1   Article
2   **Investigation of invitro Antioxidant and invivo Protective Effects of Hypericum**
3   **triquetrifolium Seed Methanol Extracts Against Cyclophosphamide-Induced Acute**
4   **Myelotoxicity, Hemotoxicity and Hepatotoxicity in Rats**
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21 **Abstract:**
22 The aim of this study was to investigate in-vitro antioxidant properties and in-vivo protective

23 effects of different concentrations of Hypericum triquetrifolium Turra. (HT) seed methanol

24 extracts against acute hepatotoxicity, myelotoxicity and hematotoxicity in rats exposed to

25 overdose of cyclophosphamide (CP). HT seed methanol extracts were tested in view of its *in-*

26 *vitro* antioxidant activities as total phenolic contents and DPPH free radical-scavenging

27 activity. To investigate *in-vivo* protective effects of HT seed methanol extracts on rat tissues;

28 tested animals were divided into nine groups. Three groups only were treated with HT

29 extracts (25, 50 and 100 mg/kg HT) for 6 days. Three groups were pre-treated with the extract

30 of HT (25, 50 and 100 mg/kg HT) for 6 days and on the last day they were injected with

31   single dose of CP (150-mg/kg body weight). Two groups were used as control groups and one

32   group was only treated with CP (150 mg/kg) on the 6th day. The toxic effects of CP and

33   protective effects of HT extracts on the nucleated cells which were produced by bone marrow

34   and serum alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase

35   (LDH), oxidative stress index (OSI) levels were investigated biochemically. Additionally,

36   liver tissue samples were examined histopathologically. Our results show that HT seed

37   methanol extract has high total phenolic content and antioxidant activity. Over dose CP

38   administration caused hepatotoxicity, myelotoxicity and hematotoxicity on rat. Whereas, 25,

39   50 and 100 mg/kg HT plus CP administered groups showed significant protective effects on

40   nucleated cells. And 25, 50, 100 mg/kg HT plus CP treated groups showed an important

41   decrease on serum ALT, ALP, LDH and OSI levels when compared with CP treated group.

42   Our results showed that the administration of different HT doses with high doses of CP

43   significantly reduced hepatotoxicity, myelotoxicity and hematoxicity on rats.

44

45   **Keywords:** Cyclophosphamide, cytoprotective, hepatotoxicity, hematotoxicity, *Hypericum*
46   *triquetrifolium* Turra., myelotoxicity, rat.

47

48   **1. Introduction**

49   Chemotherapeutic drugs are generally toxic to healthy cells and multiple organs so they cause

50   critical side effects such as myelosuppression [1]. In spite of the wide spectrum of application,

51   the usage of Cyclophosphamide (CP) is generally limited due to its undesirable toxic side

52   effects, including hepatotoxicity [2,3] and myelosuppression/immunotoxicity which limit its

53   usefulness [2-4]. Moreover, CP causes a significant reduce on erythrocyte, leucocyte,

54   thrombocyte and bone marrow nucleated cells [5].

55   CP, an alkylating chemotherapeutic prodrug, is metabolized by liver cytochrome P450

56   enzymes namely, CYP3A4 and CYP2B6 demontsrate active therapeutic and cytotoxic

57   metabolites [6] and diffuse out of the hepatocytes into the plasma and are distributed

58   throughout the body and generates two active metabolites; phosphoramide mustard (PAM)

59   and acrolein (ACR).While CP's immunosuppressive and antineoplastic effects are related

60   with PAM, ACR is responsible for its undesirable toxicity [7]. ACR produces highly reactive

61   oxygen species (ROS) and oxidative stress in hepatocytes [8,9]. Therefore; it interacts with

62   protein amino acids and causes changes in enzyme's structure and function, and effects to the

63   tissue antioxidantive defense mechanism [10]. CP-depended toxicity is a consequence of a

64   mitochondrial dysfunction and ends up with a decrease of adenosine triphosphate owing to

65   nitrosative and oxidative stress [4].

66   CP is used in combination with different detoxifying and protective agents with the aim of

67   decreasing or removing its adverse toxic effects [11]. Extracts of herbal meds are indicated

68   with beneficial therapeutic impressions such as antioxidant, anticancer, antiinflammatory,

69   antimicrobial effects [12]. The antioxidants may minimize the toxic side effects of

70   chemotherapeutic agents and may supply the use of more effective high doses of the

71   anticancer drugs [4].

72   The *Hypericum* species known as ''Clusioid clade'' are a member of the *Hypericaceae*

73   family, belonging to the large clade of generally tropical plants [13]. The methanolic extract

74   of *Hypericum* species are particularly rich in bioactive compounds, such as pseudohypericin,

75   hypericin, naphthodianthrones, phloroglucinol derivatives, flavonoids, adhyperforin,

76   hyperforin, tannins, essential oils, xanthones, procyanidins, and some other water-soluble

77   components which possess a broad order of biological features [14,15].

78   *Hypericum triquetrifolium* Turra. (HT) belongs to the *Hypericaceae* family and it has been

79   used for its sedative, antiseptic, anti-inflammatory, antihelminthic and effects with traditional

80   methods [16]. Moreover, HT includes hyperoside, chlorogenic acid, quercetin, quercitrin,

81   rutin, kaempferol, phenolic and flavonoid compounds which are important for antioxidant

82   capacity [17,18] That's why, HT posesses significant antioxidative activities that might be

83   beneficial in inhibiting or decreasing the progress of various oxidative stress-induced

84   diseases. Even so, its effects against hepatotoxicity, myletotoxicity and hematotoxicity have

85   not been unspecified in detail. For these reasons, we examined the protective effects of HT

86   seed methanol extract on CP-related toxicity of the hepatic cells, bone marrow nucleated cells

87   and peripheral blood cells of rats in the present study.

88

89   **2.   Materials and Methods**

90   2.1. Drug and chemicals

91   CP (Endoxan, Cyclophosphamide Monohydrate, C0768) was acquired commercially from

92   Sigma-Aldrich, Taufkirchen, Germany. CP (500 mg) was dissolved in 25 mL bidistilled water

93   to its own appropriate concentration prior to the injection, sequentially, and was stocked at 4

94   °C before use. Single dose CP (150 mg/kg b.w.) was intraperitoneally administered rats.

95   2.2. Herbal material

96   The herbs were reaped at seeding stages in August and September, 2015 from where its wild

97   population is located Zınar, Mardin, Turkey. Voucher specimens were conserved at the

98   Herbarium of Mardin Artuklu University (2015-3-MAU). 20 g of seed were ground into

99   powder. Seed powder was preserved in a glass flask with 200 mL (99%) methanol at room

100   temperature. Extraction process was repeated 3 times under magnetic stirrer. Before the

101   solvent was removed on rotary evaporator under vacuum it had been filtrated. Then about 4 g

102   of the crude methanol extracts of HT seeds were supplied and saved in light-hindered glass

103   vials at -20 ºC prior to the experiments.

104   The test samples (crude methanol extract) were prepared by dissolving them in 0.2% dimethyl

105   sulfoxide (20 ml in 10 ml saline (0.9% NaCl)) (DMSO) (Sigma-Aldrich, Germany) solution

106   to obtain concentration of 25, 50 and 100 mg/kg in a flask. The flask was partially immersed

107   into the ultrasonic bath for 3 minutes at 30 ºC to provide effective dissolving. 0.5 ml 0.2%

108   DMSO and serum physiologic (SF) were used as positive controls.

109   2.3. Determination of Antioxidant Capacity

110   2.3.1. Total phenolic assay

111   The amount of total phenolic content in seed methanol extract was determined according to

112   the Folin-Ciocalteu method [19]. Briefly, 0.2 mL of sample solution (2 mg/mL) was added

113   into a test tube containing 1 mL of Folin–Ciocalteu reagent and 2 mL of Na_2CO_3 (7.5%). The

114   final volume was brought up to 7 mL with deionized water. After 2 h of incubation at room

115   temperature, the absorbance was measured at 765 nm with a spectrophotometer (perkin elmer

116   lambda 25 UV/Vis). The results were expressed as gallic acid (standard) equivalents (GAE).

117   The equation calculated as y = 0.002X-0.051(R^2 = 0.995) according to linear calibration

118   curve.

119   2.3.2. Scavenging Activity on DPPH Radical

120   The free radical scavenging activities were quantitatively tested with using a 2.2-diphenyl-1-

121   picrylhydrazyl (DPPH) based on Shimada et al., (1992) method [20]. Briefly, 0.1 mM

122   solution of DPPH in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml of

123   each extract solution at different concentrations (25–500 μg). The mixture was shaken

124   vigorously and allowed to stand at room temperature for 30 mins. Then the absorbance was

125   measured at 517 nm with a spectrophotometer (Perkin Elmer lambda 25 UV/Vis). Lower

126   absorbance of the reaction mixture was indicated as higher free radical scavenging activity.

127   Butylated Hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) were used as the

128   standard antioxidant (positive controls) for free radical scavenging activity test. The radical

129   scavenging activity was calculated with used below formula:

130   DPPH scavenging activity (%) = $(A_{517\ of\ control} - A_{517\ of\ sample}/A_{517\ of\ control}) \times 100$ [21].

131   2.4. Experimental design

132   Experimental materials (wistar albino rats) were provided commercially from Kobay

133   Experimental Animals Lab. San. Tic. A.S.S., and our study was applied in accordance with

134   the approbation of ESOGU Experimental Animals Ethic Comitte's (ethical approval code:

135   444-1/2015). The rats were nourished in a suitable environment as it should be (12 hours

136   light/dark) with drinkable water tap and pellet feed. Experimental albino rats (healthy, male,

137   Wistar, 3-4 months old, weight 200 - 240 gr) were divided in 9 groups (7 per grup) including

138   the control group. 1st group (control) was administered with 0.5 ml serum physiologic (SF);

139   2nd group was administered with single dose 150 mg/kg CP on the 6th day of experiment. 3,

140   4 and 5th groups were administered with 25, 50 and 100 mg/kg HT extracts respectively for

141   six days; 6, 7 and 8th groups were administered with 25, 50 or 100 mg/kg HT for six days and

142   on 6th day a single dose of CP was injected, group 9 was treated with 0,5ml - 0.2% DMSO).

143   On 7th day, all the rats were sacrificed. Before the injections and sacrifice, the animals were

144   weighed again. Then, according to ethical guidelines, the rats were sacrificed by cardiac

145   puncture with ketamine/xylazine anesthesia and the samples of blood were collected.

146   2.5. Histological measurements

147   Hepatic tissues were stained in 10% formaldehyde solution. Through routine histologic

148   preperation, tissue samples were embedded in paraffin, then 5.0   thick serial sections,

149   which were kept with Hematoxylin–Eosin, were done. After all, histopathological properties

150   were examined. The consequences were assayed with Variance of One Way Analysis and

151   Kruskal-Wallis test to score varying unnatural distribution. The differences between the

152   groups were found important if $p<0.001$ and if $p>0.05$, not statistically important.

153   2.6. Immunohistochemistry

154   Sections of cardiac tissues were deparaffinized and rehydrated routinely. Antigen retrieval by

155   citrate buffer (pH 6.0) was done by heating the sections in a microwave at 700 W for 10 min.

156   After blocking with 3 mL/L H_2O_2 and swine serum, sections were incubated with the primary

157   antibodies, directed against Bcl-2 (Abcam), Bax (Abcam) and caspase-3 (Thermo) at dilutions

158   of Ultravisionquanto detection system (Thermo Scientific), respectively.

159   2.7. Analysis of blood and bone marrow samples

160   The samples of blood were taken with cardiac puncture via the ketamine/xylazine anesthesia

161   then the rats were killed on the 7th day. After rat femurs dissection bone marrow was taken

162   with serum physiologic into a test tube, bone marrow samples were homogenized. Then, bone

163   marrow nucleated cells and peripheric blood cells were calculated with a cell counter

164   (Coulter).

165   2.8. Measurement of hepatic marker enzymes level

166   Rats blood samples were centrifuged at 3000 rpm for 10 mins and examined for alanine

167   transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) hepatic

168   enzymes. The activities of ALT, ALP and LDH were measured in serum by commercial kits

169   provided by spectrum diagnostic (Cairo, Egypt), seriatim.

170   2.9. Measurement of Oxidative Stress Index (OSI)

171   Oxidative Stress Index (OSI) was calculated by taking total oxidant level (TOL)/ Total

172   antioxidant level (TAL) proportion. For this, the unit of TAL value was changed from the

173   mmol Troloxequivalent/L type to µmol Troloxequivalent/L type. OSI was calculated as

174   below: OSI= [(TOL, µmol H_2O_2 equivalent/L) / (TAL, µmolTroloxequivalent/L) x 100]

175   formula [22].

176   2.10. Statistical analysis

177   The antioxidant test data were expressed as mean±standard deviation (SD). The result of the

178   biochemical and histological test were signified as means ± S.E.M. Statistical analysing was

179   performed by One Way Analysis of Variance and Kruskal-Wallis One Way Analysis of

180   Variance on Ranks Test. $p<0.001$ was received as considerable statistical importance. All

181   experiment was applied at least three times.

182   **3.   Results and Discussion**

183   The total phenolic content of seed methanol extract of HT was found as 179.52±0.52 μg

184   GAE/mg extract. Inhibition (%) of DPPH free radical scavenging activity of seed methanol

185   extract of HT (25 to 500 μg/mL concentrations) and positive controls (BHA and BHT) were

186   shown in detail in Table 1.

187   The decrease in the concentration of DPPH radicals was due to the scavenging ability of the

188   rich antioxidant contents of the *Hypericum triquetrifolium* Turra. (HT) seed methanol extract.

189   Linear increase in activities was observed with high concentrations (250, 500 μg/mL). These

190   values were found very close to the BHT and even higher than BHA which are most known

191   syntetic antioxidants. Our results show that the crude seed methanol extract of HT has strong

192   anti-oxidant activity when compared with BHA and BHT. These activities of plant extracts

193   mainly attribute to the presence of rich phenolic compounds.

194   **Table 1:** Effect of seed methanol extract of *Hypericum triquetrifolium* Turra. on the inhibition of
195   DPPH free radical.

<div align="center">% İnhibition of DPPH free radical</div>

Concentrations (μg/mL)	Standards		Sample
	BHA	BHT	HT methanol
25	67.60±0.08[*]	53.36±0.06	14.71±0.05
50	79.34±0.09	78.71±0.09	27.23±0.06
100	85.13±0.13	85.13±0.11	65.10±0.12
250	85.91±0.06	87.01±0.10	83.72±0.14
500	85.91±0.06	87.94±0.09	87.48±0.11

196   ***Data are presented as mean values; ±standard deviation (SD) of triplicate values.**

197   Histopathological assessments of our study show that hepatic tissues of the 100 mg/kg HT

198   and 0.2% DMSO groups have normal histology like control (0.5 ml saline) group. Although

199   there are no major defects in the liver of 150 mg/kg CP rats, some of the rats have small

200   bleeding, edema sites, vacuolization, ballooning and focal area of hepatic necrosis in the

201   hepatocytes (Fig. 1A, 2A, 3A). Similar results were demonstrated in former researches

202   [23,24]. No obvious disturbances are seen in liver tissues of HT plus 150 mg/kg CP rat groups

203   and on the contrary, the more HT dose increases, the liver has more normal histology like

204   control. We could infer that HT importantly improved CP caused cellular damage and

205   inflammation of hepatocytes with antioxidant and cytoprotective properties (Fig. 3A). Paralel

206   to our study, experimental evidences show that oxidative stress is the reason for CP

207   hepatotoxicity and also histopathological examinations demonstrated that CP causes serious

208   damage to liver tissue and increases in liver injury score [25,26].

209   Cancer chemotherapy is the most frequent method to treat malignant cells but hepatotoxicity

210   and myelosuppression are undesirable widespread side effect. Chemotherapy works on active

211   cells, such as cancer cells and also on some healthy blood cells. Side effects happen when

212   chemotherapy damages these healthy cells. So, we need to have new and more effective ways

213   to minimize the side effects of chemotherapeutic drugs without losing chemotherapeutic

214   effectiveness. Parallel to this report, in a research on the effects of cancer therapy on cancer-

215   related side effects it is found that 80-96% of patients receive chemotherapy [17]. Some of

216   clinical and preclinical studies report that a combination regimen of chemotherapeutic drugs

217   with chemopreventive drugs can be helpful to relieve chemotherapy induced systemic

218   toxicity, likewise to ensure recommended administration of chemotherapeutics for inquired

219   process [4,27]. Also at our previous study we demonstrated that antioxidant compounds have

220   pharmacological activity and limit toxicity [4].

221   Apoptosis assessments showed that Bcl-2, caspase-3 and bax immunohistochemistry results

222   have differences in liver tissue sections. Caspase-3 activity, elevations of Bax and a decline of

223   Bcl-2 were observed in CP induced rat livers when compared with control. In the earlier

224   investigations, parallel to the present results, was seen CP induced liver tissue apoptosis

225   [24,28]. In the livers of rats that received HT was seen dose-dependent reductions of Bax, and

226   caspase-3 density, and rise in Bcl-2, when compared with the rats that received CP plus HT

227   (Fig. 2B,C,D; 3B,C,D). Also, in the CP plus HT treated rats, apoptotic cells were observed

228   more common than in the other groups. We may infer that the anti-apoptotic activity of HT is

229   generally a reflection of its anti-inflammatory and antioxidant properties (Fig. 1,2,3).
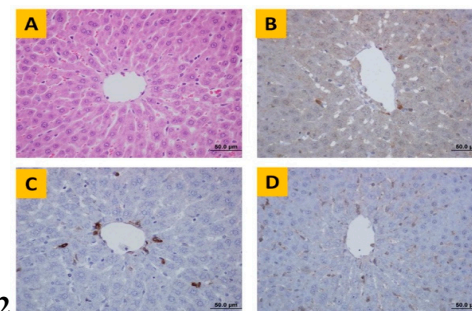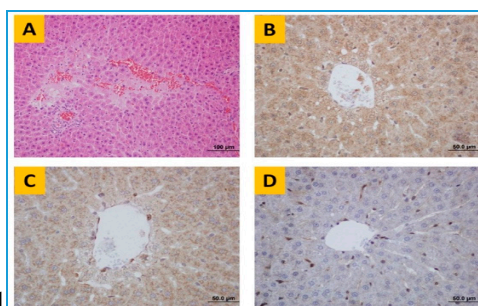
230   **Fig.1**   **Fig.2**
231
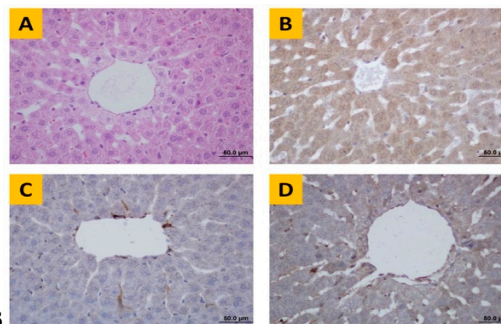
232   **Fig.3**
233   **Fig. 1-** 150 mg/kg CP group **A:** Hematoxylin–Eosin, **B:** caspase-3, **C:** Bcl-2, **D:** Bax **Fig. 2-** 100 mg/kg HT
234   group **A:** Hematoxylin–Eosin, **B:** caspase-3, **C:** Bcl-2**, D:** Bax. **Fig. 3-** 150 mg/kg CP+100 mg/kg HT group **A:**
235   Hematoxylin–Eosin, **B:** caspase-3, **C:** Bcl-2, **D:** Bax.
236

237   CP is a cytostatic drug which non-specifically affects to not only cancer cells but also normal

238   healthy cells with high proliferation capacity by binding to DNA and interfering with the cell

239   cycle [29]. Chemotherapeutic drugs-induced damage on the immune system is a major side

240   effect. In agreement, our results showed a significant ($p<0.001$) fall in the 150 mg/kg CP

241   group when compared with control [Table 2, Fig. 4]. The bone marrow's damaged or loss of

242   stem cells cannot regenerate new blood cells and results in thrombocytopenia and

243   leucopoenia. 25, 50, 100 mg/kg HT+CP (specially 50 mg/kg HT+CP) groups were found to

244   have a significant increase in the bone marrow nucleated cells values ($p<0.001$) in comparison

245   CP group [Table 2, Fig. 4]. According to these results, we may assert that HT and its active

246   compounds are great candidates for alternative adjuvant chemotherapy in decreasing the CP

247   immunotoxicity. In the DMSO control group, bone marrow nucleated cell values showed no

248   significant differences from the control group (0.5 ml saline) ($p>0.05$) (Table 2, Fig. 4).

249   **Table 2.** The effects of 25, 50,100 mg/kg doses of *Hypericum triquetrifolium* Turra.' s (HT) numbers
250   of erythrocytes, leucocytes, thrombocytes and bone marrow nucleated cells assessments on 150 mg/kg
251   CP toxicity in rats in multiple comparision

	Erythrocytes (X10³ ml)	$p<0.001$	Leucocytes (X10³ ml)	$p<0.001$	Thrombocytes (X10³ ml)	$p<0.001$	Bone marrow (X10³ ml)	$p<0.001$
1.Control (SF)	8.423 ±0.51	1-4, 1-5, 1-6, 1-8, 1-9	6.55± 0.80	1-2, 1-3, 1-4, 1-5, 1-6, 1-8	718.57± 57.71	1-2, 1-6	33.91±2.50	1-2
2. 150 mg/kg CP	7.87±0.32	2-8	2.02±0.22	2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9	340.00±17.36	2-3, 2-4, 2-5, 2-7, 2-9	5.59±0.44	2-3, 2-4, 2-5, 2-8, 2-9
3. 25 mg/kg HT	8.04±0.33		10.98±1.33	3-4, 3-6, 3-7, 3-8, 3-9	666.29±38.42		37.65±1.20	3-6, 3-7, 3-8
4. 50 mg/kg HT	7.73±0.38		13.86±1.03	4-5, 4-6, 4-7, 4-8, 4-9	666.57±52.68		38.44±1.39	4-6, 4-7, 4-8
5. 100 mg/kg HT	7.62±0.36		10.99±1.18	5-6, 5-7, 5-8, 5-9	693.29±123.80		33.01±1.63	
6. 25 mg/kg CP+HT	7.43±0.30		4.29±0.57	6-9	546.00±47.43	6-9	10.99±1.34	
7. 50 mg/kg CP+HT	7.97±0.40	7-8	5.23±0.64	7-9	671.71±48.58		12.35±1.27	
8.100 mg/kg CP+HT	7.21±0.30		4.87±0.64	8-9	576.71±58.41	8-9	9.95±0.34	
9. Control (DMSO)	7.74±0.34		7.53±0.53		741.00±68.34		35.40±0.99	

252   (p<0.001: statistically significant differences, (p>0.05): no statistically significant differences).
253

254   In the present study, we observed that the number of erytrocytes decreased in CP group in

255   comparison control. In the 50 mg/kg HT+CP group, the number of erytrocytes increased,

256   furthermore it got closer to the control despite of CP toxicity ($p<0.001$). According to the

257   results it could be concluded that dose of 25 mg/kg HT wasn't enough to prevent CP toxicity

258   when compared with CP group (Table 2, Fig. 5). On the other hand dose of 100 mg/kg HT is

259   toxic for erytrocyte cells because of showing no cytoprotective effect. We could conclude

260   from the results that dose of 50 mg/kg HT+CP is the optimum protective dose on erythrocyte

261   cells (Table 2, Fig. 5).

262   There was a statistical significant decrease in the number of leucocytes in the groups of CP

263   and we thought that it could be because of high dose CP (Table 2, Fig. 6). In agrement with

264   our result, it was obtained that there was seen a significant ($p<0.001$) decrease in the number

265   of leucocyte cells in the CP administered rat group [30]. The number of leucocyte cells

266   showed a significant increase in the CP plus 25, 50, 100 mg/kg HT groups when compared

267   with 150 mg/kg CP ($p<0.001$). From these results it could be infered that all dose of CP+HT

268   increased the number of leucocyte and 50 mg/kg HT+CP is the optimum dose on leucocyte

269   cells (Table 2, Fig. 6).

270   The number of thrombocytes was decreased ($p<0.001$) in the CP group in comparison with

271   control and 25, 50, 100 mg/kg HT groups (Table 2, Fig. 7). Similar to our study Merwid et al.

272   (2011) reported that CP which is an anticancer or immunosuppressive drug significantly

273   decreased thrombocyte and leucocyte cells, however the usage of high dose CP is limited

274   because of its toxicity [30]. The effect of CP on the erytrocyte cells was not so clear as in the

275   case of leucocyte and thrombocytes. The current study made it clear that CP lessened the

276   number of leucocyte and thrombocyte more than erythrocyte cells. Whereas the number of

277   thrombocyte cells increased in the 25, 50 and 100 mg/kg CP plus HT and this rise was

278   especially in the 50 mg/kg HT+CP group. And these results were found to be statistically

279   significant ($p<0.001$) (Table 2, Fig.5,6,7). In the DMSO group, the number of erytrocyte,

280   leucocyte and thrombocytes showed no significant change from the control group ($p>0.05$)

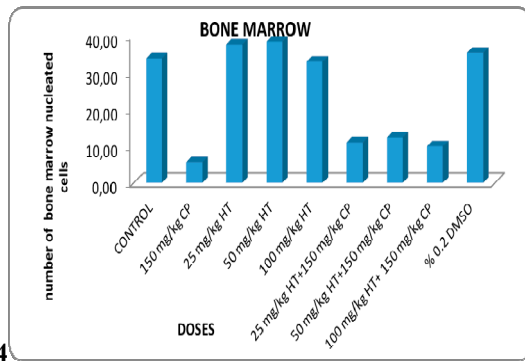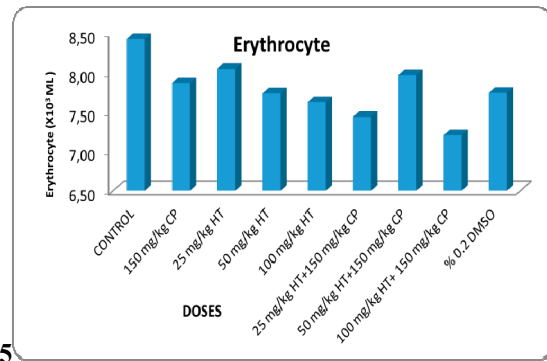281   (Table 2, Fig.5,6,7).

282

283   **Fig.4**

Fig.5
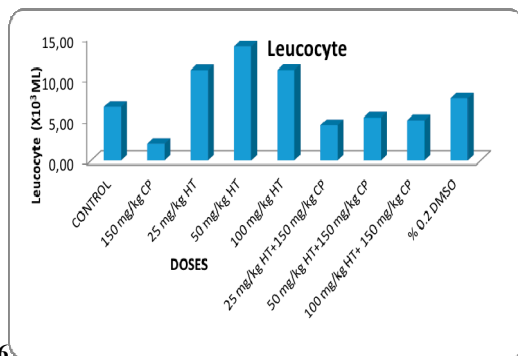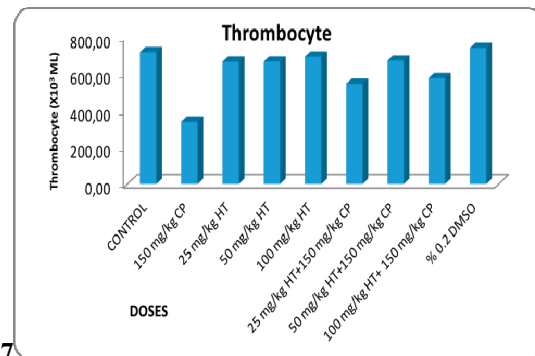

284   **Fig.6**

Fig.7

285   **Fig. 4,5,6,7**- Number of erytrocyte (Fig. 5), leucocyte (Fig. 6), thrombocyte (Fig. 7) cells of 150 mg/kg CP, 25-
286   50-100 mg/kg HT, 25-50-100 mg/kg HT+ 150 mg/kg CP, % 0.2 DMSO applied experimental groups
287

288   The usage of CP, which is used widely in clinical settings as a chemotherapeutic agent, is

289   severely limited by its physiological side effects, such as hepatotoxicity and

290   myelosuppression [31]. CP-treated hepatotoxicity causes cell injury as a result of the cytosolic

291   enzymes (ALT, ALP, LDH) and leaches out leading to their raised levels in the blood (Table

292   3; Fig. 8,9,10). The serum values of ALT, ALP and LDH are most widely used clinical

293   markers of hepatocellular toxicity. So, the effect of CP metabolism on the liver function

294   enzymes was evaluated and serum ALT, ALP and LDH levels were found high in the CP

295   groups. In several researches it was demonstrated that, CP hepatotoxicity was associated with

296   elevated ALT levels on mice [3] and rats [25,32]. It was demonstrated that there was an

297   increase in ALT, ALP and LDH in CP- induced hepatocyte injury as well. And this was

298   evidenced by the significant ($p<0.001$) increase in serum ALT and ALP when compared with

299   the control group [26]. On the other hand, CP plus 25, 50, 100 mg/kg HT treatment,

300   especially CP plus 25 and 50 mg/kg HT doses, reduced the serum levels of ALT, ALP and

301   LDH (Table 3, Fig. 8,9,10).

302

303   **Table 3.** The demonstration of the effects of 25, 50,100 mg/kg doses of *Hypericum triquetrifolium* Turra.'s (HT
304   ) biochemical assessments (ALT, ALP, LDH, OSI) on 150 mg/kg CP toxicity in rats in multiple comparision

	ALT (U/L)	*p*<0.001	ALP (U/L)	*p*<0.001	LDH (U/L)	*p*<0.001	OSI(TOS/TASx100)	*p*<0.001
1.Control (SF)	12.77±0.98	*1-2, 1-5, 1-6, 1-7	49.02±5.35	1-2, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9	349.67±23.26	1-2, 1-5, 1-6	0.0156±0.0009	1-2, 1-6, 1-7, 1-8
2. 150 mg/kg CP	26.17±2.28	2-3, 2-4, 2-5, 2-7, 2-8, 2-9	89.85±5.32	2-3, 2-4, 2-5, 2-7, 2-8, 2-9	514.29±15.04	2-3, 2-4, 2-5, 2-7, 2-8, 2-9	0.2431±0.0155	2-3, 2-4, 2-5, 2-6, 2-7, 2-8, 2-9
3. 25 mg/kg HT	13.09±1.54	3-5, 3-6, 3-7	50.64±4.19	3-4, 3-5, 3-6, 3-7, 3-8	397.79±44.04	3-5, 3-6	0.0165±0.0014	3-6, 3-7, 3-8
4. 50 mg/kg HT	11.94±1.99	4-6, 4-7	67.87±6.00	4-5, 4-6, 4-8, 4-9	393.01±27.44	4-5, 4-6	0.0161±0.0006	4-6, 4-7, 4-8
5. 100 mg/kg HT	9.21±0.91	5-6, 5-7, 5-9	28.61±2.72	5-6, 5-7, 5-8, 5-9	293.42±17.34	5-6, 5-7, 5-8, 5-9	0.0152±0.0006	5-6, 5-7, 5-8
6. 25 mg/kg CP+HT	25.34±2.32	6-7, 6-8, 6-9,	83.92±3.75	6-7, 6-8, 6-9	472.69±31.03	6-7, 6-8, 6-9	0.1900±0.0074	6-8, 6-9
7. 50 mg/kg CP+HT	17.65±1.38	7-8, 7-9	72.61±4.50	7-8, 7-9	382.91±31.78		0.1321±0.0095	7-8, 7-9
8.100 mg/kg CP+HT	11.72±1.62		58.65±3.77		350.66±21.37		0.0899±0.0040	8-9
9. Control (DMSO)	12.35±1.71		52.76±3.37		353.49±29.87		0.0155±0.0009	

305   (*p*<0.001: statistically significant differences, (p>0.05): no statistically significant differences).

306

307   Cellular antioxidant defense system is a major defense against CP-induced oxidative stress

308   [33,34]. ACR, which leads to a breach in the normal antioxidantive defense system and can

309   result in uninterrupted ROS accumulation, attacks to the tissue antioxidant enzymes. In the

310   150 mg/kg CP group, level of OSI increased when compared with the control group. This

311   might result from a decrease in antioxidant status, as it was evidenced by the substantial

312   decline in the CP-treated group. 25, 50, 100 mg/kg HT prevented the CP toxicity and we can

313   explain this with the decrease in OSI level (Table 3, Fig. 11). So, the prevention of OSI

314   increment and enhancement of the antioxidant enzymes play a central role in the

315   cytoprotective activity of HT against CP. In the same way our study is supported by numerous

316   studies and in all these studies the herbs are obviously play a crucial role with antioxidant and
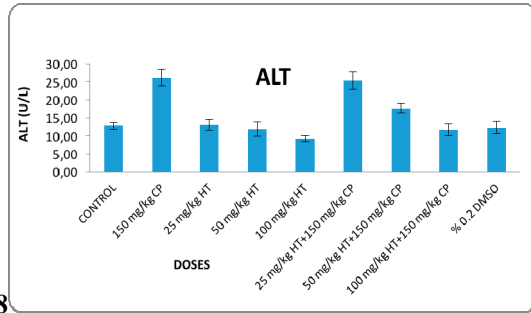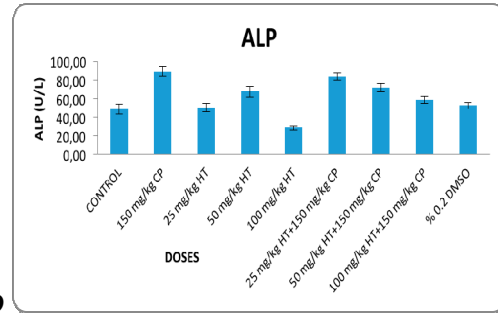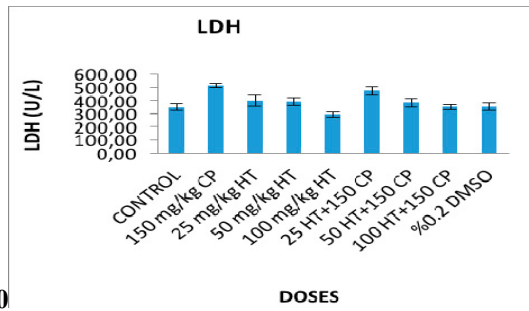
317   antitumoral features.

318   **Fig.8**

319
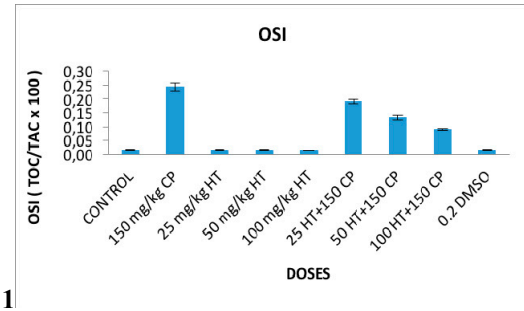
320   **Fig.10**   **Fig.11**

321

322   **Fig. 8,9,10,11**- ALT, ALP, LDH, OSI levels of 150 mg/kg CP, 25-50-100 mg/kg HT, 25-50-100 mg/kg

323   HT+150 mg/kg CP and % 0.2 DMSO applied experimental groups

324

325   **4. Conclusion**

326   Therapeutic effects of anticancer agents are associated with adverse side effects because of

327   the toxicity they cause. Even though CP is very commonly prefered as a chemotheputic drug,

328   its toxic side effects limit high dose usage. So, we are in need of new effective agents in order

329   to protect the normal tissue from chemotherapy-related toxicity without protecting the tumour

330   and tumour growth stimulation features. Therefore, the combination of the treatment regimens

331   with antioxidantive and cytoprotective features can be useful in the protection of healthy cells

332   and tissue against CP-treated oxidative damage. From our results, it could be concluded that

333   HT seed methanol extracts show high antioxidant activity and protective effect on blood, bone

334   marrow nucleated cells and liver tissue despite of high dose CP without limiting its

335   chemotheputic effectiveness. Based on our study findings, it could be proposed that all dose

336   of HT especially 50 mg/kg HT plus CP are strong candidates in preventing the CP-induced

337   myelotoxicity, hemotoxicity and hepatotoxicity.

342   **Author Contributions:** Concept - S.C.Y.; Design - S.C.Y.; Supervision - S.C.Y., C.K.,

343   Resource -S.C.Y.; Materials - S.C.Y., C.K., A.A; Data Collection and/or Processing - S.C.Y.,

344   C.K.; Analysis and/or Interpretation - S.C.Y., C.K., A.A; Literature Search - S.C.Y., C.K.;

345   Writing - S.C.Y.; Critical Reviews - S.C.Y., C.K.

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347   authors.

348

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