study the general histological changes.

2) Mallory trichrome stain to detect changes in collagen content in different glands.

3) **Immunohistochemical staining** using:

a. Monoclonal antibodies to Ki-67 as a marker of cell proliferation (Lazăr et al., 2010).

b. Monoclonal antibodies to cleaved Caspase-3 as a marker of cell apoptosis (Said et al., 2004).

4) Quantitative morphometric analysis

The following parameters were examined using Leica Qwin 500 Image analysis (Hessen, Wetzlar, Germany):

For each parameter, measurements were taken in five randomly selected non-overlapping fields per slide from five slides of each animal.

(1) Area percentage of collagen in the Mallory trichrome stained sections: it was measured from sections from all groups using an objective lens of magnification 4 (a total magnification of 40).

(2) Area percentage of Caspase-3: it was measured from sections from all groups using an objective lens of magnification 10 (a total magnification of 100).

(3) Number of KI-67 immunopositive cells: interactive counting of immunopositive cells was done using an objective lens of magnification 40 (total magnification of 400).

Statistical analysis:

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc, USA). For quantitative data, the mean and standard deviation were calculated. ANOVA (Analysis of variance) was used to test the difference about mean values of measured parameters among groups, multiple comparison between pairs of groups were performed using Post hoc test. For interpretation of results of tests of significance, significance was adopted at $P \leq 0.05$. ***(Emsley et al., 2010).***

3. Results

Group I (control group): Both subgroups showed that SMG was tightly related to the corresponding SLG 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 intercalated ducts, striated ducts, granulated ducts 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. (fig. 1-3)

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 blood vessels (bl.vs) were noticed. In Subgroup IIb (2 weeks after MTX injection), all previous features became more obvious with appearance of cellular infiltrations and apoptotic bodies. Both subgroups IIa & IIb showed an obvious increase in the thickness of collagen in trabeculae and around ducts, KI 67 reaction was detected in few nuclei of acinar and ductal cells in both subgroups IIa & IIb, with no remarkable difference from the control.

Caspase-3 reaction, in both subgroups IIa & IIb, revealed increased reactivity compared to control group in all glands. The increase of apoptosis in subgroup IIb was more remarkable compared to subgroup IIa in all glands. (figs. 4-9)

Sections of group III (green tea) and group IV (chamomile) showed picture close to normal control sections in all glands. Both groups III & IV showed no remarkable difference in collagen content in trabeculae and around ducts compared to control group. 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. In addition to that; Caspase-3 reaction was faint in all glands of group III and negative reaction in group IV. (fig. 10-12)

In group V (mixed MTX and GT group) subgroup Va (prophylactic) and subgroup Vb (therapeutic) 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. Collagen fibers were apparently decreased in subgroup Va & Vb in all glands.

There was an increase in the number of positive Ki-67 immunostained nuclei in subgroup Va (prophylactic).

Therapeutic group (subgroup Vb), showed few proliferating cells in all glands.

Caspase-3 immunoreactivity in prophylactic (subgroup Va) showed decrease in reaction in all glands. Also, therapeutic group, (subgroup Vb) showed decrease in anti-Caspase 3 stained area. (figs. 13-18).

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

In prophylactic and therapeutic subgroups (VIa & VIb), all glands showed decrease in collagen in Mallory trichrome stained sections.

Ki-67 immunohistochemical stained sections revealed increase in proliferation mainly in duct cells of SMG & PG & SLG in both subgroups VIa & VIb.

Caspase-3 immunostainined sections in both subgroups VIa & VIb showed decrease in apoptosis in all glands compared to control (figs. 19-24).

Morphometric Statistical Results:

ANOVA among groups as regard mean area % of collagen, mean number of positive nuclei (ki-67 stain) and Mean area% of caspase 3 reactions showed that, in each parameter, there was statistically significant variance among different groups were $P < 0.05$.

So comparison between each two groups in these parameters was performed using Post Hoc test and with significant differences found between many groups in different parameters. These results were summarized in tables (1, 2 and 3) and charts (1, 2 and 3):

Table (2) & Chart (1) summarize the results of the mean Area % of collagen.

Table (3) & Chart (2) summarize the results of the mean number of positive nuclei (ki-67 stain) immunoreactivity.

Table (4) & Chart (3) summarize the results of the mean area% of caspase 3 immunoreactivity.

Table (2): The mean area % of collagen in salivary gland in different studied groups

Mean & SD	Group 1 Control	Group 2A	Group 2B	Group 3	Group 4	Group 5A	Group 5B	Group 6A	Group 6B
Submandibular	1.37±0.57	5.69±2.15	8.67±1.76	2.35±0.70	1.06±0.27	3.72±0.40	4.59±0.41	2.24±0.64	5.68±1.29
Parotid	0.77±0.08	9.23±1.21	13.21±3.71	1.41±0.58	1.54±0.45	3.80±0.52	3.43±1.21	6.59±0.65	5.45±2.38
Sublingual	1.52±0.62	5.30±1.23	6.80±1.95	0.63±0.23	1.73±0.55	3.49±1.07	3.93±1.25	1.38±0.21	1.99±0.71

Table (3): Number of positive nuclei immunostained with ki 67 in salivary glands in different studied groups:

Mean & SD	Group 1 Control	Group 2A	Group 2B	Group 3	Group 4	Group 5A	Group 5B	Group 6A	Group 6B
Submandibular	1.60±0.55	1.40±0.54	1.20±0.45	1.40±0.55	0.00±0.00	3.00±1.58	0.80±0.44	2.40±1.14	1.80±0.83
Parotid	0.00±0.00	0.80±0.44	2.00±0.70	0.80±0.44	0.00±0.00	4.80±1.92	1.20±0.45	4.40±1.67	2.60±0.55
Sublingual	1.80±0.44	1.60±0.55	1.40±0.55	1.60±0.55	0.00±0.00	2.20±0.84	0.60±0.20	1.80±1.30	1.40±1.14

Table (4): The mean Area % of Caspase- 3 immunoreactivity in salivary glands in different studied groups

Mean & SD	Group 1 Control	Group 2A	Group 2B	Group 3	Group 4	Group 5A	Group 5B	Group 6A	Group 6B
Submandibular	0.09±0.03	15.56±2.06	18.97±3.18	0.07±0.02	0.08±0.02	4.30±0.11	7.80±0.94	8.74±0.67	5.24±0.86
Parotid	0.01±0.005	11.16±2.75	29.01±3.78	0.31±0.11	0.02±0.009	5.01±0.98	11.65±2.72	5.86±0.86	6.61±1.73
Sublingual	0.52±0.19	9.11±1.28	14.58±3.04	0.65±0.19	0.09±0.03	7.76±0.61	6.55±0.60	9.10±0.62	10.66±0.42

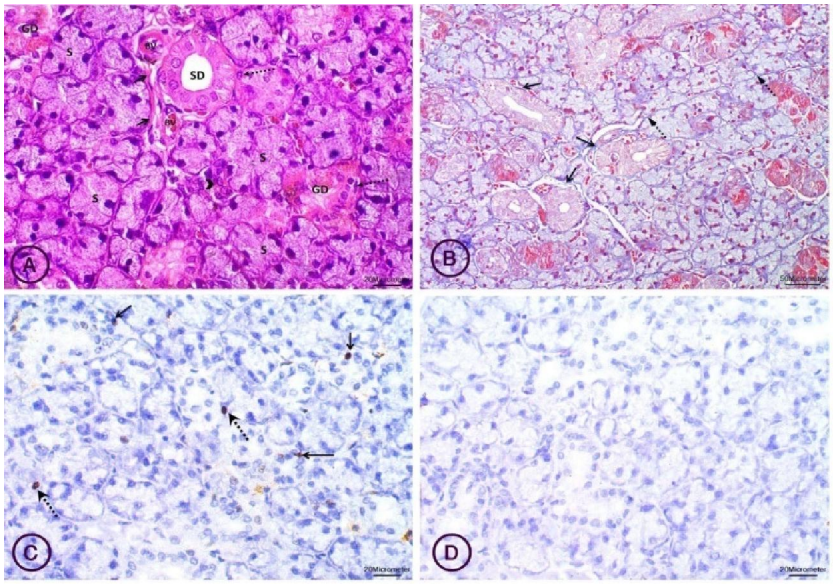

Fig. 1

(Fig.1): Photomicrographs of SMG in control group (I):

(A) sections of SMG stained with H & E show serous acini (S) lined with pyramidal cells with rounded nuclei. Acini separated with very fine CT (arrows). Striated ducts (SD), granular ducts (GD) and intercalated ducts (arrow head) in-between acini. Myoepithelial cells (dotted arrows) are also noticed.
(**B**) in sections stained with Mallory trichrome thin collagen fibers appear separating acini (dotted arrows) and around ducts (arrows).
(**C**) few cells appear with Ki 67 positive nuclear immunoreactivity in the acini (dotted arrows) and ducts (arrows).
(**D**) there is negative cytoplasmic Caspase 3 reaction in acinar and ductal cells.
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

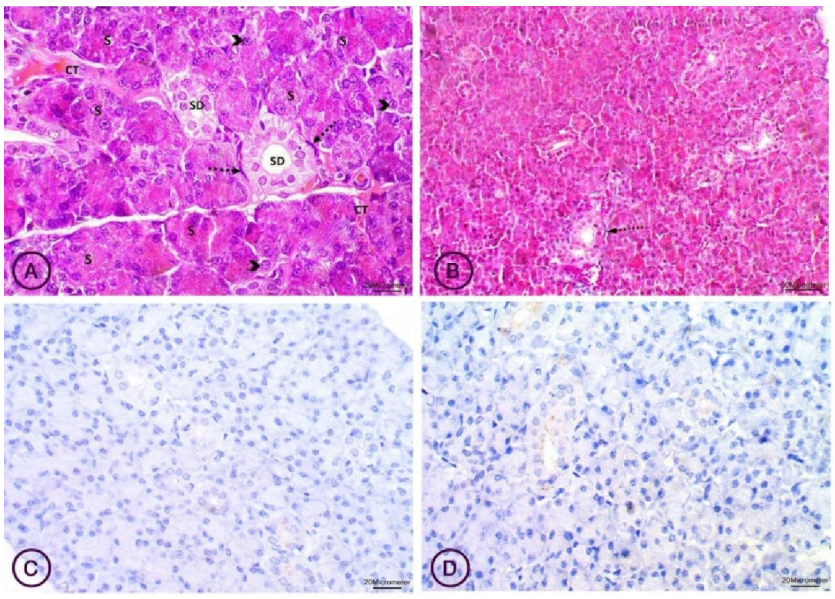

Fig. 2

(Fig.2): Photomicrographs of PG in control group (I):

(**A**): sections of PG stained with H & E show serous acini (S) lined with pyramidal cells with vesicular nuclei (arrows head). Many striated ducts (SD) and fine CT trabeculae (CT) are also seen. Meoepithelial cells are also seen around duct (dotted arrows).
(**B**): in sections stained with Mallory trichrome very thin collagen fibers appear around ducts (dotted arrows).
(**C**): there is negative nuclear Ki 67 immunoreactivity in the acini and duct.
(**D**): negative Caspase 3 cytoplasmic reaction in acinar and ductal cells.
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

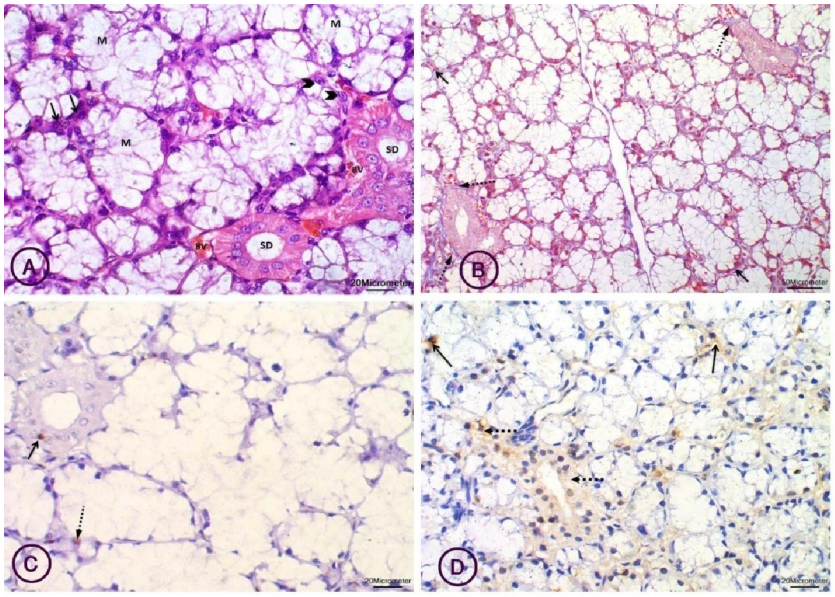

Fig. 3

(Fig.3): Photomicrographs of SLG in control group (I):

(**A**): sections of SLG stained with H & E show mucous acini (M) lined with cubical cells with flat nuclei. Some acini show serous demilune (arrows) and myoepithelial cells surround acini (arrow head). Striated ducts (SD) blood vessels (BV) are also seen.
(**B**): in sections stained with Mallory trichrome very thin collagen fibers separating acini (arrows) and around ducts (dotted arrows).
(**C**): there is positive Ki 67 nuclear immunoreactivity in few acini (dotted arrows) and ducts (arrows).
(**D**): mild cytoplasmic Caspase3 immunoreactivity in few acini (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

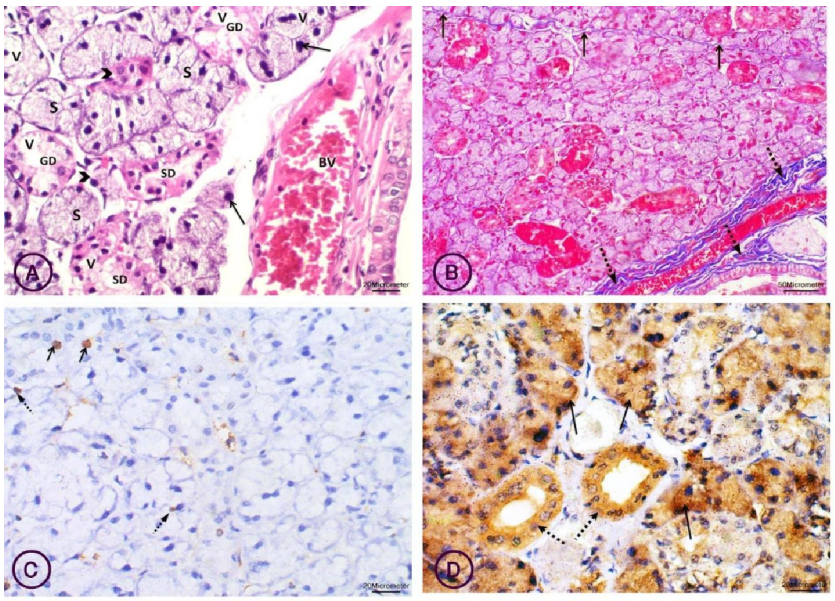

Fig. 4

(Fig. 4): Photomicrographs of SMG in MTX treated rats (subgroup II a):

(**A**) sections of SMG stained with H & E show disrupted serous acini (S), granular ducts (GD) and striated ducts (SD) with many vacuolations (V) and darkly stained apoptotic nuclei (arrows). Granular ducts (GD) also showing loss of granules. Most acini and ducts appeared degenerated (arrow head). Dilated congested bl.vs (BV) are also noticed.
(**B**) in sections stained with Mallory trichrome there is increased collagen deposits around ducts and around blood vessels (dotted arrows). Thin collagen fibers in CT septa (arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in some acini (dotted arrows) and ducts (arrows).
(**D**) there is strong cytoplasmic Caspase 3 immunoreactivity in the acini (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

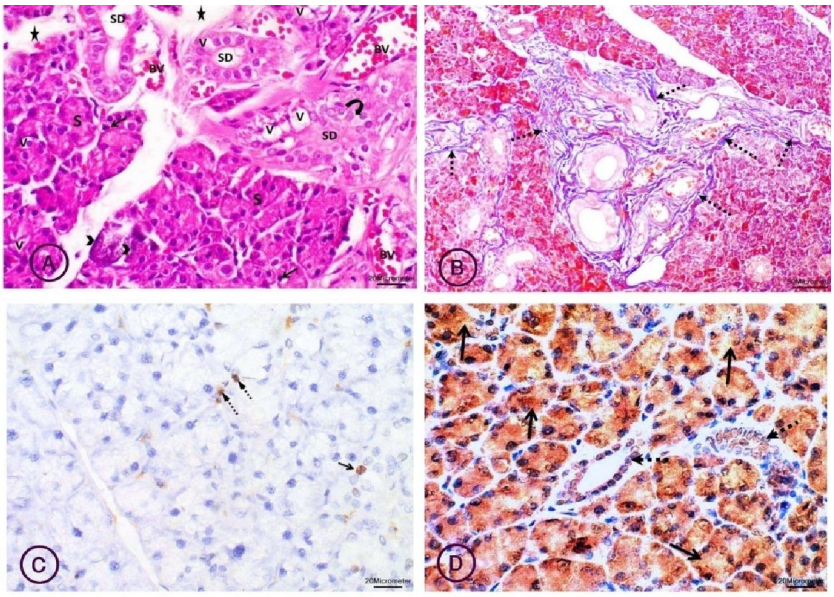

Fig. 5

(Fig.5): Photomicrographs of PG in MTX treated rats (subgroup II a):

(**A**) section of PG stained with H & E show disturbed serous acini (S) and striated ducts (SD) with many vacuolations (V). Some acini showing darkly stained nuclei (arrows). Some acini appear apoptotic (arrow head) or even completely degenerated leaving empty spaces (stars). Congested dilated bl.vs (BV) are also seen. Striated duct showing epithelial stratifications (curved arrow).
(**B**) in sections stained with Mallory trichrome there is increased collagen deposits around ducts (dotted arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in few acini (dotted arrows) and ducts (arrows).
(**D**) there is strong Caspase 3 cytoplasmic immunoreactivity in most acini (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

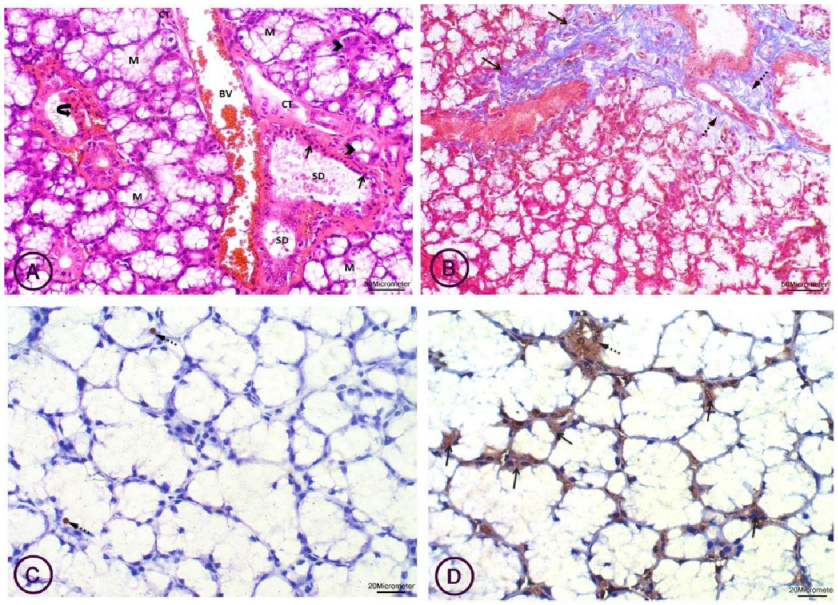

Fig. 6

(Fig.6): Photomicrographs of SLG in MTX treated rats (subgroup II a):

(**A**) section of SLG stained with H & E show thick connective tissue (CT) and dilated congested bl.vs (BV) distributed between mucous acini (M). Some acini and ducts are showing degeneration (arrows head). Striated ducts (SD) are appearing dilated with distorted epithelium and stagnant secretion. Duct epithelium is showing stratification (curved arrow) and some of duct nuclei are appearing pyknotic (arrows).
(**B**) in sections stained with Mallory trichrome there is thick collagen fibers in trabeculae (arrows) and around ducts (dotted arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in few acini (dotted arrows).
(**D**) there is strong Caspase 3 cytoplasmic immunoreactivity in many acini or around (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

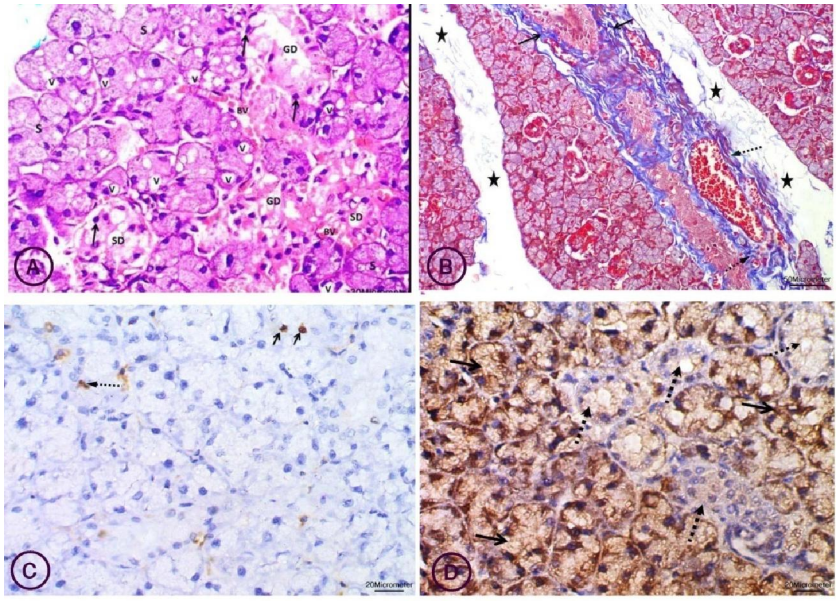

Fig. 7

(Fig. 7): Photomicrographs of SMG in MTX treated rats (subgroup II B):

(**A**) sections of SMG stained with H & E show clear vacuolations (V) in cytoplasm of most sreousacini (S). Striated ducts (SD) and granular ducts (GD) are appearing vacuolated, degenerated and lined with pyknotic nuclei (arrows). Congested blood vessels (BV) are also seen.

(**B**) in sections stained with Mallory trichrome there is increased collagen deposits around ducts (arrows) and bl.vs (dotted arrow) with wide trabeculae (stars).

(**C**) There is positive Ki 67 nuclear immunoreactivity in few acini (dotted arrows) and ducts (arrows).

(**D**) there is strong cytoplasmic Caspase 3 immunoreactivity in most acini (arrows) and mild reaction in ducts (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

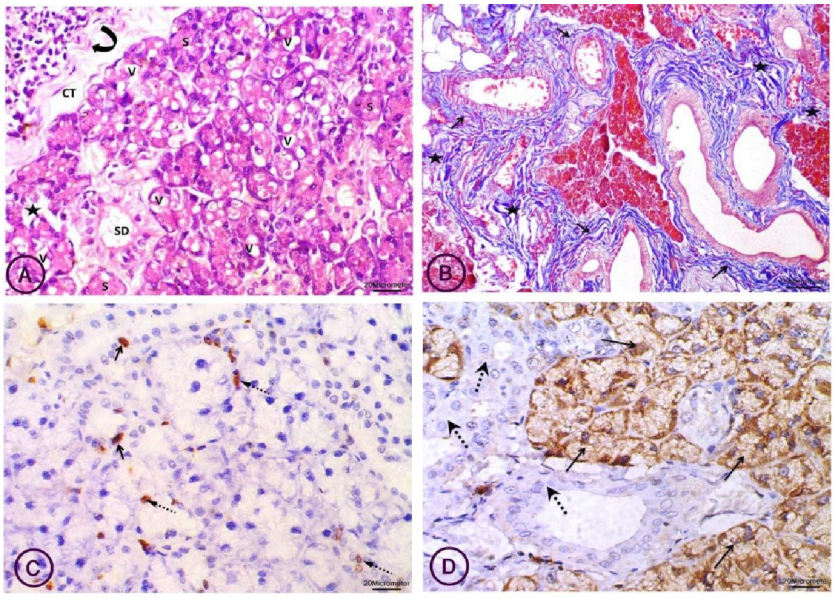

Fig. 8

(Fig.8): Photomicrographs of PG in MTX treated rats (subgroup II B):

(**A**) section of PG stained with H & E show disturbed serous acini (S) with prominent vacuolations (V). Completely degenerated acini leaving empty space (star). Striated ducts (SD) are also disturbed. Cellular infiltrations (curved arrow) and thick trabeculae (CT) are also noticed.

(**B**) in sections stained with Mallory trichrome there is significantly increased collagen fibers deposits in trabeculae (stars) and around ducts (arrows).

(**C**) There is positive Ki 67 nuclear immunoreactivity in some acini or around (dotted arrows) and ducts (arrows).

(**D**) there is very strong Caspase 3 cytoplasmic immunoreactivity in most acini (arrows) and mild reaction in some cells of ducts (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

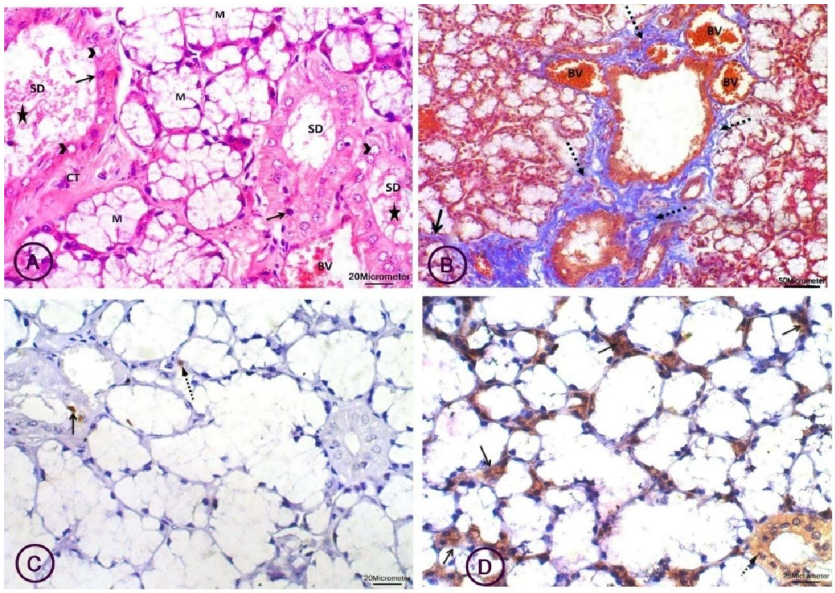

Fig. 9

(Fig.9): Photomicrographs of SLG in MTX treated rats (subgroup II B):

(**A**) section of SLG stained with H & E show mucous acini (M) and Striated ducts (SD) that showing irregularity and containing stagnant secretion (stars). Some duct nuclei appeared pyknotic (arrows) or karyolitic (arrow head). Thick CT is also seen around ducts (CT).
(**B**) in sections stained with Mallory trichrome there is increase in collagen fibers deposits in trabeculae (arrows) and around ducts (dotted arrows). Dilated congested bl.vs (BV).
(**C**) There is positive Ki 67 nuclear immunoreactivity in few acini or around (dotted arrows) and ducts (arrows).
(**D**) there is strong Caspase 3 cytoplasmic immunoreactivity in most acini and around (arrows) and moderate reaction in ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

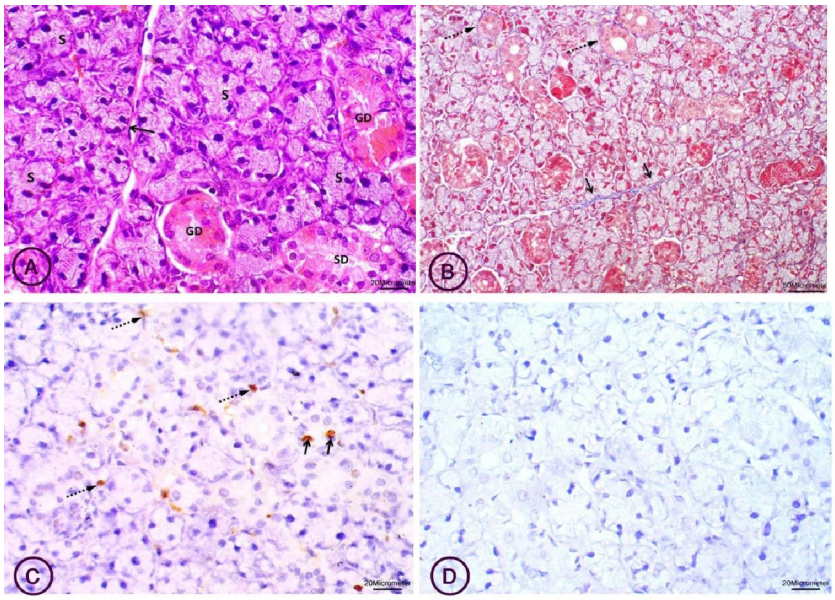

Fig. 10

(Fig. 10): Photomicrographs of SMG in GT treated rats (group III):

(**A**) sections of SMG stained with H & E show serous acini (S) with basal rounded nuclei. Many striated ducts (SD) and granular ducts (GD) are also seen. Fine CT fibers in trabeculae (arrows).
(**B**) in sections stained with Mallory trichrome thin collagen fibers in trabeculae between acini (arrows) and around ducts (dotted arrows).
(**C**) few cells appear with Ki 67 positive nuclear immunoreactivity in some acinar epithelial cells (dotted arrows) and ductal cells (arrows).
(**D**) there is negative cytoplasmic Caspase 3 reaction in acini and ducts.
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

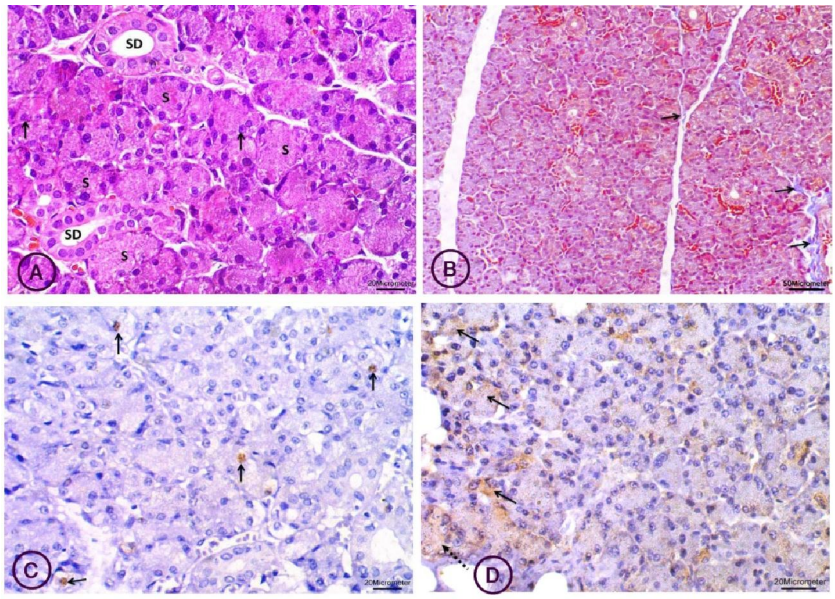

Fig. 11

(Fig. 11): Photomicrographs of PG in GT treated rats (group III):

(**A**): sections of PG stained with H & E show serous acini (S) with rounded vesicular nuclei (arrows). Many striated ducts (SD) are also seen.
(**B**): in sections stained with Mallory trichrome thin collagen fibers in trabeculae (arrows).
(**C**): there is positive nuclear Ki 67 nuclear immunoreactivity in few acini (arrows).
(**D**): mild Caspase 3 cytoplasmic immunoreactivity in few acini (arrows) & ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

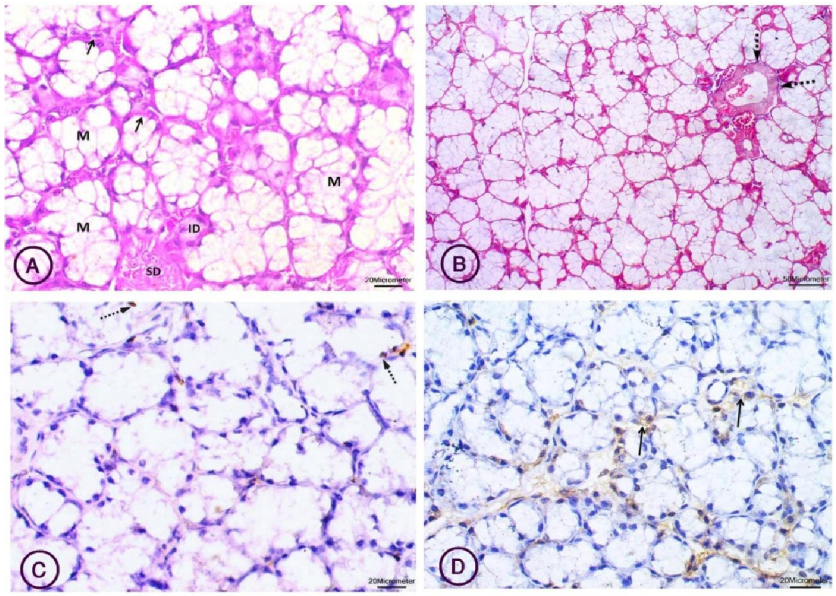

Fig. 12

(Fig. 12): Photomicrographs of SLG in GT treated rats (group III):

(**A**): sections of SLG stained with H & E show regularly arranged mucous acini (M), intercalated duct (ID) and striated ducts (SD). Many acini show serous demilune (arrows).
(**B**): in sections stained with Mallory trichrome very fine collagen fibers around duct (dotted arrows).
(**C**): there is positive Ki 67 nuclear immunoreactivity in few acini (dotted arrows).
(**D**): faint positive cytoplasmic Caspase3 cytoplasmic immunoreactivity in few acinar cells (arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

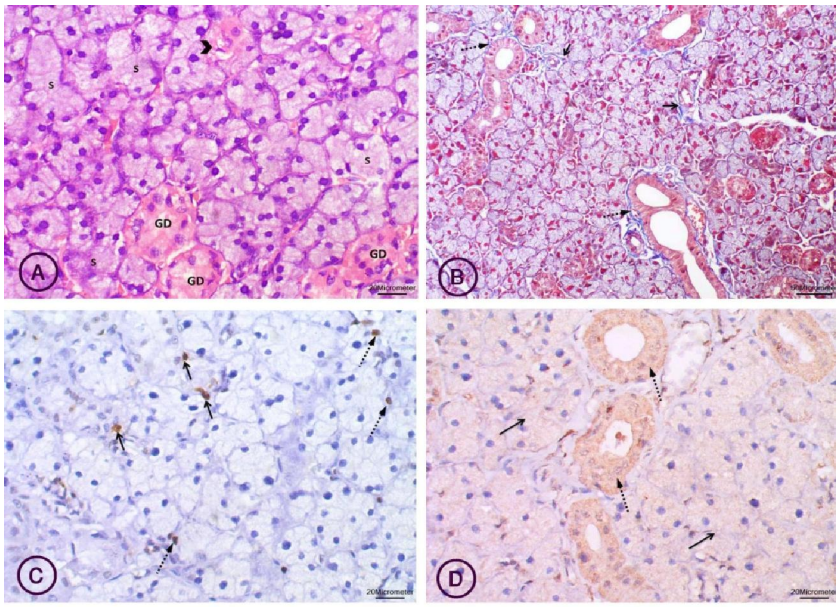

Fig. 13

(Fig. 13): Photomicrographs of SMG in prophylactic MTX & GT treated rats (subgroup V a):

(**A**) sections of SMG stained with H & E show regularly arranged serous acini (S), many granular ducts (GD). Few ducts are degenerating (arrow head).

(**B**) in sections stained with Mallory trichrome there is thin collagen fibers in septa separating acini (arrows) and around ducts (dotted arrows).

(**C**) There is positive Ki 67 nuclear immunoreactivity in some of acini (dotted arrows) and ducts (arrows).

(**D**) there is mild positive cytoplasmic Caspase 3 immunoreactivity in most acini (arrows) and moderate reactions in ductal cells (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

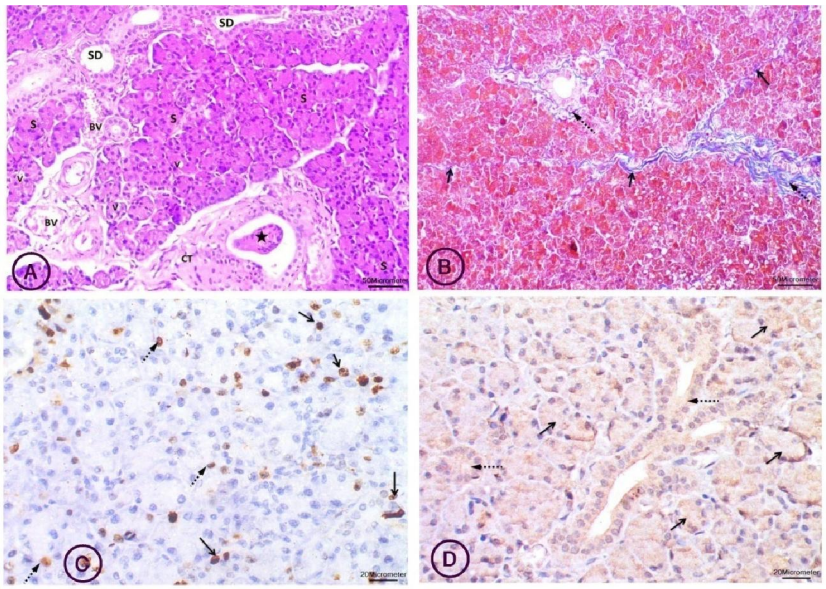

Fig. 14

(Fig. 14): Photomicrographs of PG in prophylactic MTX & GT treated rats (subgroup V a):

(**A**) section of PG stained with H & E show serous acini (S), many striated ducts (SD) dilated congested bl.vs (BV). Some acini are showing vaculations (V). Thick connective tissue around duct (CT) and some ducts containing stagnant secretion with exfoliated cells (star).

(**B**) in sections stained with Mallory trichrome there is thin collagen fibers in septa between acini (arrows) and slightly thickened collagen fibers around ducts.

(**C**) There is positive Ki 67 nuclear immunoreactivity in many acini (dotted arrows) and ducts (arrows).

(**D**) there is moderate Caspase 3 reaction in acini (arrows) and ducts (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

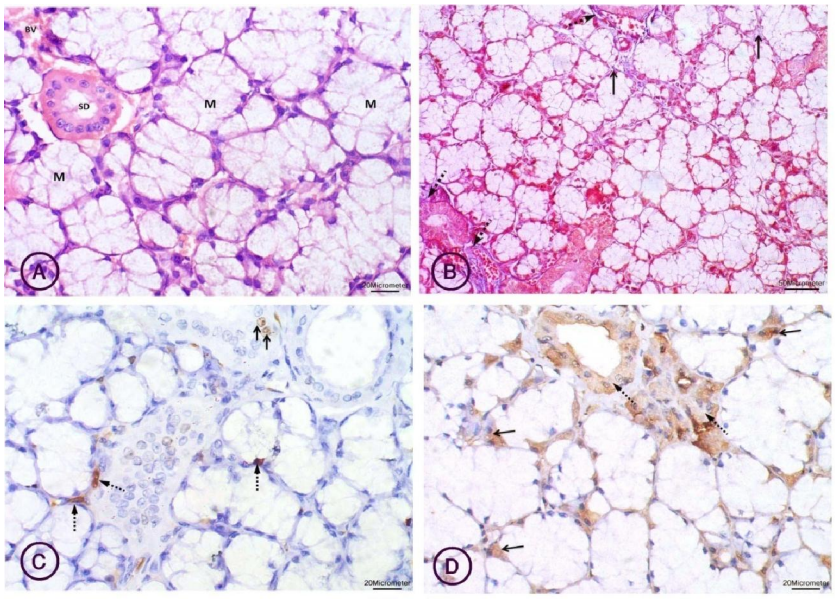

Fig. 15

(Fig. 15): Photomicrographs of SLG in prophylactic MTX & GT treated rats (subgroup V a):

(**A**) section of SLG stained with H & E show mucous acini (M), striated ducts (SD) and bl.vs slightly dilated (BV).
(**B**) in sections stained with Mallory trichrome there is thin collagen fibers in septa around acini (arrows) and around ducts (dotted arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in few acini and around (dotted arrows) and ducts (arrows).
(**D**) there is strong positive Caspase 3 cytoplasmic immunoreactivity in some acini (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

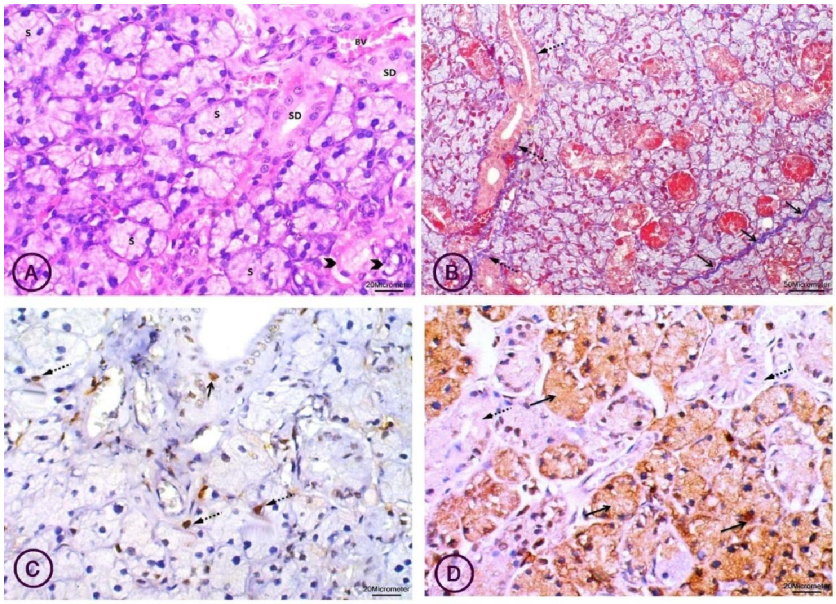

Fig. 16

(Fig. 16): Photomicrographs of SMG in therapeutic MTX & GT treated rats (subgroup V B):

(**A**) sections of SMG stained with H & E show regularly distributed serous acini (S) and many striated ducts (SD). Few acini and ducts showing degeneration (arrow heads). Blood vessels appear dilated & congested (BV).
(**B**) in sections stained with Mallory trichrome there is clear collagen fibers between acini (arrows) and around ducts (dotted arrows).
(**C**) There is positive Ki 67 nuclear immunoreaction in some acini (dotted arrows) and ducts (arrows).
(**D**) there is strong positive Caspase 3 cytoplasmic immunoreaction in many acini (arrows) and mild reactions in ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

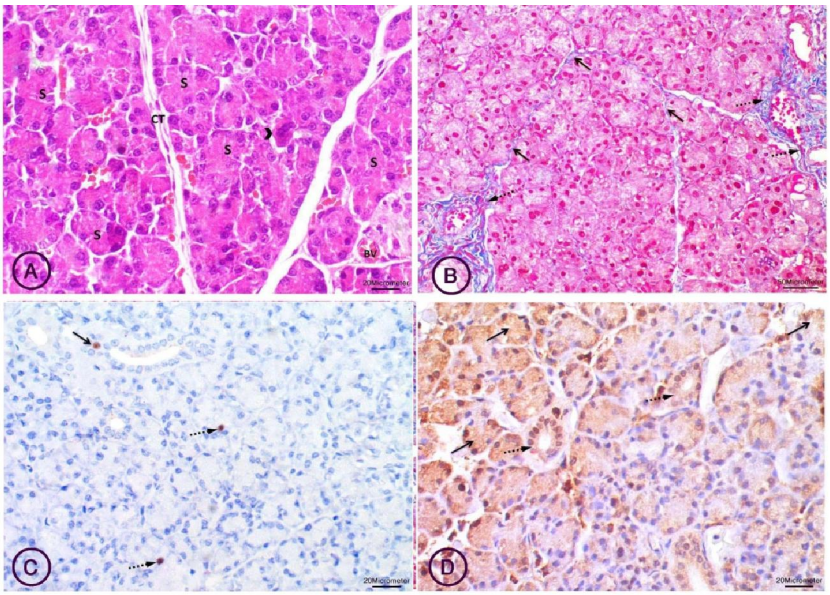

Fig. 17

(Fig. 17): Photomicrographs of PG in therapeutic MTX & GT treated rats (subgroup V B):

(**A**) section of PG stained with H & E show serous acini (S) with some disruption. Few acini appear degenerated (arrow head). Bl.vs appears slightly congested (BV) with clear connective tissue septa (CT).

(**B**) in sections stained with Mallory trichrome there is thin collagen fibers in septa between acini (arrows) and slightly thickened around ducts (dotted arrows).

(**C**) There is positive Ki 67 nuclear immunoreaction in few acini (dotted arrows) and ducts (arrow).

(**D**) there is moderate Caspase 3 cytoplasmic immunoreaction in some acini (arrows) and ducts (dotted arrow).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

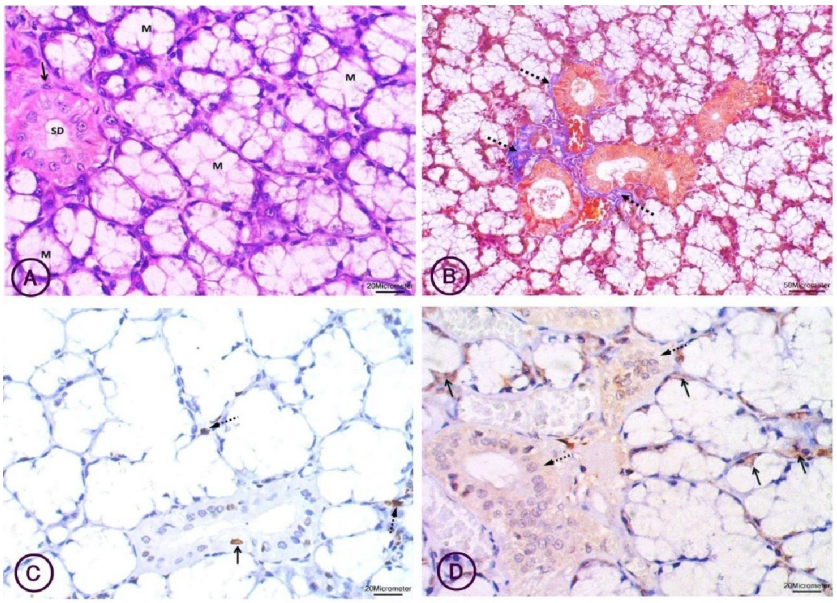

Fig. 18

(Fig. 18): Photomicrographs of SLG in therapeutic MTX & GT treated rats (subgroup V B):

(**A**) section of SLG stained with H & E show normally structured mucous acini (M) and striated ducts (SD). Myoepithelial cell (arrow) appears prominent around striated duct.

(**B**) in sections stained with Mallory trichrome there is slightly thickened collagen fibers around ducts (dotted arrows).

(**C**) There is positive Ki 67 positive nuclear immunoreaction in few acini (dotted arrows) and ducts (arrows).

(**D**) there is moderate Caspase 3 cytoplasmic immunoreactivity in many acini (arrows) and mild reaction in ducts (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

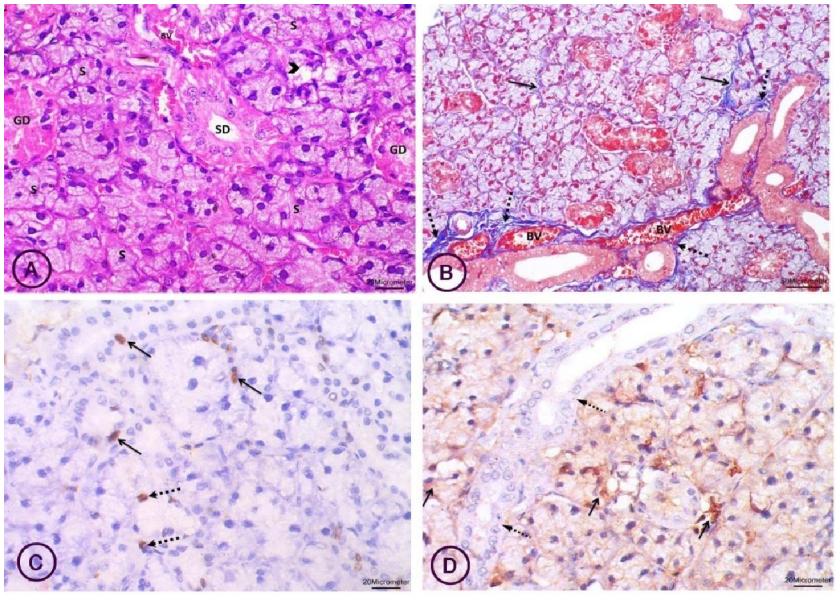

Fig. 19

(Fig. 19): Photomicrographs of SMG in prophylactic MTX & chamomile treated rats (subgroup VI a):

(**A**) sections of SMG stained with H & E show serous acini (S), few of them appearing degenerated (arrow head). Striated ducts (SD) and granular ducts (GD) appear normal while bl.vs (BV) showing minimal congestion.

(**B**) in sections stained with Mallory trichrome there is thin trabecular collagen fibers (arrows) and slightly thickened fibers around ducts (dotted arrows) with dilated congested bl.vs (BV).

(**C**) There is positive Ki 67 nuclear reaction in many acini (dotted arrows) and ducts (arrows).

(**D**) there is moderate to strong positive cytoplasmic Caspase 3 immunoreactivity in acinar reaction (arrows) and negative ductal reaction (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

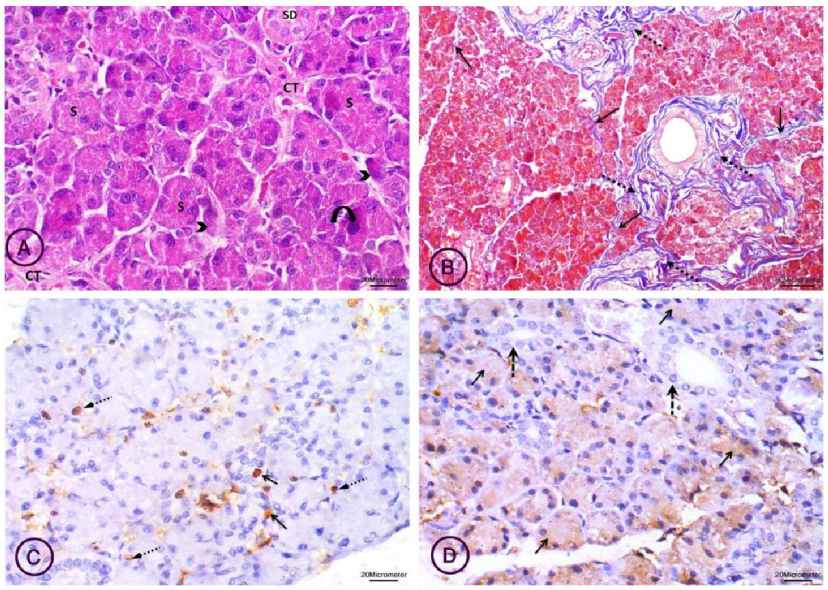

Fig. 20

(Fig. 20): Photomicrographs of PG in prophylactic MTX & chamomile treated rats (subgroup VI a):

(**A**) section of PG stained with H & E show serous acini (S) and striated ducts (SD) separated with trabeculae (CT). Few acini showing degeneration (arrow head) with appearance of apoptotic bodies (curved arrow).

(**B**) in sections stained with Mallory trichrome there is clear collagen fibers in septa between acini (arrows) and thickened fibers around ducts (dotted arrows).

(**C**) There is positive Ki 67 nuclear immunoreactivity in many acini (dotted arrows) and ducts (arrows).

(**D**) there is moderate Caspase 3 reaction in many acini (arrows) and mild reaction in ducts (dotted arrows).

[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

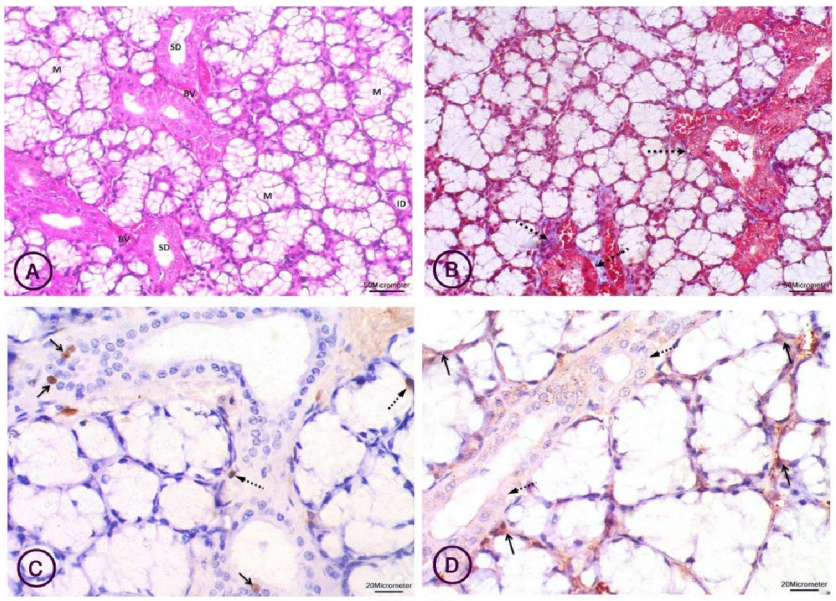

Fig. 21

(Fig. 21): Photomicrographs of SLG in prophylactic MTX & chamomile treated rats (subgroup VI a):
(**A**) section of SLG stained with H & E show regular mucous acini (M) separated with intercalated duct (ID), striated ducts (SD) and bl.vs which slightly congested (BV).
(**B**) in sections stained with Mallory trichrome there is thin collagen fibers around ducts (arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in few acinar (dotted arrows) and some ductal cells (arrows).
(**D**) there is strong positive cytoplasmic Caspase 3 reaction in many acinar cells (arrows) and mild reaction in ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

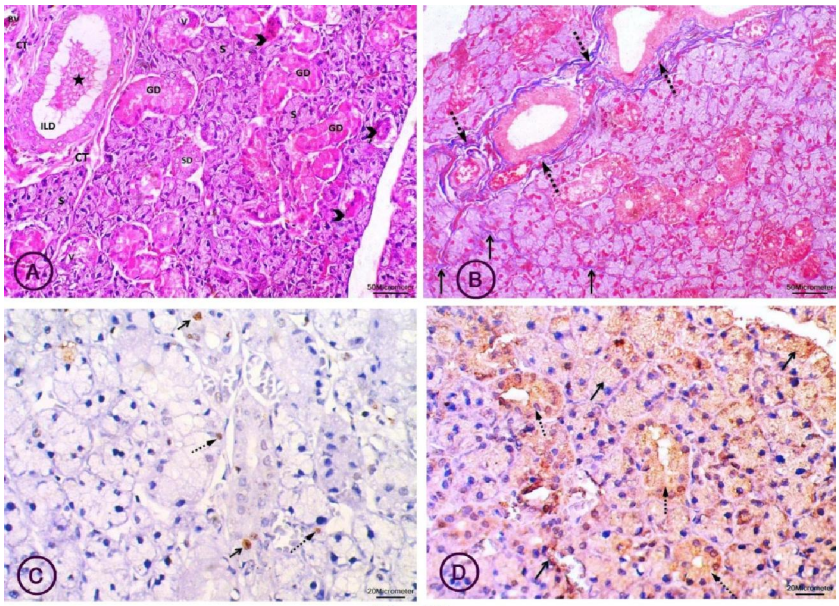

Fig. 22

(Fig. 22): Photomicrographs of SMG in prophylactic MTX & chamomile treated rats (subgroup VI B):
(**A**) sections of SMG stained with H & E show serous acini (S) with some irregularities. Acini separated with striated ducts (SD), many granular ducts (GD), congested bl.vs (BV) and connective tissue (CT). Some degenerated ducts (arrows head) are also shown. Vacuolations (V) are also seen in few cells. Interlobar duct (ILD) showing dilatation and stagnant secretion (star).
(**B**) in sections stained with Mallory trichrome there is fine collagen fibers in septa (arrows) and slightly thickened around ducts (dotted arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in acini (dotted arrows) and ductal cells (arrows).
(**D**) there is moderate cytoplasmic Caspase 3 immunoreactivity in many acini (arrows) and strong reaction in ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

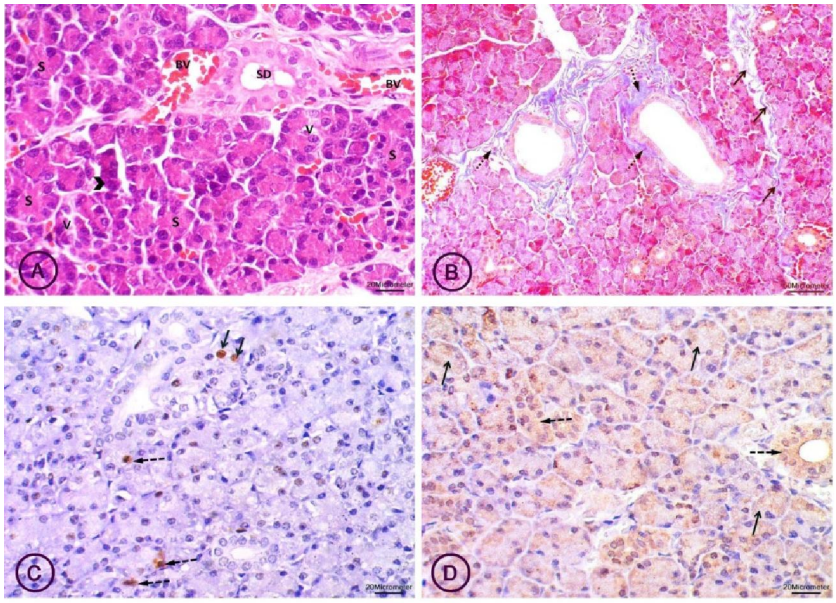

Fig. 23

(Fig. 23): Photomicrographs of PG in prophylactic MTX & chamomile treated rats (subgroup VI B):
(**A**) sections of PG stained with H & E show serous acini (S), dilated congested bl.vs (BV) around striated duct (SD). Some acini showing vacuolations (V) and few of them are degenerated (arrow head).
(**B**) in sections stained with Mallory trichrome there is clear septa (arrows) and slightly thickened collagen fibers around ducts (dotted arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in some acini (dotted arrows) and ducts (arrows).
(**D**) there is moderate cytoplasmic Caspase 3 immunoreactivity in many acini (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

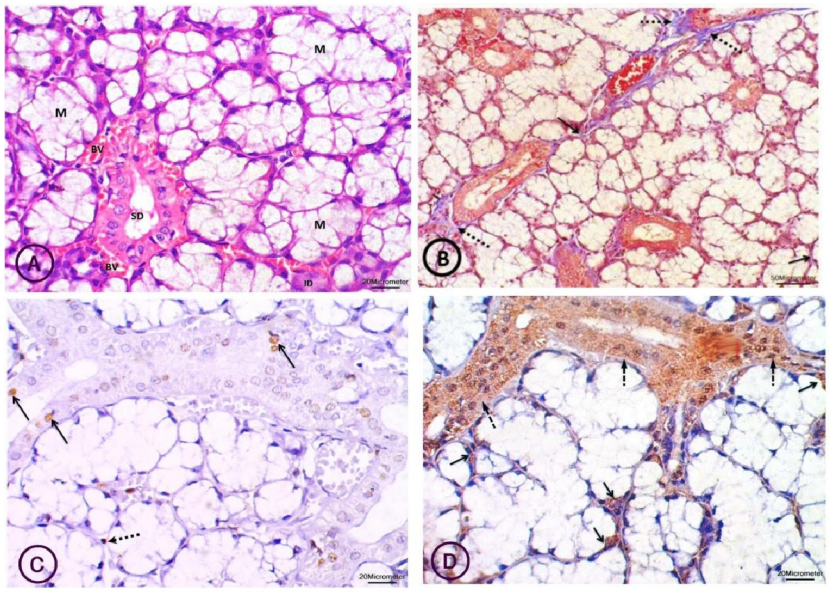

Fig.24

(Fig. 24): Photomicrographs of SLG in prophylactic MTX & chamomile treated rats (subgroup VI B):
(**A**) sections of SLG stained with H & E show mucous acini (M) separated with many striated ducts (SD), intercalated duct (ID) and slightly congested bl.vs (BV).
(**B**) in sections stained with Mallory trichrome there is thin collagen fibers around ducts (dotted arrows) and between acini (arrows).
(**C**) There is positive Ki 67 nuclear immunoreactivity in immunostaining reaction in few acinar (dotted arrows) and some ductal cells (arrows).
(**D**) there is strong cytoplasmic Caspase 3 immunoreactivity in positive cytoplasmic reaction in many acini (arrows) and ducts (dotted arrows).
[A: H & E x400; B: Mallory x200; C: Ki-67 x400; D: Caspase x400]

4. Discussion

Saliva and salivary glands are believed to be the massive relationship between oral and general systemic health ***(Elham & Mahmoud, 2017).*** Major salivary glands are paired glands. Approximately 90% of total saliva is produced by the parotid and submandibular glands while sublingual and minor salivary glands together produce only 10% of the saliva volume ***(Ho et al., 2011)***.

MTX is used for a wide range of neoplastic disorders ***(Jensen et al., 2008).*** And many other indications as autoimmune diseases ***(Dadhania et al., 2010)***. Its use is restricted by the severe side effects, so finding ways to diminish its side effects is an important scientific challenge. ***(Widdifield et al., 2018*** & ***Yu et al., 2018).***

MTX induced toxicity based on systemic oxidative stress. Therefore, antioxidant anti-inflammatory properties of green tea and chamomile may protect against these oxidative stress ***(Braicu et al., 2013)***.

This study was designed to evaluate the possible prophylactic and therapeutic effects of chamomile versus green tea on a methotrexate induced injury on the major salivary glands.

In the current study, MTX induced toxic effects in the form of acinar and ductal cell vacuolization and apoptosis in the acinar cells with pyknosis in the nuclei were in agreement with what was shown by ***Ali et al., (2014)***. this cellular vacuolization and complete replacement of some acinar cells by large vacuoles, could be explained by the accumulation of lipid droplets to be unutilized fatty acids resulted from cellular dysfunction as reported by ***El-Agamy et al., (2014)***.

These vacuolations were also referred by ***Leitão et al., (2011)*** to the presence of large residual bodies (secondary lysosomes) containing partially degraded fragments of damaged epithelial cells and mitochondrial swelling identified in both acini and ducts.

The observed marked dilatation and congestion of the blood vessels appeared in MTX group were reported by ***Zahawi, (2015)*** in SMG of rabbits after chemotherapy such dilatation and congestion might be due to inflammatory reaction associated with MTX treatment that increase transendothelial permeability as reported by ***Garipardic et al., (2010)*** *and* ***Abeer et al., (2017)***.

Dilatation of ducts with stagnant secretion was an obvious finding in MTX group. This dilatation of ducts suggested the pathological effect of MTX on myoepithelial cells embracing them with failure of expelling the secretion into the oral cavity as a result of glandular dysfunction as reported by ***Moheb et al., (2014***).

One of the characteristic finding of MTX treated group **(IIa & IIb subgroups)** in SMG in the present work was the presence apoptotic bodies that was previously reported by ***Ali et al., (2014)***.

As regards chemotherapy increased cell apoptosis***, Al-Moula et al., (2012)*** approved that the apoptotic bodies greatly increased in tissues which have been subjected to chemotherapy while they are found in small numbers in normal tissues.

After 2 weeks of MTX injection, areas of cellular infiltrations in all glands were observed. This was in agreement with ***Abeer et al., (2017)*** who found mononuclear infiltrations in parotid gland after MTX treatment.

It could be suggested that cellular infiltrations in the present study due to the effect of oxidative stress as ROS damage the connective tissue as well as DNA and cell membrane leads to stimulation of macrophages, neutrophil infiltration and proinflammatory cytokine release. Leading to increased sub-epithelial vascularity as reported by **Sonis et al., (2007) and Cure et al., (2015).**

Sections of **group II** (MTX group) in all glands showed increased collagen deposition. Fibrosis was more obvious after 2weeks of MTX administration**.**

Which could be explained by oxidative stress and inflammation as reported by ***Braicu et al., (2013***) who stated that oxidative stress could release inflammatory cytokines, differentiation of cells to myofibroblasts and deposition of extracellular matrix components.

On the other hand, ***Al-Refai et al., (2014***) referred the increase in collagen following MTX administration to acinar and ductal cell deaths followed by connective tissue replacement.

As regarding the exaggerated effect of a single dose of MTX injection, in the current study, pathological findings became more obvious in MTX group **(subgroup IIb)** after 2 weeks of MTX injection compared to **subgroup IIa**.

Another explanation by ***Vardi et al., (2013)*** who reported that MTX makes cells more vulnerable to ROS by reducing the production of nicotinamide adenine dinucleotide phosphate (NADPH) which is important in the antioxidant defense system. They added that MTX had toxic effects on epithelial cells evidenced by increased number of apoptotic cells.

In **MTX group,** both **subgroups II (a & b),** all glands showed positive Ki-67 (proliferating marker) immunoreaction in few acinar and ductal cells. This was statistically non-significant in subgroup IIa (after one week) compared to control group indicating the low rate of cellular proliferation while subgroup II b in PG showed statistically significant increase compared to control group. This is in agreement with the results of ***Al-Refai et al., (2014***) who proved that methotrexate treated SMG showed mild positive Ki-67

immunoreactions in the nuclei of acinar and ductal cells.

In accordance, ***Nishimura et al., (2010)*** reported decreased Ki-67 value following MTX administration as it restricted the proliferative ability of the epithelium and this may explain the salivary impairment with decreasing the whole salivary flow rate.

As regards Caspase-3 (apoptotic marker) immunoreactivity in **MTX group,** both **subgroups**, showed positive cytoplasmic Caspase-3 immunoreaction in acinar and ductal cells in all glands. In **subgroup IIb,** there was statistically significant increase compared to **subgroup IIa.** This could be explained by disruption of protein synthesis through depletion of foliate co-factors and cytolysosome formation; an evidence of apoptosis process ***(Al-Moula et al., 2012***).

Histological examination of major salivary glands in rats administered green tea (GT) showed a picture more or less similar to that of the control with fine collagen fibers. Regarding Ki-67 and anti-Caspase 3 area % immunohistochemical staining were statistically non-significant compared to control group in all glands.

The results of this study confirmed by ***Hininger et al., (2009)*,** who proved that consumption of green tea has many beneficial effect on human health, particularly polyphenols (catechins) and their derivatives that retard various forms of cancers due to its antioxidant, antimutagenic and anticarcinogenic properties.

In the current study sections of salivary glands in chamomile treated group showed a picture more or less similar to that of the control ***Srivastava & Gupta, (2007)*** reported that chamomile extracts were shown to cause less growth inhibitory effects on normal cells and induce apoptosis in cancer cells but not in normal cells at similar doses.

The present study demonstrated the prophylactic and therapeutic effect of green tea on MTX induced toxicity in major salivary glands. This was evidenced by histological, immunohistochemical, morphometric and statistical results.

As examination of the SMG & PG & SLG of **group V**, mixed MTX and GT group, **prophylactic (Vasub group)** showed improvement compared to MTX group. However, few acinar and ductal cells were still affected. Also, in **therapeutic group (subgroup Vb),** all glands showed significant improvement compared to MTX group.

This was in agreement with ***Hafez, (2006)*** and ***Al-Refai et al., (2014)*** who proved that the combined treatment of MTX and green tea extract ameliorated the histological changes in salivary gland tissue induced by MTX alone.

In the current study, the protective and therapeutic effect of GT referred to its anti-oxidant property as reported by ***Namita et al., (2012)*** who approved that EGCG is the most active and abundant polyphenol in green tea which has potent antioxidant properties by attenuation of oxidative stress, scavenging ROS and increasing the level of glutathione in several studies in animal and cell culture models.

In addition, ***Panat et al., (2016)*** stated that GT phenolic constituents have the ability to suppress the chain reactions of lipid peroxidation and to reduce proinflammatory nitric oxide-generated mediators.

On the other hand, ***Thakur et al., (2012*)** proved that green tea has anti angiogeneic property. They noticed that the connective tissue showed significant decrease in the number of dilated and congested blood vessels compared to the MTX treated group resulting in reduction in the blood flow to the oral mucosa and decreasing the availability of chemotherapeutic agents to the oral mucosa.

Collagen fibers were apparently decreased in both **subgroup Va** (prophylactic GT & MTX**)** and **subgroup Vb** (therapeutic GT & MTX**)** in comparison with MTX group. These findings were proved morphometrically. In all glands, collagen area % was statistically highly significant decrease compared to subgroup IIb (2weeks after MTX) while this was statistically non-significant compared to subgroup IIa (1week after MTX) except in PG.

Zhen et al., (2007) referred the antifibrotic effect of green tea to EGCG which is decreasing the synthesis of type I collagen and reducing the oxidative stress.

In addition, the antifibrotic effect of GT related to its anti-oxidant and anti**-**inflammatory effectasits catechins effectively eliminate free radicals and decrease the production of inflammatory cytokines as IL-6 and TNF-α by inhibiting the action of transcription factors as reported by ***Monika et al., (2017)*** and ***Jia et al., (2018).***

Immunohistochemical results showed increase in the number of positive Ki-67 (proliferating marker) immunostained nuclei in **subgroup Va** (prophylactic GT & MTX). This was statistically significant increase in SMG compared to **subgroup IIb** (2 weeks after MTX) and **Vb** (therapeutic GT & MTX). In PG, the increase was statistically significant compared control group and IIa subgroup (1week after MTX).

This was in accordance with ***Al-Refai et al., (2014)*** who found that the epithelium restores its integrity and revealed marked improvement with a non-significant increase in the index of Ki-67 in SMG of MTX and green tea treated group. They suggested that both the undamaged parenchymal and ductal cells attempts for regeneration and proliferation.

There generative effect of GT could be due to its flavonoids and polyphenols which had ability to stabilize and preserve the integrity of the cell membrane, stimulating cellular regeneration and protein synthesis to repair damaged tissues (***Pezeshki et al., 2016).***

On the other hand, **subgroup Vb** (therapeutic GT & MTX)**,** in the present study showed few proliferating cells in all glands which were statistically non-significant compared to control. While in PG & SMG there was statistically significant decrease compared to **subgroup Va** (prophylactic GT & MTX).

In the present study, the most proliferating cells were observed to be in duct cells and this was confirmed by the work of ***Kishi et al., (2006)*** who showed that experiments of ductal obstruction in rat salivary glands demonstrated that regeneration of salivary glands originates from putative multipotent salivary gland stem cells (SGSCs) residing in the ductal compartment. They possess self-renewal capacity, high proliferation and multipotent differentiation activity and seem to exist in salivary glands even after irradiation.

Furthermore, study on salivary gland development by ***Kagami et al., (2008)*** suggest that cells in the ducts close to acini seem to provide all cell types necessary for formation of ductal structures and acini. Accordingly, SGSC are considered to be present in the intercalated ducts.

As regards Caspase-3 immunoreactivity in **group V,** mixed GT & MTX, **prophylactic (subgroup Va)** showed statistically significant decrease compared to subgroups IIa & IIb (MTX group) in all glands**.** In **subgroup Va (prophylactic),** all glands showed statistically significant increase in area % of Caspase-3 compared to control groups.

On the other hand, **subgroup Vb** (therapeutic GT & MTX group) showed statistically significant decrease anti-Caspase 3 area % compared to both subgroup II a & b (1 & 2 weeks after MTX) in SMG. While in PG & SLG, there was statistically significant decrease compared to subgroup IIb (2weeks after MTX) only. Apoptosis marker showed statistically significant increase compared to control groups in all glands. In SMG & PG of **subgroup Vb**, there was statistically significant decrease compared to **subgroup Va**.

Increase in the antiapoptotic activity of green tea in the glands in relation to the methotrexate group was in accordance with ***Al-Refai et al., (2014)*** and ***Sawyer & Ratain (2001)***. This antiapoptotic effect of GT could be suggested by improvement of the antioxidant capacity and reduction in the release of apoptotic relating proteins (***Al-Basher, 2017)***

In the current work, when we used chamomile as a prophylactic with MTX in **subgroup VIa,** sections of all glands showed obvious improvement with minimal bl.vs congestion, few degenerated acini and few apoptotic bodies compared to MTX group. Also, in **subgroup VIb** (therapeutic chamomile & MTX group)**,** all glands showed improvement with minimal cytoplasmic vacuolations and bl.vs congestion compared to MTX group. This improvement could be attributed to reducing methotrexate-induced oral mucositis and accelerating oral ulcers healing as shown by ***Mazokopakis et al., (2005)*** and ***Martins et al., (2009)***.

In the current study, the improvement that occurred with chamomile extract administration could be due to its antioxidant effect (***Shukla & Gupta, 2010)***. The other suggestion is the role of chamomile and its constitutes in reducing IL-6 and TNF-α production that in turn would suppress the inflammatory activity (***Drummond et al., 2013***).

It could be suggested that antifibrotic effect of chamomile related to its antioxidant and cytoprotective properties (***Bhaskaran et al., 2012)***

In addition, the protective effect of chamomile could be attributed to apigenin which is a flavonoid that causing reduction of the pro-fibrosis (collagen 1A1 and fibronectin) and pro-inflammation (IL-6 and IL- 8) ***(Mrazek et al., 2015*)**. Apigenin also attenuate inflammation through inhibition of cyclooxygenase-2 and 5-lipoxygenase leading to inhibition of prostaglandin release. Moreover, apigenin augments apoptosis of recurrently activated human T cells and inhibit cytokine-induced adhesion protein expression in endothelial cells ***(Lampropoulos et al., 2013)***.

Regarding the ability of chamomile for regeneration proved by Ki-67 immunohistochemical staining, chamomile has the ability to stimulate epithelial regeneration ***(Kyokong et al., 2002)*** and it attenuates oxidative-induced cell damage in cells and improve differentiation and regeneration of the cells (***Woon-Won, 2014).***

In the present work, the proliferation in parotid gland was more than the other salivary glands. This was in accordance with ***Takahashi et al., (2001)*** who proved the high percentage of myoepithelial cell proliferation in parotid gland during atrophy. They referred that to the number of MECs as the MECs of the rat PG are less than those of SMG. Thus, they need to proliferate more than those of submandibular gland.

Shah et al., (2016) added that MECs associated with the intercalated ducts of the parotid gland are greater in number and longer than those of the submandibular gland.

The present work showed proliferation mainly in duct cells and cells outside acini in extracellular matrix (ECM). This was in accordance with result of ***Carpenter et al., (2009)*** who proved that there is Stem/ progenitor cells in salivary glands which reside

in the intercalated ducts (ID) and played a role in regeneration of the gland after injury.

Regarding the anti-apoptotic activity (as shown by decrease in Caspase-3 immunoexpression); chamomile extracts were shown to cause minimal growth inhibitory effects on normal cells and induced apoptosis in cancer cells but not in normal cells at similar doses ***(Srivastava & Gupta, 2007)***.

This was in disagreement with ***Al-Refai, (2014)*** who found that when chamomile extract was taken for seven days, it can cause cytotoxic and damaging effect to the jejunum and increase apoptosis. These observations were not seen in the current study as we used chamomile for 15 days without any toxic effect.

5. Conclusion

Methotrexate (MTX), chemotherapeutic drug, has major cytotoxic effects on salivary glands. Green tea has a protective effect against these cytotoxic effects and its use for prophylaxis (before MTX injection) is more effective than its use as a therapeutic (after MTX injection).

Chamomile also proved to have a protective effect against these cytotoxic effects as it decreases fibrosis and apoptosis with increase in proliferation and regeneration of cells mainly in the parotid gland with nearly the same effects when used either as a prophylactic or a therapeutic and its use as a therapeutic may be the same or slightly more effective than green tea.

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