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The combinatorial treatment with Kaempferol and β-sitosterol attenuates the hematological and lipid profile alterations induced by cisplatin in rats.

Islam Elebshany, Hala Abdel-Azeem, Zeinab Attia*, Mohamed Shahen*

Department of Zoology, Faculty of Science, Tanta University, Tanta 31527, Egypt

***Corresponding authors**:
Dr. Zainab Attia, Ph.D
Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
Email: zainab.attia@science.tanta.edu.eg

Dr. Mohamed Shahen, Ph.D
Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
Email: Mshahen@science.tanta.edu.eg

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Abstract

Chemotherapeutic agents are in use for cancer, however, these agents showed severe side effects on vital organs upon treatment. This study aimed to evaluate the protective effect of the combinatorial treatment with Kaempferol (Kpf) and β-sitosterol (Bs) in hematological parameters and lipid profile alterations induced by cisplatin (Cis) toxicity in rats. Sixty male Sprague-Dawley rats were divided into five groups (N = 12). The first group (Gp1) served as a negative control. From Gp2 to Gp5, rats were fed on a high-fat diet (HFD) for 4 weeks, then Gp2 was injected with a single dose of Cis (7mg/kg B.Wt). Gp3, Gp4, and Gp5 were injected with Cis as in Gp2, then administered with Kpf (50mg/Kg B.Wt), Bs (50mg/Kg B.Wt) or Kpf /Bs as in Gp3, Gp4, and Gp5, respectively. Blood samples were collected in heparinized and non-heparinized tubes for hematological and lipid profile investigations. The results showed that Cis treatment led to a significant decrease in the cellular compartments of blood and increased the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Cis treatment also caused an increase in the total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C), atherogenic index (Risk 1), lipoprotein (a), and decrease the high-density lipoprotein (HDL-C). The treatment with a combination of Kpf/Bs after Cis ameliorated the above-mentioned hematological and lipid profile alterations.

Keywords: Chemotherapeutic agents, Cisplatin, Hematological parameters, Kaempferol, β-sitosterol, Lipid profile, Alterations

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Introduction

Cisplatin (Cis), a platinum anti-cancer agent, is used in many solid tumor treatments, such as head, kidney, neck, bladder, lung, testis, and ovary cancers (1). It has been reported that the cardiotoxicity caused by Cis is mainly manifested in changes of electrocardiogram (ECG), arrhythmia, acute myocardial infarction, and autonomic cardio-vascular dysfunction (2). Nevertheless, in Cis side effects, a strong body of evidence demonstrates that two important factors are inflammation and excessive reactive oxygen species (ROS) generation (3). Because β-sitosterol (Bs) and cholesterol share structural similarities, it is thought to function by preventing the small intestine from absorbing cholesterol, which can lower blood levels of bad

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cholesterol, or low-density lipoprotein (LDL-C). Bs may reduce the levels of apolipoprotein by decreasing the amount of cholesterol that is transported by high-density lipoprotein (HDL-C) (4, 5). Cis, as an antineoplastic drug, is widely used in the clinic, accompanied by such side effects as nephrotoxicity, and hepatotoxicity (6).

In addition to its interaction with cellular DNA, the changes in various biochemical enzymatic parameters, immune response, and cell surface, have also been observed (7, 8). Enzymatic changes have also been implicated in the mechanism of action of Cis (8). Triglycerides, cholesterol, phospholipids, fats, and steroids are examples of lipids, which are crucial biological components of bodily tissues and organs (9). Lipoproteins transport lipids throughout the bloodstream. Blood levels of cholesterol are elevated in atherogenic individuals who carry cholesterol indicating the existence of elevated atherogenic lipoproteins that could get stuck in the subendothelial region and be subjected to oxidation and scavenging by artery macrophages, which would cause endothelial dysfunction, and the formation of fatty and atherosclerotic (10, 11). Atherogenic lipoprotein levels that are higher encourage atherogenesis (12). When it comes to forecasting the risk of atherosclerotic cardiovascular disease (ASCVD), apolipoprotein levels and non-high-density lipoprotein cholesterol (non-HDL-C) are more reliable indicators than testing the cholesterol carried by atherogenic low-density lipoproteins (LDL-C) (13). Every atherogenic lipoprotein has one apolipoprotein molecule. Non-HDL-C is the total amount of cholesterol carried by atherogenic lipoproteins (non-HDL-C is calculated as total cholesterol minus HDL-C).

Kaempeferol (Kpf) is a kind of flavonoid that widely exists in all kinds of vegetables and fruits. Kpf has anti-inflammatory and antioxidant activities (14). It has a role in vascular smooth muscle contraction therefore; it may provide novel treatment to improve heart function for hypotrophy and heart failure (15). Bs is a compound discovered to be present in numerous plants (16). It is one of the common phytosterols that are immuno-modulating, anti-inflammatory, anticancer activity, and non-alcoholic fatty liver disease prevention. It competes with cholesterol for absorption due to similarity in their structure therefore used as an anti-hyper lipidemic agent (17). Several natural products showed potential ameliorative effects against chemotherapeutic toxicities (18). Owing to the limited number of studies, in male albino rat models, this study aimed to assess the ameliorative effects of Kpf and Bs or their combination against side effects induced by Cis in rats.

Materials and Methods

Chemicals

Cisplatin (10 mg/vial) was acquired from Mylan Institutional LLC in the United States. Kaempeferol and β-sitosterol were purchased from Sigma-Aldrich (USA).

Animals

Sprague-Dawley rat strain at (150 ± 10 g), 6-7 weeks old were bought in Dokki, Giza, Egypt from the Holding Company for Biological Products & Vaccines (VACSERA). The experimental protocol was authorized by the Tanta University Faculty of Science's Research Ethical Committee (REC) and the Institutional Animal Care Committee with approval number IACUC-SCI-TU-0169**.**

Experimental groups

Rats were grouped into five groups (N=12) and left under normal conditions with free access to food and water. The rats were housed in typical laboratory settings for the current investigation. (25±2°C; 65±5% relative humidity; 12/12 hr. light cycle). The rats were kept in polypropylene cages and received the standard pellet diet. The control group (Gp1) was given a typical, well-balanced meal. Gp2 (Cis -group) received a single intraperitoneal (i.p.) injection of Cis (7 mg/kg) after receiving a high-fat diet (HFD) for four weeks (19, 20). Gp3 was injected i.p. with Cis and then orally received Kpf for 4 weeks every day with 50 mg/kg/day (21). Gp4 was injected i.p. with Cis and then orally received Bs for 4 weeks (50 mg/kg/day) (22). Gp5 was injected i.p. with Cis and

then orally received a combination of Kpf and Bs for 4 weeks (50 mg/kg/day).

Blood samples

All rats were fasted and then sacrificed following isoflurane anesthesia, which took place after four weeks. Blood samples were collected in two vials, one with an anticoagulant for hematological analysis. Complete blood count (CBC) was counted by an automated method using a Sysmex 550 automated hematology analyzer. Blood samples were collected without anticoagulant to estimate different biochemical parameters of lipid profile, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and lipoprotein (a) which was estimated as described by Cohn et al. (1988) (23), Foster and Dumns (1973)(24), and Friedewald et al. (1972) (25).

Statistical analysis

Mean ± SD was used to express the data. ANOVA and Dennett's test as a post hoc test were used to compare treatment groups statistically with controls. p-values less than 0.05 were regarded as significant.

Results

Kaempferol and β-sitosterol attenuated hematological parameters.

Severe leukopenia for W.B.C.s count ($p \leq 0.05$) was reported in Gp2 (Cis-alone.). Treatment with Kpf (Gp3) group 3 or with Bs (Gp4) partially ameliorated the effect of the treatment with Cis. The treatment with a combination of Kpf and Bs significantly increased the WBCs count when compared to Gp2. The R.B.C.'s count was significantly decreased in Gp2, Gp3, and Gp4 in comparison with Gp1 (control) ($p \leq 0.05$). The treatment with a combination of Kpf and Bs (kaempferol and β-sitosterol) after Cis-injection restores the R.B.C.s count. Hb%. The HCT% value was significantly decreased in Gp2, Gp3, and Gp4 when compared to Gp1($p \leq 0.05$). The mean corpuscular volume (MCV) was significantly increased in Gp2 and Gp3 when compared to Gp1. Treatment with Bs (Gp4) or with a combination of Kpf and Bs (Gp5) restored the percentage of MCV close to normal value. The mean corpuscular hemoglobin (MCH) was significantly increased in Gp2 when compared to Gp1 ($p \leq 0.05$). The treatment with Kpf and Bs restored the level of MCH. The mean corpuscular hemoglobin concentration (MCH-C) was significantly ($p \leq 0.05$) increased in Gp2 when compared with Gp1 (control). Moderate thrombocytopenia was reported in Cis-treated rats (Gp2), Cis/Kpf (Gp3), and Cis/Bs (Gp4) when compared with the control group (Gp1). Treatment with a combination of Kpf/Bs after Cis treatment restored the platelets count close to normal value (Table 1).

Kaempferol and β-sitosterol attenuated dyslipidemia:

The results showed that the treatment with Cis increased the levels of total cholesterol (TC) and triglycerides (TG) in Gp2 when compared with Gp1($p \leq 0.05$). The treatment with Kpf after Cis injection led to a significant decrease in the levels of TC and TG when compared to rats treated with Cis alone (Gp2) ($p \leq 0.05$). The data also showed that the treatment with Bs post-Cis-treatment did not alter the values of TC and TG when compared to Cis-treated rats alone. The treatment with the combination of Kpf/Bs after Cis treatment restored the values of TC and TG close to normal levels (Table 2) ($p \leq 0.05$). The results showed that the treatment with Cis decreased the levels of HDL-C and increased the level of LDL-C in Gp2 when compared with Gp1. The treatment with Kpf after Cis injection led to a significant increase ($p \leq 0.05$) in the level of HDL-C and a decrease in LDL-C when compared to rats treated with Cis alone (Gp2). The data also showed that the treatment with Bs post Cis-treatment did not alter the values of HDL-C and LDL-C when compared to Cis-treated rats alone. Combinatorial treatment with Kpf/Bs after Cis-treatment restored the values of HDL-C and LDL-C close to their normal values (Table 2). The data showed that the treatment with Cis increased the atherogenic index (Chol/HDL-C) in Gp2 ($p \leq 0.05$) when compared with Gp1. The treatment with Kpf, Bs, or their combination after Cis injection led to a significant ($p \leq 0.05$) decrease in this ratio when compared to rats treated with Cis alone (Gp2).

Table 1. Hematological parameters in different groups of the study

Groups	Controls	Cis	Cis/Kpf	Cis/Bs	Cis/Kpf and Bs
RBCs $\times 10^6$/ µL	8.47±0.69	4.01±0.47*	4.75±0.41*	5.3±0.8*	7.73±0.89
Hb (g/dL)	14.36±1.12	9.93±0.53*	10.29±0.9*	10.20±0.91*	12.74±1.22
Hct%	38.28±3.4	21.65±1.47*	27.79±2.56*	25.63±2.26*	36.49±3.72
MCV fL	46.21±3.99	52.92±5.49*	51.37±4.74*	46.19±5.1	47.29±3.2
MCH (Pg)	17.23±1.92	25.59±3.19*	18.23±2.09	19.03±2.6	17.33±2.4
MCHC (g/dl)	37.75±2.85	49.19±5.3*	39.23±3.19	38.20±3.33	38.18±2.68

Data represented as mean ± SD. * indicates a significant change (P<0.05) in comparison with control.; Cis: Cisplatin; Kpf: Kaempferol; Bs: β – sitosterol; RBCs: Red blood cell; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration.

Table 2. Lipid profile in different groups of the study.

Groups	Cont.	Cis	Cis/Kpf	Cis/Bs	Cis/Kpf /Bs
TC (mg/dL)	178.5±14.9	388.3±20.8*	192.2±18.34	302.8±34.8*	191.4±18
TG (mg/dL)	95.20±9.42	272.9±34.9*	104.7±12.0	184.2±12.2*	103.2±9.5
HDL (mg/dL)	69.5±8.39	30.2±4.23*	62.40±7.6	50.5±5.19*	66.0±9.8
LDL (mg/dL)	107.1±7.6	308.2±13.0*	117.4±14.7	247±19.2*	111.1±14.6
(Chol/HDL-c)	2.62± 0.49	10.11±1.00*	2.95± 0.36	4.74± 0.65*	3.06± 0.76

Data represented as mean ± SD. * indicates a significant change (p < 0.05) in comparison with control. Cis: Cisplatin; Kpf: Kaempferol; Bs: β – sitosterol; HDL: High-density lipoproteins; LDL: Low-density lipoproteins; Risk 1: Atherogenic index (Cholesterol/HDL-c).

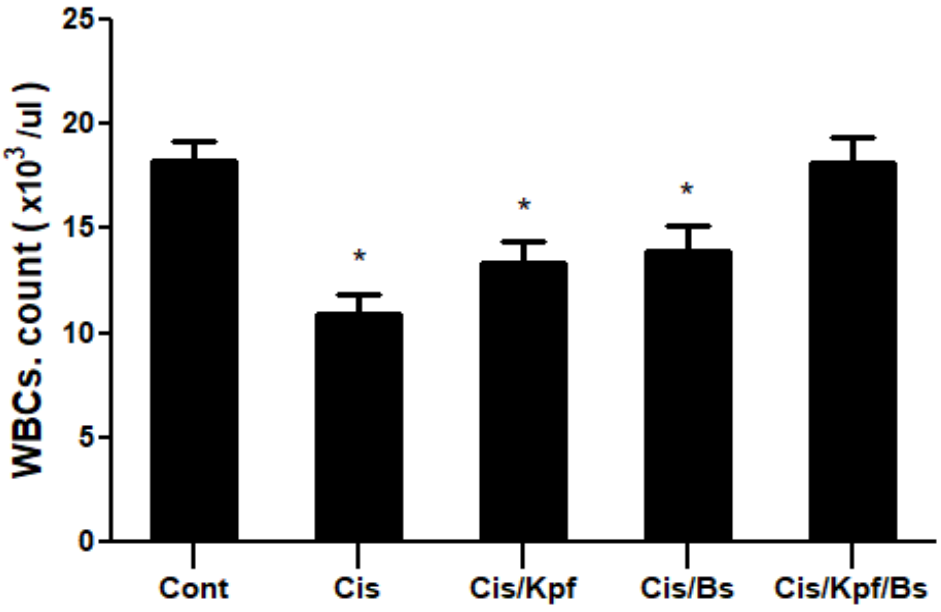

Figure 1: WBCs count in different groups of the study.

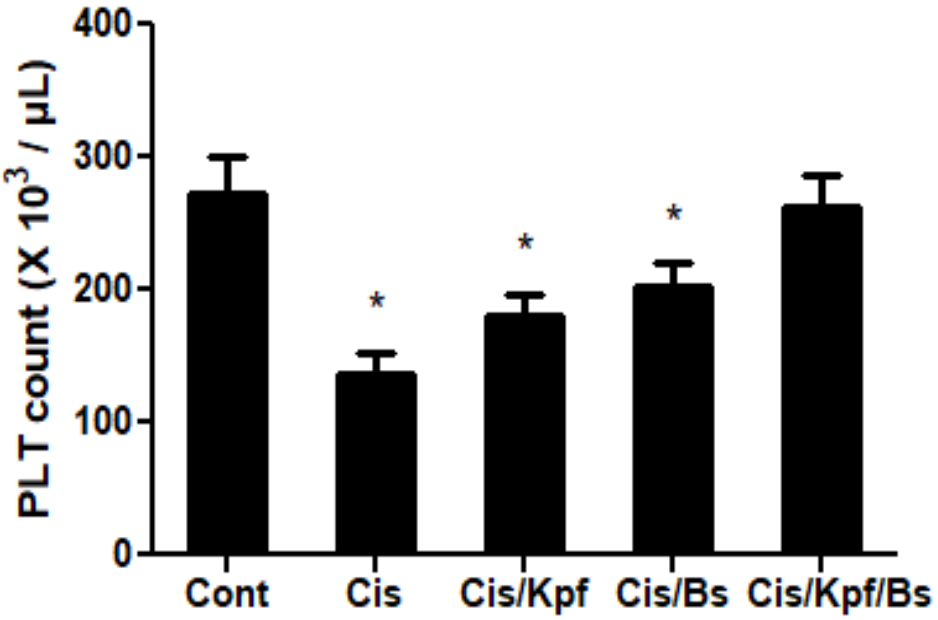

Figure 2: The total platelets count in different groups of the study.

Discussion

Cisplatin is known to have negative side effects in both clinical settings in experimental animals (26). On the Cis-induced model, this experiment examined the ameliorative effects of Kpf, Bs, and their combination by highlighting their impacts in attenuating the disturbance of hematological and lipid markers**.** Low Hb levels can increase the risk of developing cardiovascular disease (27). Hb transports oxygen throughout the body (28). When the level of HB is low, the body works hard to provide oxygen to its tissues and organs, which raises the risk of cardiovascular disease. Maintaining healthy Hb levels through a balanced diet and regular exercise is crucial since low Hb levels can also be an indication of underlying medical conditions such as iron deficiency anemia or chronic renal disease that may raise the risk of cardiovascular disease (29, 30). RBCs and platelets are both important regulators of redox balance harbouring powerful pro-oxidant and antioxidant capacities**.**

In this study, dyslipidemia occurs in Gp2 including elevated TC, TG, lipoprotein (a), and LDL-C, and decreased HDL-C levels. Treatment with Kpf and/or Bs attenuated this dyslipidemia. The lipid-lowering properties of Kpf (31) and Bs (32) have been extensively studied. Lipid-lowering effect of these compounds is regulated by competitive cholesterol absorption inhibition and by transcriptional induction of genes involved in cholesterol metabolism in both hepatocytes and enterocytes (31, 32). The obtained results showed a significant increase of LDL-C, and atherogenic index Risk 1 (CHO/HDL-C). administration of Kpf or/and Bs could modify lipid metabolism in vivo (33, 34). Most of the concerns regarding reductions in serum TC, TG, LDL-C, levels, and increases in HDL-C have been observed in Kpf supplementation. Administration of the Kpf/Bs mixture showed the most improvement in lipids profiles that reduce cardiovascular risks in rats. Kpf/Bs could reduce cholesterol levels in hypercholesterolemia (35). Results showed that apolipoprotein (a) is increased

in the Cis diseased group and that β-sitosterol succeeded in lowering the amount of lipoprotein and this agrees with (36) who suggested in his research (Intake of stigmasterol and β-sitosterol alters lipid metabolism and alleviates NAFLD in mice fed a high-fat western-style diet) that β-sitosterol lower lipoprotein. Also (37) in her research (antiatherogenic effects of flavonoid on cholesterol Efflux capacity) agree with these results as she discussed that kaempeferol has a good effect in reducing LDL-C. Insight towards, (38) disagrees with these results of his research (treatment of severe hypercholesterolemia with a combination of β-sitosterol and lovastatin) as even with a large dose of β-sitosterol, he saw no significant changes in very low-density lipoprotein, TG, TC, or high-density lipoprotein (HDL-C).

Conclusion

In conclusion, Kpf and Bs have shown great amelioration of hematological parameters and dyslipidemia in relieving Cis-induced side effects**.**

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* Authors' contributions

ZA, conceptualization of the research idea, supervising, experimentation, data analysis and interpretation, writing and editing the manuscript. **IE, HA, & MS** research design, experiments, methodology development, data collection, interpretation of results, and writing review & editing. **ZA, IA, HA & MS** methodology development, interpretation of results, data collection, writing-review & editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article. If detailed data are required, they can contact the correspondence of the study, M. Shahen (Email address: mshahen@science.tanta.edu.eg).

Declarations

Ethics approval and consent to participate.

All human cell lines used in this experiment have been approved by the appropriate ethics committee and have therefore been performed by the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. "Informed consent was obtained by the provider from all subjects and/or their legal guardian(s)". The Research Ethical Committee (REC) and The Institutional Animal Care Committee at Tanta University's Faculty of Science's Zoology Department approved the experimental protocol (No. REC/IACUC/SCI/TU/0169). There were no humans involved in this investigation**.**

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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