Hematological and Hemostatic changes in garlic, curcumin and curcumin plus garlic treated rat.

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A B S T R A C T

The present study carried out by using garlic (G), curcumin plus garlic (R+G) and curcumin (R). Four groups of rats were used each one consists of 5 rats; control group (C); which received distilled water intragastric daily for 6 weeks. garlic-treated group (G); which received garlic intragastric at a dose of 500 mg /kg B. wt. daily for 6 weeks. Curcumin plus garlic group (R+G) which received curcumin intragastric at a dose of 200 mg /kg B. wt. with garlic at a dose 500 mg /kg B. wt. daily for 6 weeks. Curcumin-treated group (R); which received curcumin intragastric at a dose of 200 mg /kg B. wt. daily for 6 weeks. Garlic-treated group showed normocytic normochromic anemia with non-significant changes in leukogram. Meanwhile, Curcumin plus garlic-treated group showed an improvement in RBC count that, reduced by garlic. Curcumin-treated group showed monocytosis. Concerning haemostatic markers, there were significant prolongations of PT, APTT and TT, as well as reduction in platelet count and aggregation percentage in both groups and in curcumin-treated group when compared with control. The results of this study demonstrate that curcumin and garlic have antithrombotic and antiplatelet effects and, their combination potentiate each other as inducing agents for haemostatic disorders.

***Keywords*:** Hemostatic markers, Garlic, Curcumin, platelet Aggregation, Hematological parameters.

(http://www.bvmj.bu.edu.eg) (BVMJ-32(2): 157-163, 2017)

1. INTRODUCTION

Garlic (*Allium Sativum*), as a medicinal plant is known to have several effects in the body. These include inhibition of platelet aggregation (*Sharma and Sunny, 1988*) and prolongation in PT and APTT with reduction in platelet count *(Alhamami et al., 2006).* Also, administration of garlic causes changes in hematological parameters as decreases in packed cell volume and hemoglobin concentration. However, the red cell count was significantly elevated as well as increased neutrophil, lymphocyte and monocytes counts *(Tende et al., 2012)*. Curcumin, a yellow pigment from *Curcuma longa*, is a major component of turmeric and one member of the ginger family. It has a wide range of pharmacological and physiological actions such as antioxidant, radioprotective, antibacterial, antifungal, antiviral, anti-inflammatory, anti-aflatoxogenic, anti-proliferative, proapoptotic and antiatherosclerotic effects (*Ghoniem et al., 2012; Kedia et al., 2014*). Curcumin showed an enhancement in the hematological parameters as *Abdel-Moneim et al., (2015)* found that rats treated with curcumin after lead intoxication showed improvement in RBCs count, Hb concentration, total leukocytic count, eosinophils and monocytes toward normal. Curcumin has better anticoagulant action as it caused significant increases in PT, APTT and inhibition the activities of thrombin and FXa *(Kim et al., 2012)*. Therefore, the aim of the study is the investigation of the changes in hematological and hemostatic parameters in experimentally treated rats by garlic (G), curcumin (R) and curcumin plus garlic (R+G).

2. MATERIALS AND METHODS

2.1. Animals and experimental design:

A total number of 20 apparently healthy adult male white Albino rats (180–190 gm) body weight. The animals were obtained from faculty of

veterinary medicine, Cairo, Egypt and housed for one week at constant environmental and nutritional conditions similar to those under which the experiment was performed for accommodation. Rats were housed in suitable cages away from any stressful stimuli, and supplied with diet and water *ad libitum*. Rats were allocated randomly into four main groups each group consists of 5 rats: control group (C); served as a control and received distilled water intragastric daily for 6 weeks. Garlic-treated group (G): received garlic via intragrastric route at a dose of 500 mg/kg B.wt for 6 weeks (*Bordia et al., 1996*). Curcumin plus garlic-treated group (R+G): received curcumin intragastric at a dose of 200 mg /kg B. wt. *(Park et al., 2000)* with garlic via intragrastric route at a dose of 500 mg/kg B.wt for 6 weeks *Bordia et al.,* (*1996*).

2.2. Chemicals:

Garlic was obtained as a tablet 500 mg garlic powder per each tablet (Tomex plus) and curcumin a golden yellow powder obtained from El-Gomhoria Company.

2.3. Reagents and kits

Commercial diagnostic kits for determination of Prothrombin time (PT), Activated Partial thromboplastin time (APTT) were obtained from Biosystems diagnostic (Germany). Thrombin time (TT) was obtained from Biomed diagnostic (Spain). Kits for Adenosine diphosphate (ADP) was obtained from Hart Biologicals (Germany).

2.4. Sampling:

Blood samples were obtained from retro-orbital venus plexus of the animals after 6 week of treatment for whole blood, plasma and platelet rich and poor plasma. Whole blood was used for hemogram evaluation (RBCs count, hemoglobin determination, hematocrit, total WBCs count and differential leukocytic count). Plasma samples were used for coagulation study (PT, APTT and TT). The collected PRP and PPP were used for platelet aggregation study by aggregometer using ADP agonists.

2.5. Clinicopathological analysis:

The hematological studies including erythrogram and leukogram were determined according to Thrall et al., (2012). While, platelets count was done by manual method using improved Neubauer hemocytometer according to Feldman et al., (2000). PT, APTT, TT and platelet aggregation were determined according to method described by Biggs and Macfarlane (1962); Hoffman and Neulendijk (1978); Key et al., (2009) and Days and Holmsen (1972) respectively.

2.6. Statistical Analysis

Statistical analysis was performed using the statistical software package for social science (SPSS) for Windows (Version 16.0; SPSS Inc., Chicago, IL). The significance of differences between the experimental groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference, then differences between individual groups were estimated using Duncan as a post hoc. Results are expressed as the mean ± standard error of mean. A *P*-value of less than 0.05 was considered significant (Kinnear and Gray 2006).

3. RESULTS

Data demonstrating the effects of garlic, curcumin and curcumin plus garlic on hemogram and hemostatic markers were presented in table (1, 2, 3 and 4). Concerning RBCs count, there was a significant reduction in rats administrated garlic when compared with control group. Meanwhile, non-significant changes in RBCs count were observed in curcumin and curcumin plus garlic - treated groups when compared with control group. On the other hand, there was a significant increase in RBCs count in curcumin plus garlic-treated groups when compared with garlic-treated group. In regard to Hb, Hct, MCV, MCH and MCHC results, there were non-significant changes in garlic, curcumin and curcumin plus garlic-treated groups when compared with control group. Also, curcumin plus garlic-treated group showed non-significant modifications when compared with garlic-treated group. There were non-significant changes in TLC and lymphocyte in garlic and curcumin-treated groups when compared with control. As well as, curcumin plus garlic-treated group revealed non-significant alterations in TLC and lymphocyte counts when compared with garlic-treated group.

There were non-significant changes in granulocytes in different treated groups when compared with control. Also, curcumin plus garlic-treated group revealed non-significant changes in granulocytes when compared with garlic-treated group. Non-significant alterations were observed in monocyte count in different treated groups when compared with control groups, but curcumin-treated group showed a significant monocytosis when compared with control group. On the other hand, monocyte count revealed non-significant changes in curcumin plus garlic-treated group when compared with garlic-treated group. PT showed significant increases in garlic and curcumin-treated groups when compared with

control group. On the other hand, curcumin plus garlic-treated group showed a significant reduction in PT when compared with garlic-treated group. Among treated groups, garlic and curcumin-treated groups showed significant reductions in percentage activity of PT when compared with control. Meanwhile, curcumin plus garlic-treated group revealed non-significant alterations in percentage activity of PT when compared with control one. Also, there were non-significant modifications in percentage activity of PT in curcumin plus garlic-treated group when compared with garlic-treated group. INR showed significant increase in garlic and curcumin treated groups when compared with control. Meanwhile, curcumin plus garlic-treated group revealed non-significant modifications in INR when compared with garlic-treated group. Garlic, curcumin and curcumin plus garlic-treated groups showed significant prolongation in APTT and R when compared with control group. Non-significant changes were observed in APTT and R in curcumin plus garlic-treated group when compared with garlic treated group.

Concerning to TT result, garlic and curcumin-treated groups showed significant increases when compared with control group. Also, there was a significant prolongation in TT in curcumin plus garlic-treated groups when compared with garlic-treated group. Garlic and curcumin-treated groups showed significant increases in R of TT when compared with control group. But, curcumin plus garlic-treated group showed non-significant modifications in R when compared with garlic-treated group.

Regarding platelet count, garlic, curcumin and curcumin plus garlic-treated groups showed significant reductions in platelet number when compared with control. On the other hand, there was non-significant modifications in platelet count in curcumin plus garlic-treated group when compared with garlic-treated groups.

Concerning to results of aggregometer, aggregation % revealed significant decreases (lower aggregation percent) garlic, curcumin plus garlic and curcumin-treated groups when compared with control. Meanwhile, there was a non-significant modification in aggregation percentage in curcumin plus garlic-treated group when compared with garlic-treated group.

Table (1): Erythrogram after 6 weeks in different experimental animal groups.

Parameters & Groups	RBCs ($x10^6/\mu l$)	Hb (gm/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (%)
C	7.11 ± 0.08^{c}	15.14 ± 0.69^{b}	$40.45\pm0.88^{b,c}$	$57.8\pm1.01^{a,b}$	21.50 ± 0.73^{b}	38.08 ± 0.76^{b}
G	6.17 ± 0.08^{a}	14.94 ± 0.25^{b}	$40.35\pm0.37^{b,c}$	$59.2\pm1.31^{a,b,c}$	21.51 ± 0.35^{b}	38.12 ± 0.71^{b}
R+G	$7.02\pm0.21^{b,c}$	15.06 ± 0.26^{b}	$40.46\pm0.27^{b,c}$	57.4 ± 0.50^{a}	21.43 ± 0.26^{b}	38.03 ± 0.37^{b}
R	$7.11\pm0.13^{b,c}$	15.52 ± 0.63^{b}	41.48 ± 0.68^{c}	$58\pm0.94^{a,b}$	$20.95\pm0.55^{a,b}$	38.06 ± 0.67^{b}

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P\leq0.05$

Table (2): Leukogram after 6 weeks in different experimental animal groups.

Parameter & Groups	WBCs ($x10^3/\mu l$)	Granulocytes ($x10^3/\mu l$)	Lymphocyte ($x10^3/\mu l$)	Monocyte ($x10^3/\mu l$)
C	$14.10\pm0.69^{b,c}$	3.09 ± 0.25^{a}	$10.18\pm0.58^{b,c}$	0.83 ± 0.02^{a}
G	$14\pm2.23^{b,c}$	3.29 ± 0.56^{a}	$9.69\pm1.66^{b,c}$	$1.02\pm0.06^{a,b}$
R+G	$14.14\pm1.69^{b,c}$	3.11 ± 0.49^{a}	$10.22\pm1.52^{b,c}$	0.82 ± 0.04^{a}
R	16.54 ± 1.47^{c}	3.51 ± 0.18^{a}	11.79 ± 1.30^{c}	1.20 ± 0.15^{b}

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P\leq0.05$

Table (3): Coagulation markers after 6 weeks in different experimental animal groups.

Parameters & Groups	PT (second)	Percentage Concentration %	INR	APTT (second)	R
C	18.16±0.50[a]	40.80±1.98[b]	1.27±0.08[a]	32.30±3.16[a]	1.14±0.04[a]
G	28.32±1.23[c]	33.60±1.36[a]	1.48±0.02[b,c]	37.95±6.83[b,c]	1.54±0.10[b,c]
R+G	22.32±1.49[b]	37±1.48[a,b]	1.31±0.01[a,b]	36.28±3.63[b]	1.56±0.18[b,c]
R	21.22±0.61[b]	35.40±1.63[a]	1.62±0.08[c]	39.95±3.11[c]	1.78±0.21[c]

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P \leq 0.05$

Table (4): Coagulation markers after 6 weeks in different experimental animal groups.

Parameters & Groups	TT (second)	R	Platelet ($x10^3/\mu l$)	Aggreg.%
C	45.62±0.56[a]	2.71±0.20[a]	657±10.55[c]	52.77±1.49[b]
G	51.98±1.16[b]	3.51±0.13[b]	579.2±17.80[b]	25.52±2.91[a]
R+G	62.15±1.59[d]	3.48±0.31[b]	584.6±20.14[b]	21.63±6.60[a]
R	56.94±0.94[c]	4.15±0.28[b]	582.6±10.33[b]	23.52±4.23[a]

Results are expressed as mean ±S.E.M. Different superscripts (a, b, c and d) within the same column indicate significant differences at $P \leq 0.05$

4. DISCUSSION

Hemostasis is an important process for a number of reasons, it maintains blood in fluid form instead of coagulated status inside blood vessel, prevent excessive blood loss after injury so that it is essential for blood to be closed circulatory system. The process of hemostasis undergoes many disorders which lead to hypocoagulation (hemorrhage) or hypercoagulation (thromboembolic disorders) *(Latimer, 2011).* Curcumin is a major component of turmeric, and is commonly used as a spice and food-coloring material. It has a wide range of pharmacological and physiological actions such as antioxidant, antifungal, antiinflammatory, antiaflatoxogenic and proapoptotic effects (*Ghoniem et al., 2012; Kedia et al., 2014*).

Hematopoietic system is considered the mirror of the body as it reflects any changes in animal or human body exposed to chemical, toxic agents and drugs (Yuan et al., 2014). There were significant reductions in RBCs count in rats' administrated garlic when compared with control group after 6 weeks resulting in normocytic normochromic anemia. This agreed with *Nakagawa et al.,* (*1980*), and this reduction in RBCs may be contributed to long garlic administration toxic to RBCs as it cause lipid peroxidation of RBCs resulted in its destruction *(Banerjee and Maulik, 2002)*. On the other hand, there was a significant increase in RBCs count in curcumin plus garlic-treated group when compared with garlic-treated group after 6 weeks. This illustrated the good effect of curcumin which overcome and minimizing changes produced by aflatoxin and garlic as curcumin enhance erythropoiesis, stabilize the cell membrane and prevent cellular damage occur by free reactive oxygen species and restore blood (*Banji et al., 2011; Sharma et al., 2011*). Meanwhile, RBCs, Hb, Hct, MCV, MCH and MCHC revealed non-significant changes in curcumin-treated group when compared with control group after 6 weeks these results in accordance with Essam and Ashraf, (2013); Abdel-Moneim et al., (2015). In regard to total and differential leukocytic count, curcumin-treated group showed a significant monocytosis when compared with control group which indicates that curcumin activates the animal's immune system leading to the increased production of leukocytes (*Yousef et al., 1999; Çetin et al., 2010*).

Concerning hemostatic parameters, curcumin-treated group showed significant increases in INR, APTT, R and R of TT (PTT ratio) after 6 weeks beside increases in PT, TT and significant decrease in percent activity of PT after 6 weeks. Also, there was significant reduction in platelet count when compared with control after 6 weeks. Those results in accordance with *Kim et al., (2012)* who explain this action as a result of methoxy group in curcumin which has a positive regulated effect on anticoagulant function of curcumin. Moreover, garlic-treated groups revealed significant increases in PT, INR, TT and R of TT, APTT and R of APTT after 6 weeks of treatment, but there were significant decreases in percent activity of PT, and reductions in platelet number in the same group when compared with control group. Our results agreed with *Aro, (1991); Alhamami et al., (2006); Rahman and Lowe, (2006); Chan et al., (2007).* The effect of garlic on PTT and TT tests may be due to the inhibition of coagulation factors involved in the intrinsic and common clotting pathway such as factor I, II, V,VIII, IX and X *(Fakhar and Tayer, 2012).* Moreover, it contains large amount of some effective components as diallyl disulfide (DADS), diallyl trisulfide (DATS) and methyl ajoene that have antithrombotic effect *(Butt et al., 2009; Omar and Al-Wabel, 2010).*

Concerning to results of aggregometer, aggregation % revealed significant decreases (lower aggregation percent) in garlic and curcumin-treated groups when compared with control. Concerning the effect of garlic, our result was in accordance with *Shah et al., (1999).* The anti-aggregatory effect of curcumin is a result of inhibition of thromboxane A2 and COX, as well as preventing synthesis and signaling of Ca^2 *(Srivastava et al., 1995).* On the other hand, the result of garlic on platelet greed with *Alhamami et al., (2006); Fakhar and Tayer, 2012)* which resulted from reduction of thromboxane formation from exogenous arachidonic acid (AA) (*Dunbabin et al., 1994*); inhibition of the phospholipase activity (*Coller, 1990*). The previous mechanisms of garlic may be attributed also to garlic contains many important components such as Allicin that inhibit platelet aggregation in vitro (*Jamaluddin et al., 1988*). Moreover, it contains other compound as β-chlorogenin and quercetin which have antiplatelet effect *(Corzo-Martínez et al., 2007).*

5. REFERENCES

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