PROPHYLACTIC ROLE OF GLUTATHIONE AND GRANULOCYTE - COLONY STIMULATING FACTOR ON 5-FLUOROURACIL INDUCED MYELOTOXICITY IN MICE

BY

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ABSTRACT

The protective effect of glutathione (GSH) and granulocyte colony stimulating factor (G-CSF) on myelosuppression induced by 5-fluorouracil (5-FU) were compared in female mice. The animals were divided into seven groups. Group 1 (10 mice) received no treatment, group 2 (10 mice) received GSH (800 mg/kg) by intraperitoneal route in daily doses for the first 7 days then left untreated for another 7 days, and group 3 (10 mice) received G-CSF (250 µg/kg) by subcutaneous route in daily doses for the first 7 days then left untreated for another 7 days. Animals in groups 1, 2 and 3 were sacrificed on day 15. Group 4 (20 mice) received a single dose of 5-FU (160 mg/kg) by intraperitoneal route in the 8th day, Group 5 (20 mice) received GSH in daily doses for the first 7 days followed by a single dose of 5-FU in the 8th day, Group 6 (20 mice) received G-CSF in daily doses for the first 7 days followed by a single dose of 5-FU in the 8th day and group 7 of animals (20 mice) received daily doses of both GSH and G-CSF for the first 7 days followed by a single dose of 5-FU in the 8th day. Animals in groups 4, 5, 6 and 7 were divided into 2 subgroups; subgroup a : 10 mice were sacrificed on day 9 i.e. 1 day after 5-FU administration and subgroup b : 10 mice were sacrificed on day 15 i.e. 1 week after 5-FU administration. Mice were sacrificed by cut throat and blood samples were obtained for determination of haematological values; haemoglobin (Hb), haematocrite value (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), as well as total (TLC) and differential (DLC) leucocyte count. Then dissection of mice was done where the right femurs were used for bone marrow cytology, while left femurs were used for bone marrow histopathology. In groups 5a given GSH pretreatment and 6a given G-CSF pretreatment (where mice killed 1 day after 5-FU dose) protection was demonstrated regarding neutropenia, bone marrow cytology and histopathology. Incomplete protection was revealed in mice received GSH pretreatment or G-CSF pretreatment and killed 1 week after 5-FU dose (groups 5b and 6b respectively), while groups 7a and 7b given combined GSH and G-CSF pretreatment revealed no protection .

INTRODUCTION

There are two major types of systemic cancer therapy : chemotherapy and biologic therapy. Cancer chemotherapeutic agents are usually discussed in groups that reflect either the origin of the drug or their predominant mechanism of action. The major classes of agents include the alkylating agents, antitumor antibiotics, plant alkaloids, antimetabolites, hormonal agonists and antagonists (Haskell, 1995).

Antimetabolites are antineoplastic agents that are structurally and chemically similar to naturally occurring compounds required for synthesis of purines, pyrimidines, and nucleic acids. Antimetabolites must be present at cytotoxic concentrations during DNA synthesis to be effective and thus tend to be cell cycle S-phase specific drugs (Richard,1992).

5-fluorouracil (5-FU), is an antimetabolites, anticancer agent which is used as an essential part for the treatment of wide range of solid tumors. It has antitumor activity against epithelial malignancies arising in the gastrointestinal tract , breast as well as the head and neck (Malet-Martino and Martino, 2002). As all the anticancer agents, 5-FU leads to several toxicities. Myelotoxicity is the major toxic effect in patients receiving bolus doses (Takimoto and Page, 2004).

The haemopoietic growth factor (granulocyte-colony stimulating factor G-CSF) was proved to shorten the duration and decrease the severity of chemotherapy induced neutropenia (Liang, 2003).

Lamson and Brignall, (2000) demonstrated the cytoprotective effect of glutathione (GSH) against chemotherapeutic agents. Kojima et al.(2003) suggested that glutathione could prevent the 5-FU-induced haemopoietic toxicities and accelerate recovery from such toxicities.

Cascinu et al.(1997) have used a combination of both G-CSF and glutathione with intensive regimen of 5-FU, cisplatin and epi-doxorubicin in advanced gastric cancer, and they confirmed high activity of the regimen, with acceptable toxicity.

The aim of the present study is to compare the protective effect of glutathione, granulocyte- colony stimulating factor or both on 5-fluorouracil induced myelotoxicity in mice.

MATERIAL AND METHODS

Drugs :
5-fluorouracil (5-FU) 5ml ampoules each contains 250mg 5-FU (Biosyn Arzneimittel GmbH Fellbach, Germany). 5-FU was diluted with distilled water to give a final concentration of 10 mg/ml. It was given in a dose 160 mg/kg by intraperito-

neal route (Friberg et al.,2000).

L-Glutathione reduced (GSH) powder, 5gm/bottle (SIGMA-ALDRICH,Inc) was obtained from Egyptian International Center Importer Cairo, Egypt. It was stored in the refrigerator. GSH was dissolved in distilled water to give a final concentration of 80 mg/ml. It was given in a dose 800 mg/kg by intraperitoneal route (Kojima et al., 2003).

Filgrastim Neupogen (Granulocyte-Colony Stimulating Factor "G-CSF") 0.5 ml prefilled syringe of 300µg/ml concentration (F.Hoffmann La Roche Ltd, Basel, Switzerland). It was stored in the refrigerator. G-CSF was diluted with distilled water to give a final concentration of 10 µg/ml. It was given in a dose 250 µg/kg by subcutaneous route (Lord et al., 2001).

Animals:

The study was carried out on 110 adult female mice with their weight ranged 27-32 gm obtained from the Animal House of Mansoura Faculty of Pharmacy. Mice were chosen all females to alleviate the gender effect on the results (Doeing et al ., 2003). The mice were housed in metallic cages , fed a standard diet and allowed unlimited access to food and water under standard laboratory conditions. After a week of acclimatization to the housing conditions the mice were divided into seven groups housed in separate cages. The

duration of the study was 15 days during which mortality among mice was recorded.

Group 1 of animals (10 mice) received no treatment and served as control group , group 2 (10 mice) received GSH (800 mg/kg) by intraperitoneal route in daily doses for the first 7 days then left untreated for another 7 days, and group 3 (10 mice) received G-CSF (250 µg/kg) by subcutaneous route in daily doses for the first 7 days then left untreated for another 7 days. Animals in groups 1 , 2 and 3 were sacrificed on day 15. Group 4 (20 mice) received a single dose of 5-FU (160 mg/kg) by intraperitoneal route in the 8th day , Group 5 (20 mice) received GSH in daily doses (800 mg/kg by intraperitoneal route) for the first 7 days followed by a single dose of 5-FU (160 mg/kg by intraperitoneal route) in the 8th day, Group 6 (20 mice) received G-CSF in daily doses (250 µg/kg by subcutaneous route) for the first 7 days followed by a single dose of 5-FU (160 mg/kg by intraperitoneal route) in the 8th day and group 7 of animals (20 mice) received daily doses of both GSH (800 mg/kg by intraperitoneal route) and G-CSF (250 µg/kg by subcutaneous route) for the first 7 days followed by a single dose of 5-FU (160 mg/kg by intraperitoneal route) in the 8th day. Animals in groups 4 , 5 , 6 and 7 were divided into 2 subgroups ; subgroup a (10 mice) in which animals were sacrificed on day 9 i.e. 1 day

after 5-FU administration and subgroup b (10 mice) in which animals were sacrificed on day 15 i.e. 1 week after 5-FU administration. Mice were sacrificed by the cut throat method.

Samples collection:

Blood samples (2ml, each) were collected in EDTA tubes from the mice cut throats after sacrification. The samples were obtained from animals of subgroups 4a,5a,6a and 7a on day 9 from start of study. The rest of the samples were obtained from animals of groups 1,2,3 and subgroups 4b,5b,6b and 7b on day 15 from the start of study.

The following haematological values were determined ; haemoglobin (Hb) g/dl, mean corpuscular haemoglobin concentration (MCHC) g/dl , mean corpuscular volume (MCV) Fl (femoliter) , mean corpuscular haemoglobin (MCH) Pcg (pictogram) measured according to the method of Riedinger and Rodak (1998), haematocrite value (Hct) % measured by microhaematocrit method (Dill and Costill,1974) as well as total (TLC) and differential (DLC) leucocyte count measured according to the method of England and Bain (1976) .

After blood sampling, animals were dissected to obtain both femoral bones. The right one was used for bone marrow cytology according to the method of Koji-

ma et al., (2003) , while the left one was used for histopathology according to the method of Anderson and Bancroft, (2002).

Statistical analysis:

Statistical analysis of data was done by using Excel program (Office 2000) and SPSS (Statistical Package of Social Science), Windows 98, computer compatible IBM. The first part of data analysis was descriptive. The mean ± S.D. were used for description of frequency.

The second part of the analysis was analytic: For quantitative data. Student's t test was used for comparison between two groups. Anova t test was used for comparing more than two groups followed by Post-Hoc test for testing the significant different one. For qualitative data Kruskal-Wallis test was used for comparing more than two groups. P value was considered significant if < 0.05.

RESULTS

I) Haematological parameters and bone marrow cytology: Table (1)

As regards TLC and bone marrow cytology it was found that groups 3 (received G-CSF), and 6a (received G-CSF before 5-FU and killed 1 day later) have higher (i.e. better) values than the control, while other groups have lower values (i.e. worse). TLC was higher also in group 5a

(received GSH before 5-FU and killed 1 day later) than the control. The peripheral leukopenia and medullary myelosuppression in this study are detected in mice received 5-FU either alone or with pretreatment of combined GSH and G-CSF and increased with progression of time. So, mice received 5-FU and killed after 1 week showed more myelosuppression than those received the same treatment but killed after 1 day.

No significant difference was detected between the studied groups as regards Hb, HCT, MCHC, MCV, and MCH.

Regarding the differential leucocytic count (DLC) it was noticed that neutrophils of all groups have lower percentages (i.e. worse) than the control whereas lymphocytes of all groups have higher percentages (i.e. worse) than the control. In addition groups 3, 6a (received G-CSF before 5-FU and killed 1 day later) and 7b (received combined GSH and G-CSF before 5-FU and killed 1 week later) showed higher eosinophil percentage than the control, while other groups showed lower eosinophil percentage than control. For monocytes percentage, the results of the present study showed that it was significantly lower in group 3 and insignificantly lower in all other groups when compared with control group. Basophils were not detected in control group and in both group 5b (received GSH before 5-FU and killed 1

week later) and 6b (received G-CSF before 5-FU and killed 1 week later). The basophils percentage in group 3 (received G-CSF) was significantly higher compared to group 2 (received GSH) .

As regards mortality rates among the studied mice no deaths were recorded in groups 1, 2, 3, 4a, 5a, 6a and 7a. The highest mortality rate (70%) was observed in both group 4b (received 5-FU only) and 7b (received combined GSH and G-CSF before 5-FU and killed 1 week later) followed by group 6b (received G-CSF before 5-FU and killed 1 week later) (60%), then group 5b (received GSH before 5-FU and killed 1 week later) (50%).

Comparison of haematological parameters and bone marrow cytology in mice of groups 4a, 5a, 6a and 7a showed no significant differences between the groups in all the compared parameters except TLC and bone marrow cytology which was significantly higher in groups 5a and 6a versus group 4a and significantly lower in group 7a versus groups 5a and 6a (Table 3).

Comparison of haematological parameters and bone marrow cytology in mice of groups 4b, 5b, 6b and 7b showed no significant differences between the groups in all the compared parameters except the bone marrow cytology which was significantly higher in group 6b versus group 4b and

significantly lower in group 7b versus group 6b (Table 4).

Comparison of haematological parameters and bone marrow cytology in mice of groups 4b, 5b, 6b and 7b (killed 1 week after receiving drugs) versus mice óf groups 4a, 5a, 6a and 7a (killed 1 day after receiving drugs) showed no significant differences except significantly lower TLC and bone marrow cytology in group 5b versus group 5a and group 6b versus group 6a , significantly lower neutrophils percentage in group 4b versus group 4a and group 7b versus group 7a and significantly higher lymphocytes percentage in group 4b versus group 4a (Table 5).

II) Histopathological findings in bone marrow :

Mice of groups (1, 2, 3, 4a, 5a, 6a and 7a) show no abnormal histopathological findings . Histopathological findings characteristic to 5-FU (hypocellularity, dilated sinusoids, interstitial haemorrhage, increased fat content and interstitial fibrosis) are detected only in groups 4b, 5b, 6b and 7b.

The survived mice of group 4b show severe hypocellularity and dilated sinusoids. Two of them show increased megakaryocytes, one shows decreased megakaryocytes (Figure 1), one shows increased fat content and interstitial haemorrhage (Figure

2) and one shows interstitial fibrosis (Figure 3).

In group 5b two out of the five (40%) of survived mice reveal mild hypocellularity , dilated sinusoids and interstitial haemorrhage. One of them has increased megakaryocytes and the other has decreased megakaryocytes (Figure 4). The other three mice show normal histopathological findings.

In group 6b two of the survived four mice (50%) have mild hypocellular bone marrow. One of them shows interstitial haemorrhage and increased megakaryocytes (Figure 5), while the other one shows decreased megakaryocytes .

In group 7b all the three survived mice have severe hypocellular bone marrow. Two of them have dilated sinusoids and increased megakaryocytes (Figure 6) and one mouse has increased fat content and interstitial haemorrhage.

DISCUSSION

5-fluorouracil has been used for the treatment of colorectal, breast and head/ neck cancer for more than 40 years and is the most commonly prescribed chemotherapeutic agent (Dobritzsch et al.,2001). Chemotherapy-induced neutropenia, the primary dose-limiting toxicity of most cancer chemotherapy agents, is associated

with numerous negative consequences, including life-threatening infections (Dale, 2003).

Glutathione is a naturally occurring endogenous antioxidant that is cytoprotective. Thus, treatment with glutathione or its precursors may protect normal cells during cancer chemotherapy (Doolittle et al.,2002). Priming has been defined as the administration of a colony stimulating factor prior to chemotherapy. There are several theoretical reasons why this schedule of administration may prove to be advantageous (Lowenberg et al., 2003).

In the present study,the insignificant differences of TLC, DLC, Hb level, blood indices and bone marrow cytology in mice received Glutathione when compared with the contol group are in agreement with the study of Kojima et al. (2003).

In the present study, higher TLC values detected in mice received G-CSF than mice of the control group is in agreement with the results of Lord et al. (2001) who suggested that significantly increased TLC values in normal mice given G-CSF reflects its stimulant effect on neutrophils granulopoiesis. The significantly lower neutrophils percentage in these mice received G-CSF than the control group can be explained by the finding of Takatani et al. (1996) who found that levels of G-CSF in serum are inversely correlated with cir-

culating neutrophils count. Thereby stoppage of G-CSF after the seventh day combined with increased neutrophils count at this time will lead to increase G-CSF elimination with decreased endogenous G-CSF level leading to fall of neutrophils percentage below the normal level.

In this study peripheral leukopenia and medullary myelosuppression detected in mice received 5-FU either alone or with pretreatment of combined GSH and G-CSF is in accord with the work of Friberg et al. (2000). The increased leukopenia and myelosuppression with progression of time noticed in the current study is in agreement with the results of Colombo et al.(2001) who found that the time of major depression for granulocytes after 5-FU was in day 7-14 in humans.

The chemoprotective effect of GSH in mice received GSH pretreatment before 5-FU and killed after 1 day is observed from absence of leukopenia or medullary myelosuppression denoting complete chemoprotection against 5-FU effect on bone marrow. These results are supported by the works of Cascinu et al. (1997) and Kojima et al. (2003).

In contradiction to these findings were the results of lerza et al. (1986) and Doyle et al. (1993) which showed failure of thiol antioxidants to protect peripheral blood elements in mice from the massive de-

crease following chemotherapy. This could be attributed to the high dose needed for chemoprotection. This explanation was supported by Prasad (2004), who stated that antioxidants are needed in high dose to be used in adjunct to chemotherapy.

Proposed mechanisms for GSH induced chemoprotection include: (1) combination of GSH with anticancer drugs to form less toxic and more water soluble GSH conjugate , (2) antioxidant and free-radical scavenging activity (Zhang et al.,1998), (3) DNA repair (Chen and Zeller,1991), (4) pumping toxic chemotherapeutics out of cells via the multidrug resistance-associated proteins (Barrand et al., 1997) and (5) decrease proliferation and differentiation of myeloid cells (Gate et al., 2004) decreasing their susceptibility to chemotherapeutic agents acting on rapidly dividing cells (De Haan et al., 1996).

Leukopenia and medullary myelosuppression detected in mice received GSH pretreatment before 5-FU and killed after 1. week denote incomplete protection. A possible explanation is that chemoprotection is maximally effective if continued during and after chemotherapy treatment as in the study of Danysz et al. (1984).

The complete chemoprotective effect of G-CSF in mice received G-CSF pretreatment before 5-FU and killed after 1 day is

noticed from absence of leukopenia or medullary myelosuppression. This finding is in agreement with that of Brockstein et al. (2000) and Danforth et al. (2003).

In contrast to these results were those obtained by Gardner et al. (2003) who found that pretreatment with haematopoietic growth factors lead to bone marrow failure after administration of chemotherapeutic agents.

In another study done by Harrison et al.(1994) it had been shown that 5-FU was able to abolish the increased numbers of primitive cells in the spleen, induced by haematopoietic growth factors administration to mice, if it was given in the middle of the 7th day haematopoietic growth factors treatment period. However, if it was administered at day 8, many stem cells had lost vulnerability to 5-FU.

Incomplete chemoprotection in mice received G-CSF pretreatment before 5-FU and killed after 1 week is denoted by presence of leukopenia and medullary myelosuppression. This result is in accord with the study of De Haan et al. (1996) where haematopoietic growth factors pretreatment could not abolish the period of major leucocytes depression in mice .

The proposed mechanism of efficacy of G-CSF pretreatment is the increased numbers of haematopoietic cells present before

chemotherapy administration. In addition, enhanced number of primitive and progenitor cells may result in reduced cycling activity, i.e. 5-FU sensitivity of these cells, due to normal physiological feed back loops (De Haan et al., 1996). The second proposed mechanism shows the importance of spacing between HGFs administration and chemotherapy administration. Time is needed until reflex inhibition of proliferation of cells occur and not exposing the cells during rapid division phase to the chemotherapeutic agent.

The combined pretreatment with GSH and G-CSF reveal no protection at all either in mice killed 1 day after 5-FU or those killed 1 week after 5-FU denoted by presence of leukopenia and medullary myelosuppression in both groups.

These results could be explained by the work of Sattler et al. (1999). They found that haematopoietic growth factors signals through the formation of reactive oxygen species (ROS) and that antioxidants e.g. N-acetyl cysteine reduced growth and viability of cells.

Also Gate et al. (2004) have shown that G-CSF was more effective at stimulating proliferation of haematopoietic cells in glutathione transferase (GST) deficient mice than in wild type. This is explained by the fact that GST interacts with and suppresses c-Jun NH2-terminal kinase [an enzyme which is responsible for increased myeloproliferation].

Cascinu et al. (1997), however contradict these findings and this could be explained by the fact that there was a separation between G-CSF and GSH as regards days of administration which could prevent the antagonism between them.

In the present study significantly higher monocytes percentage is observed in mice received G- CSF pretreatment before 5-FU and killed 1 day later versus mice received G-CSF only. This observation coincides with the findings of Gabrilove (1991). The significant decrease of monocytes percentage in mice received G-CSF versus the control group can be attributed to the fact that G-CSF level has fallen following stoppage of administration due to increased clearance by high neutrophils count which in turn will lower the monocytes percentages.

The insignificant differences detected between the studied groups as regards Hb, HCT, MCHC, MCV, and MCH denote absence of RBCs affection to which can be explained by comparing the half life of granulocytes (6-8 hours) to that of RBCs (120 days). This fact shows why the myelosuppressive drugs need longer time for producing anaemia than the time needed for neutropenia (Hoagland, 1992).

Although occurrence of deaths can be considered a drawback, however it should be put in mind that in clinical trials made on humans the complete sterile environment is difficult to be obtained specially in outpatient treatment which makes secondary infection , and not neutropenia itself, the major cause of death in humans (Dale, 2003). This could be explained by the association between increased mortality rate in mice with higher degree of neutropenia in groups 4b and 7b.

Histopathological findings characteristic to 5-FU (hypocellularity, dilated sinusoids, interstitial haemorrhage, increased fat content and interstitial fibrosis) are detected only in groups 4b, 5b, 6b and 7b. Absence of these findings in the other groups could be explained by the work of D'souza and Narayana (2002) who found that cytotoxic drug induced myelotoxicity after 24 hours was in the form of apoptosis and the damaged cells appeared as darkly stained bodies in the marrow. The presence of these cells can be detected by cytological smears, while histopathological examination cannot differentiate apoptotic cells from normal ones.

GSH can be given concurrently with chemotherapy (Kojima et al., 2003) or even after chemotherapy (Neuwelt et al., 2004), so, potentiating its protective effect. In addition GSH is a cytoprotective agent where its action is not only on bone marrow, but affecting many systems owing to its detoxifying capabilities (Doolittle et al.,2002). On the contrary haematopoietic growth factors cannot be given during the chemotherapeutic course as this will result in fatal bone marrow aplasia (Weiss and Lackman, 2002) and its protective effects are believed to be on the bone marrow only (Liang, 2003).

It can be concluded from this work that chemoprotection of GSH, do not progress to the end in mice killed one week after 5-FU dose and this can be attributed to the fact that chemoprotection is maximally effective if continued during and after chemotherapy treatment.

It can be also concluded that G-CSF pretreatment produce complete protection only in mice killed one day after 5-FU dose. This chemoprotective effect is diminished as the study proceeded in mice killed one week after 5-FU dose denoting that pretreatment combined with posttreatment of G-CSF would offer better results.

Table (1): Haematological parameters and bone marrow cytology in all studied groups

Parameter / Groups	B.M.cytology (/mm³)	Hb (g/dl)	HCT (%)	MCHC (%)	MCV (fl)	MCH (pcg)	TLC (/mm³)	DLC (%)				
								Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
1 Control	1978.5 ± 130.959	12.133 ± 0.966	37.609 ± 2.994	32.727 ± 0.699	49.872 ± 0.644	32.513 ± 0.981	3810 ± 685.484	60.8 ± 8.456	34.3 ± 9.684	1.6 ± 1.505	3.5 ± 2.223	0 ± 0
2 GSH	1852.5 ± 208.982	11.117 ± 1.361	34.457 ± 4.221	32.504 ± 0.307	50.251 ± 0.952	31.708 ± 1.121	3800 ± 1293.573	53.3 ± 8.731	42.5 ± 8.784	1.1 ± 0.737	2.7 ± 0.948	0.4 ± 0.516
3 G-CSF	2345 ± 554.000	12.181 ± 1.29	38.198 ± 4.079	32.241 ± 0.091	50.661 ± 2.618	31.529 ± 0.545	6950 ± 2867.538	45.6 ± 10.243	50.4 ± 9.754	1.7 ± 1.337	1.4 ± 0.843	0.9 ± 0.567
4a	1217 ± 288.253	10.328 ± 2.159	31.175 ± 6.011	32.927 ± 2.302	49.971 ± 6.564	30.36 ± 1.685	1980 ± 616.080	51 ± 4.320	45.2 ± 5.432	1.1 ± 0.994	1.9 ± 0.875	0.8 ± 0.788
5a	1925 ± 302.994	11.881 ± 1.226	35.482 ± 3.171	33.467 ± 3.446	47.0940 ± 4.657	29.03 ± 2.568	4170 ± 1310.682	54.1 ± 6.384	41.7 ± 6.815	1.2 ± 1.032	2.7 ± 0.948	0.3 ± 0.674
6a	2172 ± 233.942	11.871 ± 2.157	36.881 ± 6.069	31.735 ± 2.811	46.512 ± 5.484	29.232 ± 2.365	4670 ± 1695.495	54.5 ± 6.587	40.4 ± 5.853	2 ± 1.054	2.5 ± 1.080	0.6 ± 0.699
7a	1273 ± 191.546	9.8360 ± 2.135	39.802 ± 5.919	32.861 ± 1.752	48.142 ± 7.401	30.225 ± 2.351	1975 ± 300.924	51.7 ± 7.958	44.7 ± 7.846	0.9 ± 0.875	2.4 ± 1.264	0.3 ± 0.483
4b	873.333 ± 40.414	11.12 ± 1.996	34.466 ± 6.189	32.19 ± 0.103	51.633 ± 4.928	31.753 ± 0.492	750 ± 50	34.666 ± 4.509	61 ± 4.582	1 ± 0	1.666 ± 1.154	1.666 ± 1.154
5b	1310 ± 334.757	9.882 ± 0.869	30.544 ± 2.857	32.394 ± 0.302	49.754 ± 0.457	32.518 ± 0.302	1230 ± 542.678	44.4 ± 10.454	52.2 ± 9.628	0.8 ± 0.836	2.6 ± 1.516	0 ± 0
6b	1568.75 ± 391.245	10.695 ± 1.635	30.263 ± 6.063	31.318 ± 1.682	52.375 ± 4.158	30.455 ± 1.938	1875 ± 865.544	46.75 ± 9.069	50.5 ± 8.426	0.75 ± 0.957	1.75 ± 0.5	0 ± 0
7b	900 ± 100	10.69 ± 0.550	39.333 ± 7.164	32.22 ± 0.051	47.53 ± 4.291	30.19 ± 1.764	866.666 ± 305.505	34.666 ± 2.081	60 ± 2	2.333 ± 0.577	2 ± 1	1 ± 1

4a 5-FU killed after 1 day, 5a 5-FU + GSH killed after 1 day, 6a 5-FU + G-CSF killed after 1 day, 7a 5-FU + GSH + G-CSF killed after 1 day.
4b 5-FU killed after 1 week. 5b 5-FU +GSH killed after 1 week. 6b 5-FU +G-CSF killed after 1 week, 7b 5-FU+GSH+G-CSF killed after 1 week.

Table (2): Comparison of haematological parameters and bone marrow cytology in all studied groups versus control group.

Parameter / Groups	B.M.cytology (/mm³)	Hb (g/dl)	HCT (%)	MCHC (%)	MCV (fl)	MCH (pcg)	TLC (/mm³)	DLC (%) Neutrophils	Lymphocytes	Eosinophils	Monocytes
Gp2 vs control	↓ 0.703	↓ 0.170	↓ 0.172	↓ 0.509	↑ 0.866	↓ 0.141	↓ 1.000	↓ 0.18	↑ 0.145	↓ 0.643	↓ 0.458
Gp3 vs control	↑ 0.067	↑ 0.996	↑ 0.936	↓ 0.395	↑ 0.541	↓ 0.059	↑ 0.002**	↓ 0.003**	↑ 0.002**	↑ 0.982	↓ 0.010**
Gp 4a vs control	↓ 0.0001**	↓ 0.336	↓ 0.135	↑ 1.000	↑ 1.000	↓ 0.267	↓ 0.0001**	↓ 0.076	↑ 0.039*	↓ 0.977	↓ 0.175
Gp 5a vs control	↓ 1.000	↓ 1.000	↓ 0.990	↑ 0.998	↓ 0.952	↓ 0.435	↑ 0.987	↓ 0.489	↑ 0.001**	↓ 0.977	↓ 0.913
Gp 6a vs control	↑ 0.723	↓ 0.092	↓ 1.000	↓ 0.983	↓ 0.870	↓ 0.110	↑ 0.346	↓ 0.572	↑ 0.646	↑ 0.995	↓ 0.755
Gp 7a vs control	↓ 0.0001**	↓ 0.092	↑ 1.000	↑ 1.000	↓ 0.998	↓ 0.198	↓ 0.0001**	↓ 0.126	↑ 0.059	↓ 0.875	↓ 0.650
Gp 4b vs control	↓ 0.0001**	↓ 0.993	↓ 0.990	↓ 1.000	↑ 1.000	↓ 1.000	↓ 0.0001**	↓ 0.0001**	↑ 0.0001**	↓ 0.994	↓ 0.487
Gp 5b vs control	↓ 0.0001**	↓ 0.313	↓ 0.244	↓ 1.000	↓ 1.000	↓ 1.000	↓ 0.0001**	↓ 0.003**	↑ 0.206	↓ 0.898	↓ 0.945
Gp 6b vs control	↓ 0.145	↓ 0.889	↓ 0.290	↓ 0.973	↑ 0.996	↓ 0.690	↓ 0.006**	↓ 0.040*	↑ 0.013*	↓ 0.906	↓ 0.405
Gp 7b vs control	↓ 0.0001**	↑ 0.935	↑ 1.000	↓ 1.000	↓ 0.999	↓ 0.673	↓ 0.0001**	↓ 0.0001**	↑ 0.0001**	↑ 0.978	↓ 0.736

* significant if P <0.05.   ↑ = higher

** highly significant if P <0.01   ↓ = lower

Table (3): Comparison of haematological parameters and bone marrow cytology in mice killed 1 day after receiving: 5-FU (gp 4a), 5-FU preceeded by GSH pretreatment (gp 5a), 5-FU preceeded by G-CSF pretreatment (gp 6a) and 5-FU preceeded by combined GSH and G-CSF pretreatment (gp 7a).

	Gp 5a vs gp 4a	Gp 6a vs gp 4a	Gp 6a vs gp 5a	Gp 7a vs gp 4a	Gp 7a vs gp 5a	Gp 7a vs gp 6a
TLC (/mm³)	↑ 0.0001**	↑ 0.0001**	↑ 0.911	↓ 1.000	↓ 0.0001**	↓ 0.0001**
Neutrophils %	↑ 0.987	↑ 0.973	↑ 1.000	↑ 1.000	↓ 0.998	↓ 0.993
Lymphocytes%	↓ 0.977	↓ 0.868	↓ 0.868	↓ 1.000	↑ 0.991	↑ 0.925
Eosinophils %	↑ 1.000	↓ 0.608	↓ 0.338	↓ 1.000	↓ 0.999	↓ 0.338
Monocytes %	↑ 0.913	↑ 0.983	↓ 1.000	↑ 0.995	↓ 1.000	↓ 1.000
Basophils %	↓ 0.662	↓ 0.998	↑ 0.972	↓ 0.662	↓ 1.000	↓ 0.972
Hb (g/dl)	↑ 0.540	↑ 0.549	↓ 1.000	↓ 0.999	↓ 0.189	↓ 0.194
HCT (%)	↑ 0.628	↑ 0.257	↑ 0.999	↑ 1.000	↑ 0.263	↑ 0.070
MCHC (%)	↑ 1.000	↓ 0.948	↓ 0.698	↓ 1.000	↓ 0.999	↑ 0.963
MCV(fl)	↓ 0.942	↓ 0.851	↓ 1.000	↓ 0.997	↑ 1.000	↑ 0.999
MCH (pcg)	↓ 0.837	↓ 0.928	↑ 1.000	↓ 1.000	↑ 0.903	↑ 0.965
B.M cytology (/mm³)	↑ 0.0001**	↑ 0.0001**	↑ 0.411	↑ 1.000	↓ 0.0001**	↓ 0.0001**

* significant if P <0.05.   ↑ = higher
** highly significant if P <0.01   ↓ = lower

Table (4): Comparison of haematological parameters and bone marrow cytology in mice killed 1 week after receiving: 5-FU (group 4b), 5-FU with GSH pretreatment (group 5b), 5-FU with G-CSF pretreatment (group 6b) and 5-FU with combined GSH and G-CSF pretreatment (group 7b).

	Gp 5b vs gp 4b	Gp 6b vs gp 4b	Gp 6b vs gp 5b	Gp 7b vs gp 4b	Gp 7b vs gp 5b	Gp 7b vs gp 6b
TLC (/mm³)	↑ 0.997	↑ 0.695	↑ 0.961	↑ 1.000	↓ 1.000	↓ 0.803
Neutrophils %	↑ 0.642	↑ 0.414	↑ 1.000	↓ 1.000	↓ 0.642	↓ 0.414
Lymphocytes%	↓ 0.779	↓ 0.636	↓ 1.000	↓ 1.000	↑ 0.872	↑ 0.748
Eosinophils %	↓ 1.000	↓ 1.000	↓ 1.000	↑ 0.826	↑ 0.553	↑ 0.570
Monocytes %	↑ 0.988	↑ 1.000	↓ 0.989	↑ 1.000	↑ 0.999	↑ 1.000
Basophils %				↓ 0.915		
Hb (g/dl)	↓ 0.986	↓ 1.000	↑ 0.999	↓ 1.000	↑ 0.999	↓ 1.000
HCT (%)	↓ 0.979	↓ 0.975	↓ 1.000	↑ 0.960	↑ 0.330	↑ 0.347
MCHC (%)	↑ 1.000	↓ 1.000	↓ 0.998	↑ 1.000	↓ 1.000	↑ 1.000
MCV(fl)	↑ 1.000	↑ 1.000	↑ 0.998	↓ 0.987	↓ 1.000	↓ 0.946
MCH (pcg)	↑ 1.000	↓ 0.994	↓ 0.811	↓ 0.986	↓ 0.780	↓ 1.000
B.M cytology (/mm³)	↑ 0.305	↑ 0.016*	↑ 0.828	↑ 1.000	↓ 0.388	↓ 0.023*

* significant if $P < 0.05$ ↑ = higher
** highly significant if $P < 0.01$ ↓ = lower

Table (5): Comparison of haematological parameters and bone marrow cytology in mice of groups 4b, 5b, 6b and 7b (killed 1 week after receiving drugs) versus mice of groups 4a, 5a, 6a and 7a (killed 1 day after receiving drugs).

	Gp 4b vs gp 4a	Gp 5b vs gp 5a	Gp 6b vs gp 6a	Gp 7b vs gp 7a
TLC (/mm³)	↓ 0.384	↓ 0.0001**	↓ 0.0001**	↓ 0.527
Neutrophils %	↓ 0.026**	↓ 0.267	↓ 0.663	↓ 0.017*
Lymphocytes%	↑ 0.045*	↑ 0.388	↑ 0.346	↑ 0.059
Eosinophils %	↓ 1.000	↓ 0.999	↓ 0.545	↑ 0.504
Monocytes %	↓ 1.000	↓ 1.000	↓ 0.988	↓ 1.000
Basophils %	↑ 0.447			↑ 0.718
Hb (g/dl)	↑ 0.999	↓ 0.944	↓ 0.963	↑ 0.998
HCT (%)	↑ 0.986	↓ 0.706	↓ 0.428	↓ 1.000
MCHC (%)	↓ 1.000	↓ 0.992	↓ 1.000	↓ 1.000
MCV(fl)	↑ 1.000	↑ 0.989	↑ 0.602	↓ 1.000
MCH (pcg)	↑ 0.974	↑ 0.146	↑ 0.977	↓ 1.000
B.M cytology (/mm³)	↓ 0.488	↓ 0.0001**	↓ 0.004**	↓ 0.376

* significant if P <0.05.   ↑ = higher
** highly significant if P <0.01   ↓ = lower
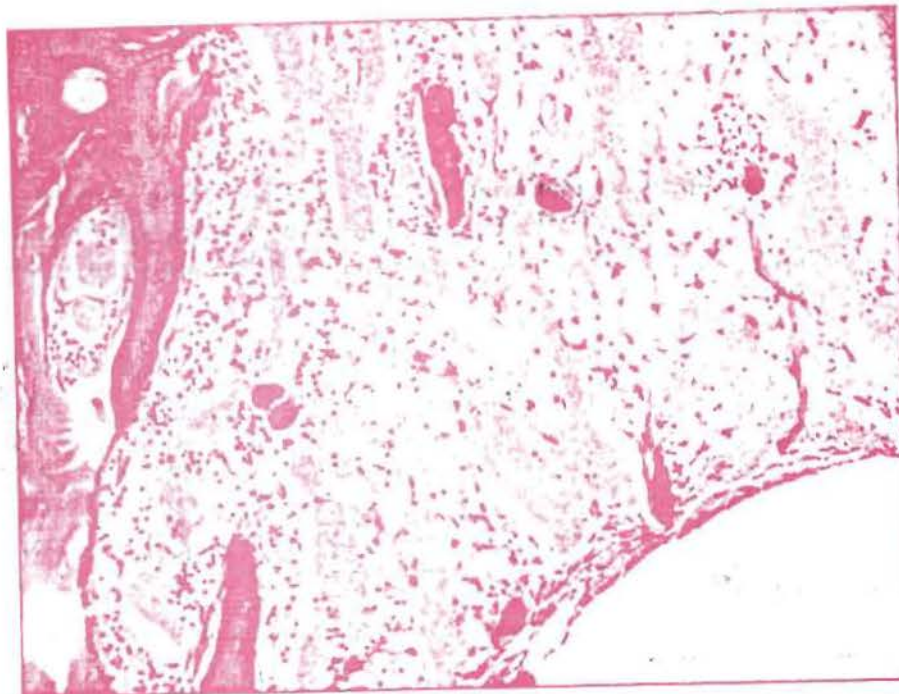
Fig. (1) : A photomicrograph showing severe hypocellularity (H), increased fat content (F), dilated sinusoids (S) and decreased megakaryocytes in bone marrow of a mouse treated with 5-FU and killed after 1 week (group 4b).   *(H&E x 100).*
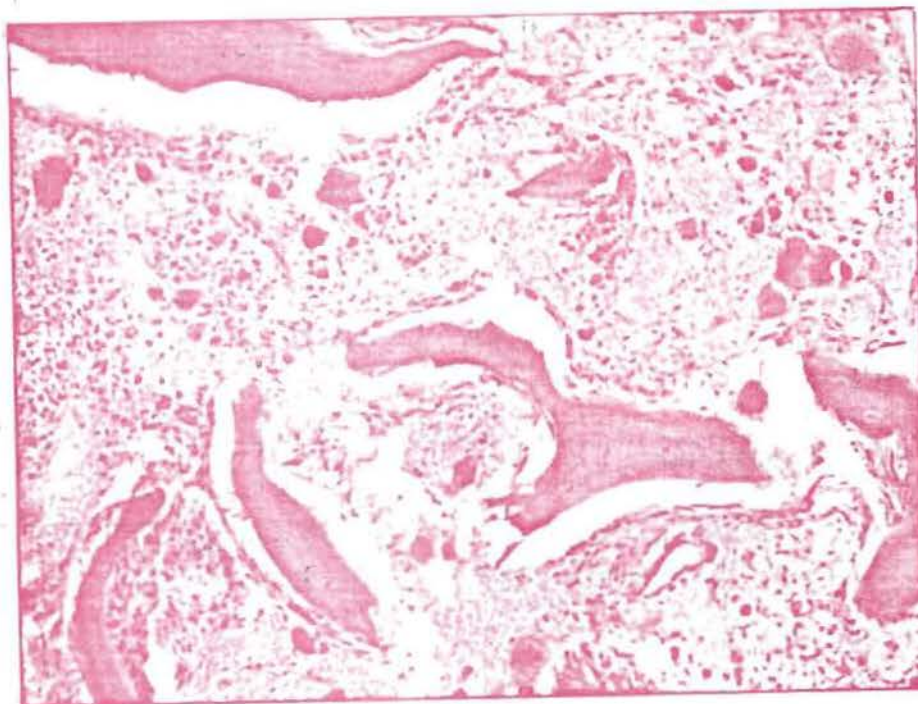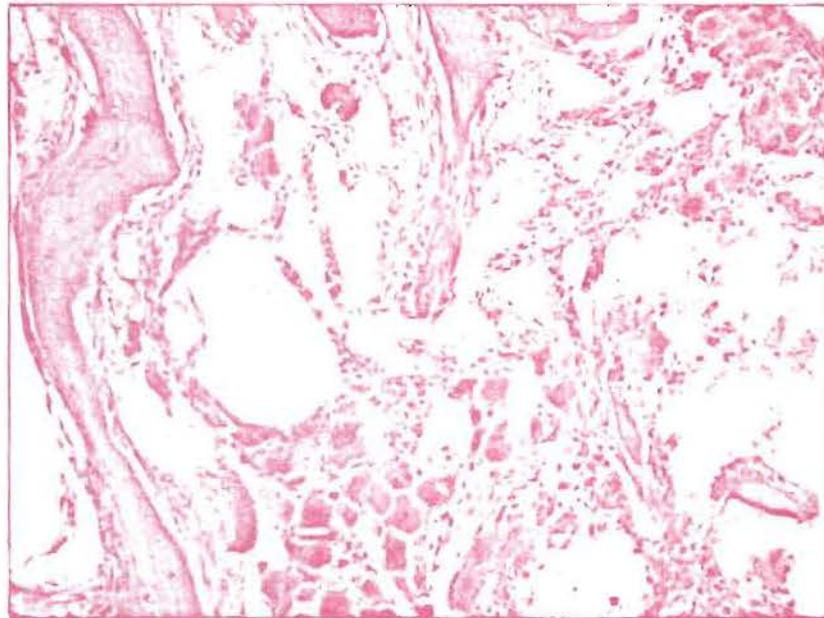

Fig. (2): A photomicrograph showing severe hypocellularity (H), interstitial haemorrhage (IH), dilated sinusoids (S) and increased megakaryocytes in bone marrow of a mouse treated with 5-FU and killed after 1 week (group 4b).   *(H&E x 100).*

Fig. (3) : A photomicrograph showing severe hypocellularity (H) and interstitial fibrosis (F) in bone marrow of a mouse treated with 5-FU and killed after 1 week (group 4b).   *(H&E x 100).*
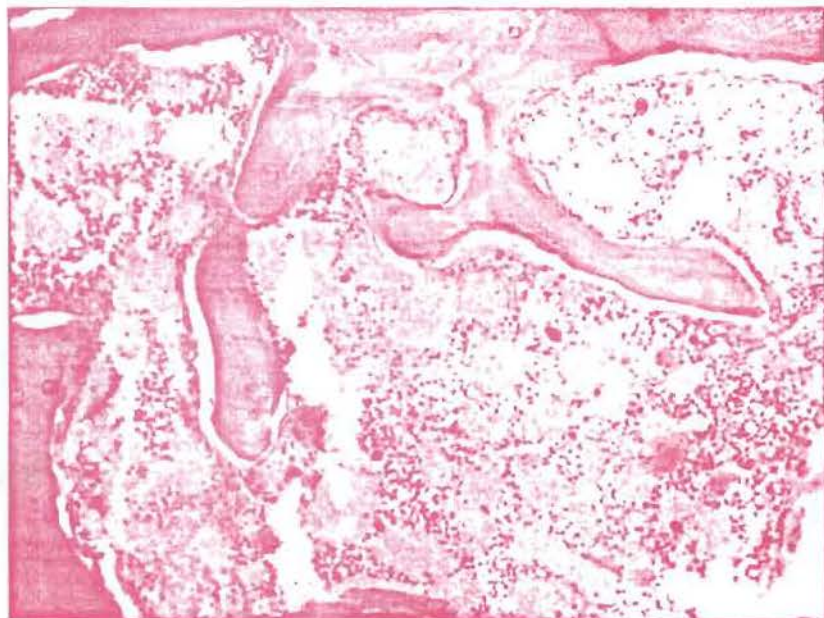

Fig. (4) : A photomicrograph showing mild hypocellularity (H), dilated sinusoids (S), interstitial haemorrhage (IH) and decreased megakaryocytes in bone marrow of a mouse treated with 5-FU + GSH and killed after 1 week (group 5b).
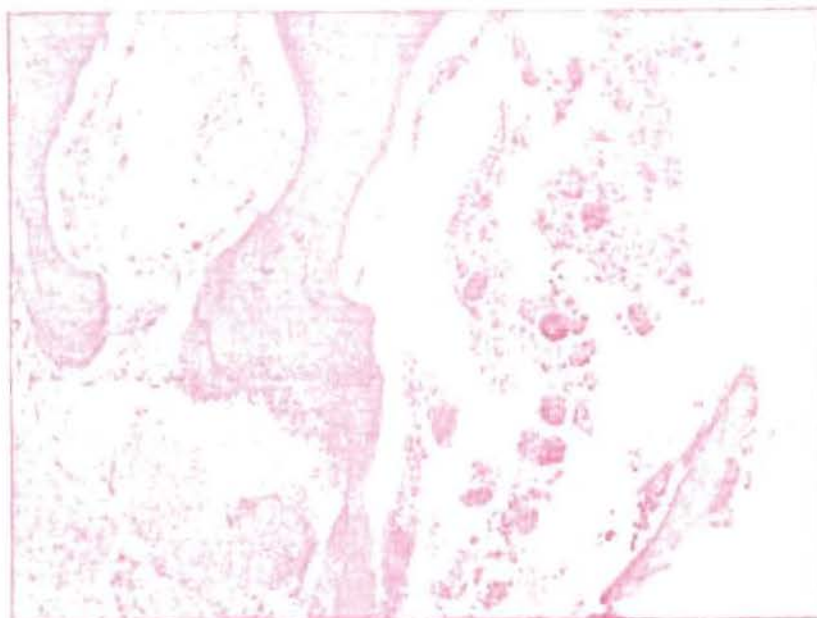*(H&E x 100).*

Fig. (5) : A photomicrograph showing mild hypocellularity (H), interstitial haemorrhage
(IH) and increased megakaryocytes in bone marrow of a mouse treated with 5-
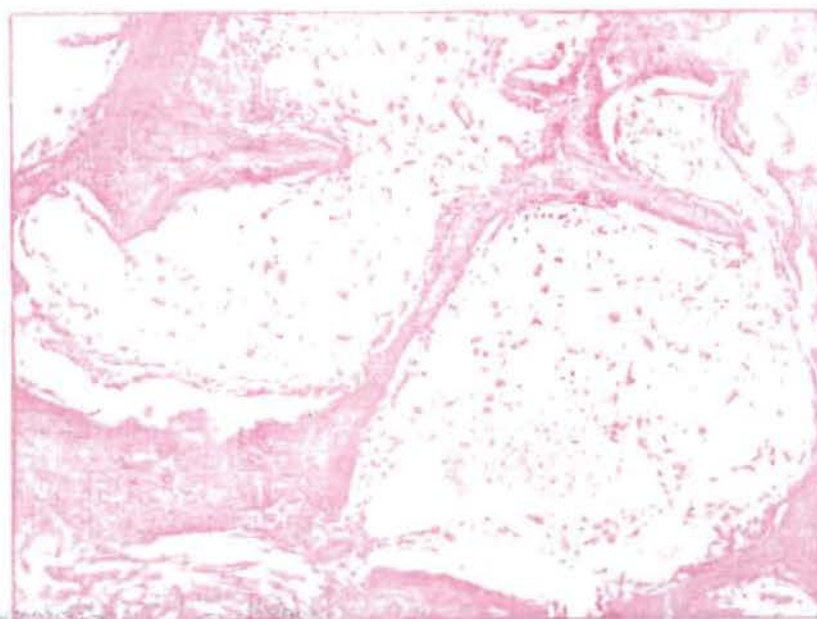FU + G-CSF and killed after 1 week (group 6b).   *(H&E x 100).*

Fig. (6) : A photomicrograph showing severe hypocellularity (H), increased fat content
(F) and interstitial haemorrhage (IH) in bone marrow of a mouse treated with
5-FU + GSH + G-CSF and killed after 1 week (group 7b).   *(H&E x 100).*

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التأثير الوقائي للجلوتاثيون وعامل النمو (محفز مزارع الخلايا المحببة) على سمية عقار 5-فلورويوراسيل على النخاع العظمى في الفئران

المشتركون في البحث

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من أقسام الطب الشرعى والسموم الإكلينيكية والباثولوجى*

كلية الطب – جامعة المنصورة

إستهدفت هذه الدراسة مقارنة التأثير الوقائي للجلوتاثيون أوعامل النمو(محفز مزارع الخلايا المحببة)أو كلاهما على سمية عقار 5-

فلورويررواسيل على النخاع العظمي في الفئران في عدد ١١٠ من إناث الفئران البالغة بعد تقسيمها الى ٧ مجموعات كالآتي: المجموعة الأولى

(عشرة فئران) لم تتلق فيها الحيوانات أي علاج وأستخدمت كمجموعة ضابطة . المجموعة الثانية (عشرة فئران) أعطيت فيها الحيوانات جرعة

يومية من الجلوتاثيون (٨٠٠ مللجرام/كجم) داخل التجويف البريتوني لمدة ٧ أيام ثم لم تتلقى بعدها أى علاج لمدة ٧ أيام أخرى . المجموعة

الثالثة (عشرة فئران) أعطيت فيها الحيوانات جرعة يومية من عامل النمو(٢٥٠ميكروجرام / كجم) تحت الجلد ٧ أيام ثم لم تتلقى بعدها

أى علاج لمدة ٧ أيام أخرى. تم ذبح فئران المجموعات الأولى والثانية والثالثة بعد ١٥ يوماً. المجموعة الرابعة (عشرون فأرأ) أعطيت فيها

الحيوانات في اليوم الثامن جرعة واحدة من 5- فلررويوراسيل (١٦٠ مللجرام/كجم) داخل التجويف البريتوني. المجموعة الخامسة (عشرون

فأرأ) أعطيت فيها الحيوانات جرعة يومية من الجلوتاثيون داخل التجويف البريتوني لمدة ٧ أيام وفى اليوم الثامن أعطيت جرعة واحدة من 5-

فلورويوراسيل داخل التجويف البريتوني . المجموعة السادسة (عشرون فأرأ) أعطيت فيها الحيوانات جرعة يومية من عامل النمو تحت الجلد

لمدة ٧ أيام وفى اليوم الثامن أعطيت جرعة واحدة من 5- فلورويوراسيل داخل التجويف البريتوني. المجموعة السابعة (عشرون فأرأ)

أعطيت فيها الحيوانات جرعة من الجلوتاثيون داخل التجويف البريتوني مع جرعة من عامل النمو تحت الجلد يوميا لمدة ٧ أيام وفى اليوم

الثامن أعطيت جرعة واحدة من 5- فلورويوراسيل داخل التجويف البريتوني . قسمت المجموعات من الرابعة حتى السابعة إلى مجموعتين

فرعيتين كل منها عشرة فئران (أ) والتى ذبحت فئرانها في اليوم التاسع أى بعد يوم واحد من جرعة 5- فلورويوراسيل و (ب) والتى

ذبحت فئرانها في اليوم الخامس عشر أى بعد إسبوع من جرعة 5- فلورويوراسيل. تم جمع عينات الدم من جميع الفئران بعد ذبحها لعمل عد

كلي وتمايزي لكرات الدم البيضاء. ومستوى الهيموجلوبين ومناسب الدم لها على النحو التالى: المجموعات الفرعية (٤أ ، ٥أ ، ٦أ ، ٧أ) في

اليوم التاسع و المجموعات (١،٢،٣) و المجموعات الفرعية (٤ب ، ٥ب ، ٦ب ، ٧ب) في اليوم الخامس عشر. بعد جمع عينات الدم تم

تشريح الفئران واستخراج عظمتي الفخذ من كل الحيوانات وتم عمل الفحص الخلوى للنخاع باستخدام العظمة اليمنى والفحص الهستوباثولوجى للنخاع باستخدام العظمة اليسرى. و كانت النتائج على النحو التالى : وجد تأثير وقائى ملحوظ للجلوتاثيون على الفئران التى تناولته قبل اعطاؤها ٥-فلوروريوراسيل و تم ذبحها بعد ذلك بيوم واحد كما وجد تأثير وقائى ملحوظ لعامل النمو على الفئران التى تناولته قبل اعطاؤها ٥-فلوروريوراسيل و تم ذبحها بعد ذلك بيوم واحد ولكن لم يوجد تاثير وقائى ملحوظ لأى من الجلوتاثيون أو عامل النمو على الفئران التى ذبحت بعد أسبوع وعند الجمع بين الجلوتاثيون و عامل النمو.

وعلى ضوء هذه النتائج يوصى الباحثون باستخدام الجلوتاثيون أو عامل النمو فى الوقاية من التأثير الضار لعقار ٥-فلوروريوراسيل على النخاع العظمى وأيضاً باعطائهما قبل وبعد جلسات العلاج الكيميائى. كذلك يوصى بعدم الجمع بين الجلوتاثيون و عامل النمو نظرا للتأثير المضاد لكل منهما على الاخر وأيضاً اجراء المزيد من الدراسات على مضادات الأكسدة وعوامل النمو الأخرى لدراسة تأثيرها الوقائى مع زيادة زمن فترة الدراسة.