The immunological and protective role of Baicalin in male rats treated with chemotherapy (Gemcitabine)

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Abstract: Background. Baicalin is a flavonoid glycoside derived from a Chinese herbal plant (Scutellaria baicalensis) that possesses various biological properties. Baicalin has been an important medical agent with a variety of pharmacological properties. Aims of study: The current study aimed to evaluate the immunological and protective role of the herbal extract (Baicalin) in male rats treated with chemotherapy (Gemcitabine), by measuring the level of some cytokines (IL-10 & IL-12), and measuring the level of antioxidants (MDA & GSH) in the serum of rats by the ELISA technique. The study also included measuring the level of phagocytosis index and the WBC differential count. Materials and Methods: This study was conducted in the animal husbandry of the college of Veterinary Medicine / Al-Qadisiyah University from January, 2018 to June, 2018. Eighty (80) male Albino Rats (Rattus norvegicus) were used in the experiment, and were divided into eight groups, each group had 10 animals. Results: As per the statistical analysis results it was observed that there is significant difference in the study group at the level (P < 0.05). The data obtained showed a significant decrease (IL-10 & IL-12) in the T5 group treated with Baicalin in combination with chemotherapy (Gemcitabine) compared to the T1 and T2 groups treated with chemotherapy drug. Not only this, the result obtained showed the marked decrease in the study groups T1 and T2 treated with chemotherapy (Gemcitabine) compared to T3, T4, and T5 groups treated with herbal extract (Baicalin) and control group. While the antioxidant results also showed the marked increase (MDA) in group T1 and T2 treatment with Gemcitabine compared to the study groups. As well as significant decrease in (GSH) in T1 and T2 groups treated with Gemcitabine compared to T3, T4, T5, T6 and control. And the results of the statistical analysis showed a significant decrease in both lymphocytes, neutrophils, and monocytes in the T1 and T2 groups compared to all study groups. Conclusion: Baicalin has a significant preventive role in reducing oxidative stress induced by chemotherapy drugs and has a significant role in improving immune and defensive lines of the body against pathogens.

1. Introduction

Baicalin is a flavonoid that is extracted from the Chinese herbal plant, which is a member of the mint group, which is known as the (Scutellaria baicalensis, golden root, Skullcap) which grows mainly in China and Russia, and extends across the oceans to various parts of the wild [1].

Root of Scutellaria (common name: Huang-chin in China) is one among the most crucial and significant and multifunctional herbs quite popular in China and various other eastern countries. It is majorly used for treating medical conditions like hyperlipidemia, arteriosclerosis hypertension, dysentery, common cold and inflammatory diseases such as atopic dermatitis. Not only effective in these diseases but also has been proven with its results shown its cell inhibition effect on various cancer cell lines in vitro [2]-[3], and also in vivo in mouse tumor models[4]-[5]. Several in vivo and in vitro studies conducted in the past decade have shown that Baicalin has been an important medical agent with a variety of pharmacological properties such as
Baicalin extracts have strong anti-inflammatory effects. This is especially important for macrophages and immune cells. Macrophages are immune cells involved in the response to inflammation in the body. In particular, macrophages help in initiation of the inflammatory process further maintain the process and finally resolution of the inflammatory response [12]. Studies have shown that extracts from Chinese Skullcap can activate the anti-inflammatory role of macrophages. It seems that the mechanism involved is a binding activity to the estrogen receptors of macrophages [13]. Baicalin has also been shown to inhibit the activity of chemokines in inflammation. Baicalin produces a decrease in the activity of the selected chemokines. This helps to reduce the body's inflammation. This action seems to be through Baicalin’s binding to various chemokine ligands are thereby disrupting their activity [14].

2. Materials and methods

2.1 Experiment animals

This study was performed in animal husbandry of the college of the Veterinary Medicine / Al-Qadisiyah University during the period from January, 2018 to June. In the experiment used 80 male Albino Rats (*Rattus norvegicus*). In order to start the study all male rats used in the study were made to acclimatize to the animal husbandry environment before performing the experiment. The male rats were breeding in the animal house with water and standard chow according to the [15]. and all animals were treated to similar conditions in light cycle (12 hours lighting - 12 hours’ darkness) and room temperature 22-25 C°. Animal weights ranged from 175± g, while the ages ranged from 10-12 weeks. The male rats were distributed in plastic cages covered with metal coverings and a well-ventilated room. Maximum five rats were kept in single cage. Their floor was sprayed with clean wood sawing. The cages were cleaned and replaced every three days.

2.2 Experimental design

The animals were divided into eight groups as shown:

1. Control group: Distilled water is given only for two months.
2. First treatment group: Gemzar is given at a concentration of 15 mg / kg for one month [16].
3. Second treatment group: Gemzar is given at a concentration of 15 mg / kg for two months [16].
4. Third treatment group: Baicalin is given at a concentration of (30 mg / kg orally for one month) [17].
5. Fourth treatment group: Baicalin is given at a concentration of (30 mg / kg orally for two months) [17].
6. Sixth treatment group: Gemzar is given orally at 15 mg / kg body weight combination with baicalin which given orally with a concentration of 30 mg / kg at the end of the experiment.
7. Seventh treatment group: Oral gemzar with a concentration of 15 mg / kg body weight for one month, and oral baicalin given (30 mg / kg) to the end of the experiment.

8. Eighth treatment group: Oral baicalin is given with a dose of 30 mg / kg body weight for one month and then gemzar is given orally at a concentration of 15 mg / kg weight until the end of the experiment.

2.3 Sacrifice of Animals
During the complete experimental procedure all the male rats were kept under vigilance. Once the experiment was completed, all the rats either of treated and of controlled group were anesthetized (by injection of 0.3ml ketamine + 0.1 ml of xylazine/ kg b.w.), and then were dissected and then their blood samples were collected from the abdominal vein in non-heparinized tubes. Blood samples were extracted using disposable syringe by maintain aseptic technique. Each collected sample was directly was followed in sterile tube containing EDTA for WBC differential count and phagocytosis processes (during 2 hour). Blood serum samples were differentiated (by centrifugation at 3000 rpm for 5 minutes) and kept at –20 °C until assessment of (MDA, GSH), (IL-10 &12).

2.4 Methods
Kits of (IL-10 & IL-12) provided by CUSABIO company, China. The method was done according to the company's instructions. Kits of (GSH and MDA) provided by ABO company, Switzerland. The method was done according to the company's instructions. Phagocytosis was performed as per [18]. The hematological tests that included (differential WBCs counts) were done by using Auto Hematology Analyzer (NSysmex – kx21) in which the results read and printed automatically.

2.5 Dosage and solution of Gemcitabine drug (Gemzar)
Company, 2003) in 333 ml of Nacl and at a concentration of 0.9% compared to the dose given to humans (1000 mg) and saved in the refrigerator (4 C) according to (Veltkamp et al.,2008).

Dosage and solution of Baicalin
A dosage of 20 mg / Kg body weight was prepared by dissolving the required concentration depending on the body weight of the animal according to [17].

3. Results
3.1 Level of Malondialdehyde (MDA)
The results shown in Table (1) showed a significant increase (P> 0.05) in the level of MDA in the group of animals treated with the Gemzar drug for one month T1 and for two months T2 respectively compared to the control group as well as the groups (T3, T4, T5, and T6), while no significant difference was found between the T3, T4, T5 and T6 groups.

3.2 Level of Glutathione
The results of the Glutathione in Table (4-5) showed significant differences (P <0.05) in the Glutathione levels of the experimental animals in their eight groups when compared with each other, except for groups (T5 and T6), with no significant difference among them. The results showed a significant decrease of P (<0.05) in the level of glutathione in the animal's groups treated with the Gemzer drug for one month T1 and for two months T2, respectively, compared to the control group as well as the groups (T3, T4, T5, T6 and T7). The results showed a significant increase in the rate The level of glutathione for the T3 and T4 groups treated with Baicalin for one month and two months respectively compared with the other groups. The
results showed a decrease in the level of glutathione of T7 group compared to the control group and T5 and T6 group, and there is no difference between T5 and T6 group.

Table 1. Shows the Antioxidant levels of male rats treated with Baicalin and Gemzar

|     | MDA Mean ± Std.Error | GSH Mean ± Std.Error |
|-----|----------------------|----------------------|
| C.  | 10 1.6720 ± 0.07899  | 2.4310 ± 0.27522     |
| D   | 10 2.6500 ± 0.09262  | 1.7910 ± 0.05021     |
| B   | 10 3.2070 ± 0.18494  | 1.3100 ± 0.12202     |
| A   | 10 1.7010 ± 0.02234  | 2.7570 ± 0.09105     |
| C   | 10 1.6460 ± 0.03565  | 3.0540 ± 0.08579     |
| D   | 10 1.7320 ± 0.03765  | 2.5210 ± 0.07062     |
| B   | 10 1.7890 ± 0.02807  | 2.7610 ± 0.09780     |
| C   | 10 2.3470 ± 0.10636  | 1.6310 ± 0.09758     |
|     | Total 80             | 0.086                |
| L.S.D|                     | 0.16                 |

NOTE: The results represented as Mean ± SE. Differents letters refer to a significant differences
NOTE: Groups means (C= Control, T1= Gemzar 30 day, T2= Gemzar 60 days , T3= Bicallin 30 day , T4 =Bicallin 60 days , T5= combination group , T6= Gemzar30d & Baicalin 30d ,T7 =Baicalin 30d & Gemzar30d)

3.3 Interleukin-10 (IL-10)
The results showed a significant increase (P <0.05) in the concentration of IL-10 in the animals groups treated with the Gemzar drug for one month T1 and for two months T2, respectively, compared to the control group as well as the groups (T3,T4, T5, T6 and T7), while the results showed a significant increase in the IL-10 concentration of T7 group compared to control group mean as well as and (T3, T4, T5 and T6), while no significant difference between T3, T4, T5.

3.4 Interleukin-12 (IL-12)
The results showed a significant increase of (P <0.05) in the IL-12 concentration in animal's groups treated with Gemzar for one month T1 and for two months T2 respectively compared to the control group as well as the groups (T3, T4, T5, T6 and T7). While no significant difference was found among the mean groups (T3, T4, T5 and T6) among them.

Table 2. Shows the Interleukins levels of male rats treated with Baicalin and Gemzar
### Table 3. Show the phagocytosis index of male rats treated with Baicalin and Gemzar

| No. | Phagocytosis Index | Mean ± Std.Error |
|-----|--------------------|-----------------|
| C. 10 | 46.3700 ± 1.44430 | B                |
| T1 10 | 25.4400 ± 1.08589 | D                |
| T2 10 | 16.3600 ± 1.10877 | E                |
| T3 10 | 47.3900 ± 1.31626 | B                |
| T4 10 | 74.4200 ± 1.54566 | A                |
| T5 10 | 32.4000 ± 1.12940 | C                |

**NOTE**: The results represented as Mean ± SE. Differents letters refer to a significant differences

**NOTE**: Groups means (C= Control, T1= Gemzar 30 day, T2= Gemzar 60 days , T3= Bicallin 30 day , T4 =Bicallin 60 days , T5= combination group , T6= Gemzar30d & Baicalin 30d , T7 =Baicalin 30d & Gemzar30d)

### 3.5 Phagocytosis index

The results showed a significant decrease (P <0.05) in the mean of phagocytosis in animal's groups treated with Gemzar for one month T1 and for two months T2, respectively, compared to control group as well as the groups (T3, T4, T5, T6 and T7). The results showed a significant increase in the mean of phagocytosis of T3 and T4 groups treated with Baicalin for one month and two months respectively compared to the other groups, while the results showed a decrease in the mean of phagocytosis of T7 group compared to control group and T5 and T6 group. While the results showed an increase in the mean of phagocytosis of the T6 group compared to the T5 group.
### Table

|   | Count | Value (Mean ± SE) |
|---|-------|-------------------|
| T6 | 10    | 39.3600 ± 1.03668 |
| T7 | 10    | 26.0700 ± 1.40333 |

**Note**: The results represented as Mean ± SE. Different letters refer to significant differences.

**Groups means**: (C = Control, T1 = Gemzar 30 day, T2 = Gemzar 60 days, T3 = Bicallin 30 day, T4 = Bicallin 60 days, T5 = combination group, T6 = Gemzar30d & Bicallin 30d, T7 = Bicallin 30d & Gemzar30d)

#### Figures

**Figure 1**: Shows the phagocytosis index in male rats is treated with chemotherapy (Gemcitabine).

**Figure 2**: Shows the phagocytosis index in male rats is treated with Herbal extract (Baicalin).  

#### 3.6 W.B.Cs Differential counting

The results of the statistical analysis shown in Table (4) showed a significant decrease (P < 0.05) in the mean of lymphocytes in the animals treated with the Gemzar drug for one month (T1) and for two months (T2), respectively, compared to the control group as well as the groups (T3, T4, T5, and T6), while no significant difference was found between T3, T4, T5, and T6 groups. The results showed a significant decrease in (P < 0.05) in the mean of neutrophil cells in animals treated with Gemzar for one month (T1) and for two months (T2) respectively compared to control group as well as groups (T3, T4, T5, and T6). The results showed a significant increase in the mean of phagocytic cells and their ability to ingest yeast.
in the mean of neutrophil cells of T3 and T4 groups treated with Baicalin for one month and two months respectively compared to other groups. While the results showed a decrease in the mean of T7 group in comparison to control group, T5 and T6. The results of the statistical analysis shown in Table (4-7) showed a significant decrease (P <0.05) in the mean of monocytes in animal's groups treated with the Gemzar drug for one month T1 and for two months T2 respectively compared to the control group as well as the groups (T3, T4, T5 and T6). While the results indicated increase of mean in T3 group compared to T4 group, as well as increase mean of T5 group compared to the group T6.

Table 4. Shows WBCs Differential count of male rats treated with Baicalin and Gemzar

|          | Lymphocytes | Neutrophil | Monocytes |
|----------|-------------|------------|-----------|
|          | Mean ± Std.Error | Mean ± Std.Error | Mean ± Std.Error |
| C.       | 66.9840 ± 0.36346 | 22.6550 ± 1.06480 | 5.5700 ± 0.08035 |
| T1       | 48.7500 ± 0.20723 | 12.6900 ± 0.07810 | 4.7200 ± 0.05333 |
| T2       | 36.1010 ± 0.26913 | 10.0140 ± 0.15250 | 3.3800 ± 0.05538 |
| T3       | 69.1460 ± 0.22674 | 22.7390 ± 0.14151 | 6.0470 ± 0.09500 |
| T4       | 66.7460 ± 0.42082 | 21.0300 ± 0.14471 | 5.3170 ± 0.08891 |
| T5       | 69.1460 ± 0.22674 | 19.7390 ± 0.23291 | 6.6270 ± 0.01961 |
| T6       | 69.4560 ± 0.19941 | 22.8990 ± 0.17015 | 6.0800 ± 0.09127 |
| T7       | 55.1500 ± 0.14395 | 19.0100 ± 0.08876 | 5.6200 ± 0.06799 |

NOTE: The results represented as mean ± SE. Differents letters refer to a significant differences
NOTE: Groups means (C= Control, T1= Gemezar 30 day, T2= Gemezar 60 days , T3= Bicallin 30 day , T4 =Bicallin 60 days , T5= combination group , T6= Gemzar30d & Baicalin 30d , T7 =Baicalin 30d & Gemzar30d)

4. Discussion
4.1 Antioxidant Assay

The results of the current study in the rats group were treated with a Gemzar drug in T1 and T2 groups significant increase in MDA with a significant decrease in GSH. The results of this study are in agreement with previous studies on levels of antioxidants in animals treated with anti-cancer drugs treatment and other chemical compounds. Results of the current study agreed with [19]-[20].

Lipid peroxidation is an indicator of harmful oxidative stress in tissue and oxidation causes damage to cellular fat content, MDA is the final product of lipid peroxidation as an indicator of oxidative damage in various diseases [21]. Increased MDA in the tissues is indicative of oxidative stress caused by Gemzar drug.
In animal groups T3 and T4 treated with the flavonoid (Baicalin) for a month and two months, respectively, showed a decrease in MDA, and increased in GSH. While groups of animals T5 treated with Gemzar drug for a month and then Baicalin for a month, as well as group T6 treated with Baicalin combination with the chemotherapy anti-cancer drug for two months, showed significant improvement in antioxidant such as GSH and decreased MDA compare with control group. The present study agrees with [22], which mentioned that Bicalin is one of the most powerful natural antioxidants and inhibits the production of free radicals [23]-[24], and it has strong activities on eliminating the superoxide radical.

This is agreeing with other studies such as [25]-[26]. These studies indicated that the use of flavonoids such as Quercetin, where it has the ability to increase the effectiveness of glutathione, which is the main regulator of the reactions of oxidation and reduction in cell.

Baicalin also inhibits Xanthine oxidase [23], which is the main enzyme in the process of lipid peroxidation and thus prevents this process and protects the cell membrane and mitochondria. Xanthine oxidase is one of the enzymes involved in the process of generating free radicals [27].

4.2 Immunological Assay

The results of the current study showed a significant increase in both IL-10 & IL-12 in animal groups T1 and T2 treated with anti-cancer chemotherapy (Gemzar). The results of the current study were agreed with [28]-[29], which have used Gemzar drug or another chemotherapy drug in the treatment of cancer of the pancreas and colon.

Chemotherapy is described as an anti-cancer chemical drug called the therapy of cell toxicity, which destroys rapidly growing cancer cells and stops their growth and division, cancer cells divide and grow rapidly. Chemotherapy disrupts the process of dividing cancer cells and eliminating them [30].

Despite the positive benefit in the use of chemotherapy in the treatment of tumors and resistance to cancer cells, but one of the main limitations in the treatment currently available for cancer is its side effects, which works to kill the natural defense cells in the body of the patient so weaken the immune system of people infected with the disease [31].

Therefore, chemotherapy causes weakness in the immune system and its inability to protect the person. This leads to an increase level of IL-10, because the IL-10 is an immunosuppressive, and its inhibition may restore immunity later [32]. IL-10 promotes the loss of tumorigenicity and induce protection of memory cell response against tumor by NK cell and CD8 T. cell [33]. IL-10 was originally described as the growth factor of B cells and was used for therapeutic purposes to inhibit the proliferation of Th1 lymphocytes and cytokine production [34].

IL-12 is the strongest anti-tumor and anti-metastatic cytokine. It stimulates NK cells, and promotes the maturation of CTL, also induce the production of IFN which has a role as an effective molecule to initiate Th1 response [35]. IL-12 inhibits Th2 cells and their production of cytokine [36]. The high levels of IL-12 in animals treated with chemotherapy anti-cancer drug is evidence of tissue damage and the occurrence of tumors in cells organs.

While the results showed a significant improvement in the level of cytokine in both IL-10 and IL-12 in the animal groups T3 and T4 and which treated with herbal extract (Baicalin) for one month and two months respectively. As well as T6 which treated with Baicalin combination...
Gemzar for two months compare with control group. The results of the current study were agreed with each study [37]-[38].

Baicalin is one of the best and most powerful antioxidants that play a role in protecting the body and improving its immunity. Previous studies have shown that Baicalin helps in regulating the immune balance and decreases the inflammatory reaction by facilitating the proliferation of CD4 + CD29 + cells and modifying the immunosuppressive pathways [39]. As well as previous studies have shown that Baicalin plays a major role in regulating the immune response especially in autoimmune diseases and inflammation reaction by promoting the expression of Foxp3, which plays a critical role in activation of Tregs cells [40].

Baicalin has a critical role in decreasing the level of IL-12 by decreasing the NF-kB proteins expression. Studies have shown that the elevated of IL-12 leads to atherosclerosis and the development of cardiovascular complications. Baicalin is therefore considered an anti-atherosclerotic and cardiovascular disease through its role in decreasing of IL-12 level [41]. According to the cancer immune editing theory, inhibition of immune functions of the immune system through overproduction of immunosuppressive cytokines such as IL-10, it may also facilitate fighting the tumor [42]. In this current study it was observed Biacalin significantly decreases in production of IL-10.

4.3 Phagocytosis index

The results of the present study showed a significant decrease in T1 and T2 groups which treated with Gemzar drugs for one month and two months respectively. Chemotherapy causes weakness in the immune system and its inability to protect the person. This increases the level of IL-10, because IL-10 is an immunosuppressive agent, and its inhibition can subsequently restore immunity [32]. As long as the person's immune system is suppress, it will lead to inhibit of the inflammatory response person, thus decreasing the activity of macrophages, which are considered to be the most important immune cells.

While significant increases were observed in the groups of animals treated with Baicalinin T3 and T4, and there was also a significant improvement in T6 treatment group with Baicalin in combination with the Gemzar drug for two months compared to T1 and T2 groups.

Baicalin shows a moderate anti-inflammatory effect on LPS that stimulates the activation of macrophages [43]. The mechanisms show that the baicalin activates the receptor of estrogen on the macrophages [13]. When the estrogen receptor is activated on the macrophages, it causes anti-inflammatory effects by inhibition of NF-Kb activation [44]. This indicates the high level of macrophages in animals treated with Baicalin in this study.

4.4 W.B.C.s Differential counting

The results of the present study showed a significant decrease of Lymphocytes, neutrophils and monocyte in T1 and T2 groups which treated with Gemzar drugs for one month and two months respectively. The results of the current study are degree with the study [45].

Chemotherapy causes defect in the immune system and its inability to protect the man. This leads to an increase level of IL-10, because the IL -10 is an immunosuppressive, and its inhibition may restore immunity later [32]. IL-10 was originally described as the growth factor of B cells [46] and was used for therapeutic purposes to inhibit the proliferation of Th1 lymphocytes and cytokine production [34]. Thus decreasing the level of neutrophil, and
monocytes in animals treated with Gemzar drug leads to weakness in the level of the body's defenses and low inflammatory response and lack of macrophage.

While significant increases were observed in the groups of animals treated with Baicalin T3 and T4, and there was also a significant improvement in T6 treatment group with Baicalin in combination with the Gemzar drug for two months compared to T1 and T2 groups. Increase in lymphocytes is agree with the [47]. Baicalin is one of the most powerful antioxidants, this indicates that Baicalin as an antioxidant increased the immune function of the rats as shown with the number of white cells. This explains the improvement in levels of monocytes and neutrophils.

The use of antioxidants in pharmacology has been widely studied, as oxidative stress may be an important part of many human diseases, especially stroke and neurodegenerative incidents [48]. Therefore, the antioxidants are routinely added to meals, oils, nutrients and other substances to prevent free radical damage.

5. Conclusion

Baicalin has a significant preventive role in reducing oxidative stress induced by chemotherapy drugs and has a significant role in improving immune and defensive lines of the body against pathogens.

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