Ameliorating Effects of *chrysanthemum* Against Capecitabin in Albino Rats

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Abstract

Our study was carried out to evaluate the effect of *Chrysanthemum ethanolic extract* on some hematological parameters in the anticancer drug capecitabine (XELODA®) exposed male rats using comet assay and biochemical changes. Thirty-six albino rats were divided to 6 groups (6 animals each) as follows:1) capecitabine group (xeloda at dose of 30 mg/kg bw as a positive control); 2) capecitabine + *chrysanthemum* 5 mg/kg); 3) capecitabine (xeloda) + *Chrysanthemum* (10 mg/kg); 4) *Chrysanthemum* (5 mg/kg) 5) *Chrysanthemum* (10 mg/kg); 6) Control group (negative control) for 45 days. The results of this study led to the following: capecitabine (xeloda)treatment induce decrease in RBCS, leukocytes, and platelets counts, level of Hgb and Hct, while the use of capecitabine (xeloda)in combination with *Chrysanthemum* improved these alterations especially with the high dose of *Chrysanthemum*. Finally, the data suggest that the synchronous use of *Chrysanthemum* with capecitabine (xeloda)t reatment will be useful to decrease the side effects of capecitabine.

Keywords: *Chrysanthemum*- capecitabine-hematological parameters

Introduction

The use of synthetic chemotherapy in cancer treatment is insufficient due to their adverse effects which are responsible for impaired organ function,As most chemotherapeutic agents cause liver and kidney disorders. The liver is the most active organ and so responsible for the majority of drug metabolism. Most metabolically active synthetic agents against tumor cell cause oxidative stress and led to injury ofthese tissues ***(Premkumar et al., 2001)***

Capecitabine (XELODA®) is one of anti-cancer drugs. It is one of the most effective oral types of chemotherapy against recurrent breast cancer. Also, capecitabine

could widely be used for treatment of colon cancer ***(Fujii et al., 2008)***

Several studies indicated that capecitabine caused several adverse effects, among them hematological disorders, neutropenia, anemia and thrombocytopenia **(Nabavizadeh et al., 2016).**

Because of the serious and different side effects of some anti-cancer drugs, there is a great need for new therapies to cure and prevent cancer. Scientific and research interest is going towards naturally derived compounds like Plant kingdom as they are considered to have less toxic side effects compared to current treatments such as chemotherapy also some plants have protective role against side effect of some anticancer drugs ***(Greenwell and Rahman, 2015)***

The possibility of combining plant with anticancer drugs offers very valuable advantages such as the building of more efficient anticancer treatment with less side effects for example *Chrysanthemum* can be used as a protective agent against liver & kidney damages and genotoxic effect caused by anticancer drugs ***(Ahmad et al., 2015,*** and ***Linjawi, 2015)***

Chrysanthemum is a dicotyledonous plant from family Asteraceae. These herbaceous annual plants have ornamental, medicinal, environmental and industrial values. ***(Hadizadeh et al., 2022)***

Many phytochemical compounds, including flavonoids, terpenoids, polysaccharides and unsaturated fatty acids have been isolated from the genus *Chrysanthemum*. This genus has also biological features including antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-allergic, anti-obesity, immune regulation, hepatoprotective and nephroprotective activities ***(Samiei*** and ***Shakeri, 2022)*.**

Chrysanthemum is also proved to be effective in inhibiting the agglutination of blood platelets and improving the myocardial blood circulation and white cell phagocytosis, therefore it was used to treat many diseases. However, the pharmacological activity and bioactive constituents of this natural medicine are left uncharacterized ***(Liang-Yu et al., 2010).***

Concerning the Toxicity of Chrysanthemum, studies revealed that the *chrysanthemum* does not cause acute or chronic toxicity. When rats were orally administered *Chrysanthemum* extract in doses of 320, 640, and 1280 mg/kg bw for consecutive 26 weeks. There was no death occurred, no abnormal signs or change on food and water intake, the corpuscular hemoglobin (MCH), corpuscular hemoglobin concentration (MCHC)or platelet (PLT) count were in normal range, serum level of AST, ALT were in normal range. Thus, *Chrysanthemum* is considered to be safe in general in rats ***(Li et al., 2010)***

Hence, this study was designed to examine the effect of

Chrysanthemum ethanolic extract on the hematological picture in the anti-cancer drug Capecitabine exposed albino rat for 45 days using a variety of analytical tools and techniques. This aim has been achieved through measurement of RBCS, leucocyte and platelet count. Besides, the levels of hemoglobin (Hgb) and hematocrit (Hct) were estimated.

Materials and Methods

A totally of 36 healthy albino rats were used in this study. The rats weighting 80 ± 10 gm were obtained from the Laboratory Animal Resource Center Faculty of Veterinary Medicine Suez Canal University Ismailia Egypt. They were kept for 2 weeks for acclimatization. The animals were kept in stainless steel cages at normal atmospheric temperature of 27 °C ± 5 as well as 50–60% relative humidity) under good ventilation and fed a standard ration (72% corn, 27% soya bean, and 1% fish meal) with free access to water and feed.

Animals were divided to six groups (6 animals/group) as follow:1) capecitabine group (xeloda at dose of 30 mg/kg bw as a positive control) ***(Olayinka et al., 2017)***; 2) capecitabine (xeloda)+ *Chrysanthemum*(5 mg/kg); 3) capecitabine + *Chrysanthemum* (10 mg/kg); 4) *Chrysanthemum* (5 mg/kg) 5) *Chrysanthemum* (10 mg/kg); 6) Control group (negative control) for 45 days. Rats were sacrificed 24 hours after the last dose and blood samples Were taken for toxicological and biochemical investigations.

Chrysanthemum ethanolic extract prepared as following: the corolla of the *Chrysanthemum were purchased commercially. Extract prepared using 500 g dried plant via extraction with 4* L of ethanol 95 % at room temperature for 3 days. Then the solution was centrifuged, filtered, evaporated, and freeze-dried. The residue (100 mg) was then dissolved in 1 ml of water ***(Tsuji-Naito et al., 2009)***

Capecitabine (Xeloda®) 500 mg tablets were purchased from Roche Registration Inc.

By the end of the experimental period at 45 days, blood samples were taken in empty, dry, and clean tubes for serobiochemical analysis, blood samples were maintained in a water bath set on 37 °C for 15 minutes and then centrifuged at 3000 r.p.m. for 10 minutes and the clear serum was separated carefully. Other 2 mL of blood was collected in test tube contain anticoagulant EDTA and used for estimating the hemogram parameters (RBC, Hgb, Hct and TLC) were determined according to the standard techniques described by ***(Jain, 1986)***

Statistical Analysis:

Data were expressed as means ± standard error (SE). The Statistical Processor System Support (SPSS) version 10 computer program was used to evaluate all of the acquired

data. The significance of variations in mean values between control and treated rats was determined using the one-way analysis of variance (ANOVA) test followed by Duncan's post hoc test for multiple group comparisons. Statistical significance was defined as a value of $p<0.05$. ***(Tello and Crewson, 2003).***

Percentage of change:

It is the ratio between experimental and control.

values, calculated in percentage according to the following equation.

$$\% \text{ of change} = \frac{\overline{X}_1 - X_2}{X2} \times 100$$

Where:X1: The mean of measurements of the experimental groups.

X2: The mean of control group

Results

The impact of xeloda and/or *Chrysanthemum* ethanolic extract on RBCS, leukocytes, and platelets count, beside its effect on Hgb and HCT was investigated in the present study.

From the data tabulated in Table (1) and graphically represented by Figures (1, 2, 3, 4, 5) respectively, it was denoted that a significant decrease in RBCs, leukocytes and platelets count as well as the Hgb and HCT levels ($p < 0.05$) was recorded in anticancer treated rats (group 1) after 45 days of the experimental period compared with the negative control group (group 6). Anticancer (30mg) +Chrysanthemum (5 and 10 mg/kg bw) treated animals (groups 2&3) showed a significant increase ($p < 0.05$) in all previous hematological parameters compared to positive control rats. While administration of *Chrysanthemum* ethanolic extract only (5 and 10 mg/kg bwt) in groups (4&5) induced non-significant changes compared to the control negative group.

Table (1): *Impact of xeloda and/or Chrysanthemum ethanolic extract on RBCs, leukocytes and platelets count as well as the Hgb and HCT levels after 45 days of the experiment.*

Group	RBCs (10^6/ml)	Hbg (gm/ml)	Hct (%)	WBCs (10^3/ml)	platelet(10^3/ml)
1	3.683±0.0703[a]	8.616±0.975 [a]	22.866±0.785 [a]	5.433±0.284 [a]	461.83±17.135 [a]
2	4.716±0.1701[b]	10.416±1.175 [b]	31.161±0.364 [b]	10.116±0.436 [b]	549.00±13.839 [b]
3	4.933±0.0954 [b]	10.60±1.141 [b]	33.566±0.491 [b]	10.78±0.538 [b]	568.0±11.024 [b]
4	6.716±0.2315[c]	12.45±1.132 [c]	40.33±0.666 [c]	17.033±0.463 [c]	737.50±25.53 [c]
5	6.866±0.1891 [c]	12.80±1.132 [c]	40.00±0.683 [c]	17.00±0.394 [c]	771.50±37.91 [c]
6	6.516±0.3156 [c]	13.31±1.266 [c]	40.80±0.872 [c]	16.20±0.624 [c]	749.66±11.94 [c]

Values are presented as means ± S.E

Different small superscript letters indicate significancy in the same column ($p \leq 0.05$).

group 1 administrated xeloda 30 mg/kg b.w. orally

group 2 administrated orally (xeloda 30 mg/kg + chrysanthemum 5mg/kg Bw)

group 3 administrated orally (xeloda 30 mg/kg + chrysanthemum 10mg/kg Bw)
group 4 administrated orally chrysanthemum 5mg/kg Bw
group 5 administrated orally chrysanthemum 10mg/kg Bw
group 6 kept without treatment served as negative control group.

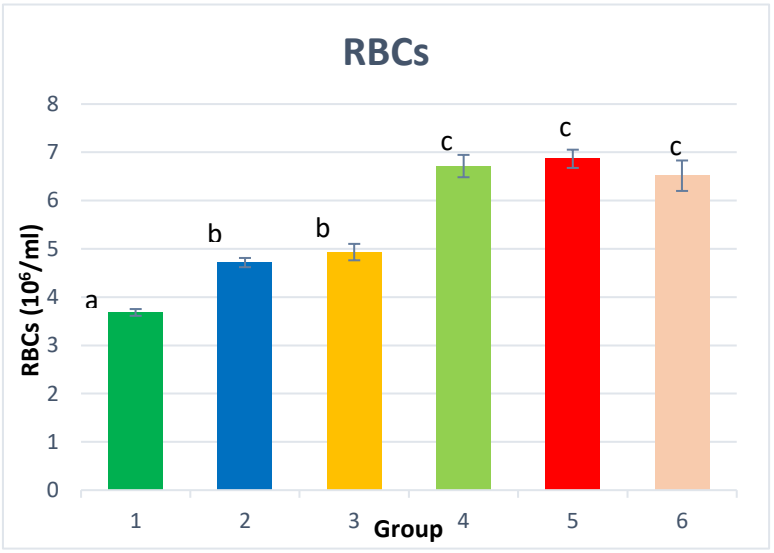

Figure (1): Impact of xeloda and/or *Chrysanthemum* treatment on count of RBCs.
Different small superscript letters Indicat significant differences

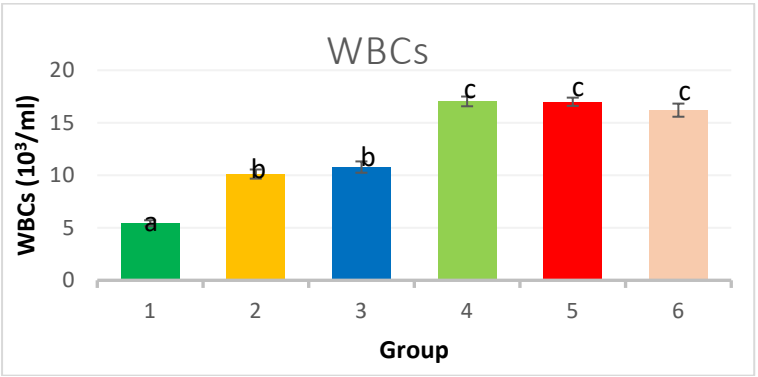

Figure (2): Impact of xeloda and/or *Chrysanthemum* treatment on count of Leukocytes.
Different small superscript letters Indicat significant differences.

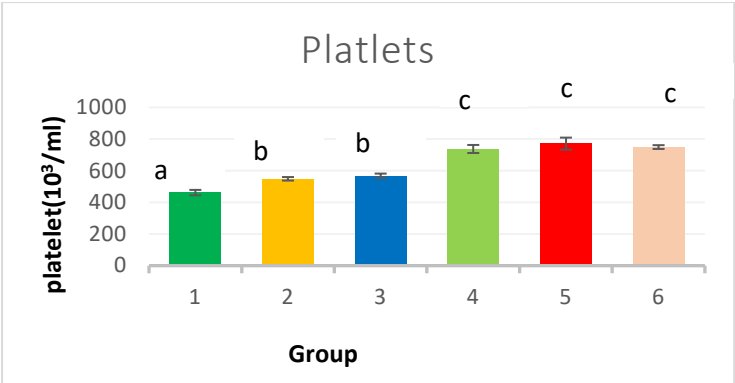

Figure (3): Impact of xeloda and/or *Chrysanthemum* treatment on count of platelets.
Different small superscript letters Indicat significant differences.

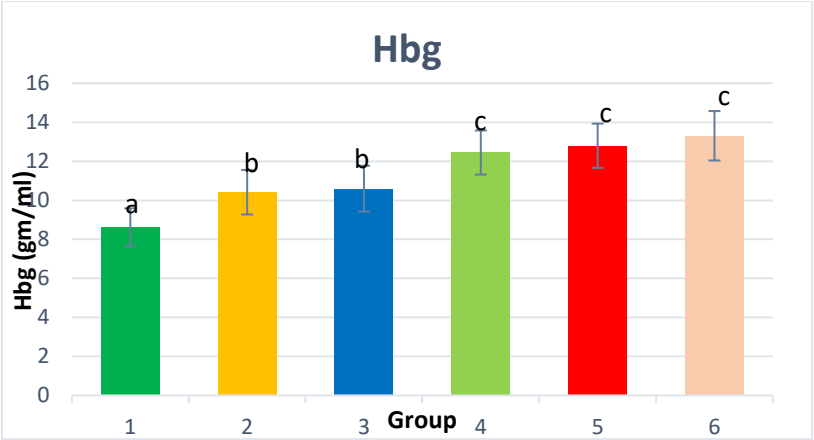

Figure (4): Impact of xeloda and/or *Chrysanthemum* treatment on the level of Hgb.
Different small superscript letters Indicat significant differences.

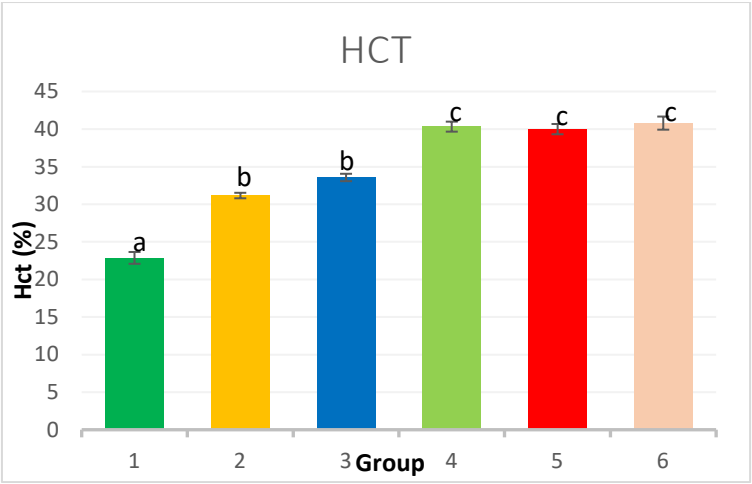

Figure (5): Impact of xeloda and/or *Chrysanthemum* treatment on the level of Hct.
Different small superscript letters Indicat significant differences.

Discussion

Our results agree with ***Nabavizadeh et al. (2016)*** Who investigated that the use of capecitabine in cancer treatment can lead to several adverse effects, among them hematological disorders, neutropenia, anemia and thrombocytopenia. Capecitabine adverse response on blood cell may be due to bone marrow depression.

The ameliorative effect of *Chrysanthemum* on the hematological picture in group 2, 3 in Table (1) and graphically represented by Figures (1, 2, 3, 4, 5) is due to volatile oil and flavonoids which are the main active components in *Chrysanthemum*. And this agree with ***Ahmad et al. (2015)*** who found that the antioxidant properties of flavonoids extracted from *Chrysanthemum* could have been responsible for its broad pharmacological effects. The alcoholic extract of *Chrysanthemum* may reduce lipid peroxidation and plays a role in protecting against damages to the cell.

In conclusion, the data suggest that the complimentary use of Chrysanthemum with capecitabine treatment will be beneficial to reduce the adverse effect of capecitabine in chemotherapy, Besides, our study confirmed the safety of Chrysanthemum under such dose and rout of administration.

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التأثيرات المحسنة لنبات الأقحوان ضد دواءالكابيستابين المضاد للسرطان فى الجرذان البيضاء

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الملخص العربى

كان الهدف من هذه الدراسة هو التحقق من التأثير المحسن للاقحوان كنبات طبيعى ضد سمية الكابيستبين (زيلودا) كدواء مضاد للسرطان

فى هذه الدراسة تم استخدام عدد 36 من الجرذان البيضاء وتم تقسيمها الى ستة مجموعات كل مجموعة منها مكونة من ستة جرذان كالتالى:

المجموعة الأولى تلقت دواء زيلودا المضاد للسرطان بالفم بجرعة 30 ملجم / كجم من وزن الجسم يوميا لمدة 45 يوما متتالية وتعتبر مجموعة ضابطة إيجابية

المجموعة الثانية تم تجريعها عن طريق الفم بدواء زيلودا30 ملجم / كجم من وزن الجسم + أقحوان 5 ملجم / كجم من وزن الجسم يوميًا لمدة 45 يومًا متتالية

. المجموعة الثالثة تم تجريعها عن طريق الفم بدواء زيلودا 30 ملجم / كجم من وزن الجسم + أقحوان 10 ملجم / كجم من وزن الجسم يوميًا لمدة 45 يومًا متتالية

المجموعة الرابعة تلقت عن طريق الفم الأقحوان بجرعة 5 ملجم / كجم من وزن الجسم يومياً لمدة 45 يوماً متتالية

المجموعة الخامسة اعطيت عن طريق الفم الأقحوان 10 ملجم / كجم من وزن الجسم يومياً لمدة 45 يوماً متتالية

المجموعة السادسة التي بقيت بدون علاج خدمت كمجموعة تحكم سلبية

امتدت مدة التجربة 45 يوما تم تجميع عينات الدم من الضفيرة الوريدية خلف مقلة العين لجميع الفئران بعد 45 يوما من بداية التجربة

بالنظر الى التحليل الاحصائى للنتائج

وجد نقص فى عدد كرات الدم الحمراء وعدد الكريات البيضاء والصفائح الدموية ومستويات الهيموجلوبين وحجم الخلايا الحمراء المكدسة بشكل ملحوظ في المجموعة 1 التى تلقت عقار الكابيستبين فقط وكان هناك زيادة معنوية فى عدد كرات الدم الحمراء وعدد الكريات البيضاء والصفائح الدموية وحجم الخلايا الحمراء المكدسة و الهيموجلوبين بشكل ملحوظ في المجموعتين3،2 (التى تم فيها الاستخدام المتزامن للمستخلص الكحولى لنبات الأقحوان مع دواء الزيلودا) مقارنة بالمجموعة 1 (مجموعة التحكم الإيجابية).

أخيرًا ، من تلك النتائج يمكننا أن نستنتج أن استخدام الأقحوان بالتزامن مع دواء زيلودا المضاد للسرطان يمكن أن يكون مفيدًا فى تقليل الآثار الضارة للكابسيتابين المضاد للسرطان (زيلودا).