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Comparative Study on Hematological Changes in Adult and Aged Rats after Curcumin Administration

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- blood indices
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Abstract

Several studies investigating the beneficial effects of curcumin administration in aging on the other hand, curcumin may have the potential to contribute to the development of anemia. So this study was designed to compare the hematological effects of curcumin administration in adult and aged rats. Materials and methods: Twelve adult rats 6 months old and twelve aged rats 20 months old were used in this study. Adult and aged rats were randomly and equally divided into four groups: control adult, curcumin-treated adult, control aged and curcumin-treated aged groups. Curcumin was administered in curcumin groups (50mg/Kg i.p. for 21 consecutive days). The rat tail bleeding time was assayed. Blood indices, platelets indices, in vitro platelets aggregation, total and differential white blood cells counts were measured. Results: Curcumin caused a significant decrease in red blood cells count and hemoglobin concentrations in aged group. Also curcumin significantly decreased hematocrit in adult and aged rats. In aged group curcumin significantly increased the platelets count and platelet indices. In adult group, it caused a significant increase in platelet indices only. Tail bleeding time and platelets aggregation significantly increased in curcumin-treated aged group versus control adult group. Conclusion: Curcumin administration in aged rats caused anemia, the cause of anemia may be the iron deficiency. Curcumin also caused an increase in platelets count and this may represents reactive thrombocytosis to the iron deficiency anemia and it prolonged the bleeding time. So these hematological sequelae of curcumin administration must be seriously taken in consideration with practical implications of curcumin in aging. Further researches are required to address the mechanisms of these effects.

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INTRODUCTION

Aging is an unavoidable, universal, biological phenomenon which characterized by a decrease in biochemical and physiological functions of most organs (1,2).The average human life expectancy has increased dramatically over the last century. The population of elderly (>65 years) is predicted to be doubled by the year 2025 due to increased health care and nutrition (3).

The hematopoietic system is modestly affected by aging, and these effects become particularly notable after age 65. There is a continuous decrease in the volume of the hematopoietic marrow with age, which does not cause significant alterations in either granulocytes or monocytes. A slight decrease in mean hemoglobin levels (less than 1.0 g/dl in the sixth through eighth decades) in men occurs(4).Among humans, anemia increases with age manifested by decreases in hemoglobin, red blood cells (RBCs), and hematocrit(3). The lymphocytes count tends to decrease in the blood. The platelets count does not change with age but enhanced in vitro reactivity to platelet-aggregating agents has been observed (4).

Curcumin is the phytochemical derived from the rhizome of Curcuma longa, present in the spice turmeric and it gives Indian curry its yellow color. Curcumin has been used as a wound-healing agent and for treating a variety of diseases in traditional Indian and Chinese medicine. Curcumin has attracted the attention of researchers as an agent capable of inhibiting the proliferation of cancer cells. Also curcumin raises interest as an agent of potential use in therapy of many diseases including cardiovascular diseases, Alzheimer's disease, rheumatoid arthritis and metabolic syndrome. The amount of data documenting beneficial effects of curcumin in protecting against different diseases, particularly those which are related to age are increasing (5)**.** Studies demonstrated that curcumin has antioxidant, anti-inflammatory, antiapoptotic, antiproliferative, anticancer, antidepressant, immunomodulatory and neuroprotective effects on humans and laboratory animals (1). These variable effects are leading to a growing number of studies investigating the beneficial effects of curcumin administration in aging in human and animals (6-10). On the other hand, it has been demonstrated that curcumin has a dramatic effect on hematologic parameters in mice consuming a low-iron diet and causing a reduction in hematocrit and hemoglobin, so curcumin may have the potential to contribute to the development of anemia in patients with marginal iron status or those exhibiting the anemia of cancer and chronic diseases (11). So, as the prevalence and incidence of anemia increase with age (12), it may be an important issue to consider the hematological sequelea of curcumin administration in elderly. Also many in vitro studies demonstrated that curcumin inhibited platelets aggregation (13,14).

So this study was designed to compare the hematological effects of curcumin administration in adult and aged rats.

MATERIALS AND METHODS

Animals:

Twenty four male albino Sprague-Dawely rats: twelve adult rats 6 months old (equivalent to18 years old in human) and twelve aged rats 20 months old (equivalent to 50 years old in human) (15, 16), purchased from center for experimental animals, Faculty of Veterinarian Medicine,

Zagazig University were used in the study. All rats were left to acclimatize for one week prior to the experiment and were housed in plastic cages maintained at controlled room temperature (22-24 ◦C) with 12 hour diurnal (day and night change) with free access to water and standard laboratory animal diet throughout the study. Adult rats were randomly and equally divided into two groups: control adult and curcumin-treated adult groups. Aged rats were randomly and equally divided into two groups: control aged and curcumin-treated aged groups.

Methods:

Curcumin administration:

Curcumin $[HOC_6H_3\ (OCH_3)\ CH{=}CHCO]_2CH_2$, molecular weight 368.38 (Bio Basic Canada INC.) was dissolved in 50% dimethyl sulfoxide (DMSO, Sigma) (17) and it was administered intraperitoneally (i.p.) at a dose of 50 mg/kg to curcumin-treated adult and curcumin-treated aged rats. Both, control adult and control aged groups were i.p. treated with the vehicle. All injections were injected once daily for 21 consecutive days (8).

Tail bleeding time assay:

30 min after the last curcumin or vehicle injection, rats were anesthetized. The rat tail bleeding time was assessed by severing 3 mm from the tail tip with a razor blade and gently drying the wound with filter paperat 30-s intervals. Tail bleeding time was defined as the time from incision until the first arrest of bleeding (no bleeding for 30 s)(18).

Measurement of blood indices:

Two milliliter of blood were withdrawn from retro-orbital venous plexus from each anesthetized rat and equally divided into an EDTA-containing eppendorff and sodium citrate-containing eppendorff. The blood samples in EDTA-containing eppendorffs were used for performing following tests: red blood cells (RBC) count, hemoglobin concentration (Hb), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cells distribution width (RDW), total and differential white blood cells (WBC) count, platelets count, mean platelet volume (MPV), platelet crit (PCT)and platelets distribution width (PDW). Blood testing was carried out using the hematology fully automated cell counter **(**Heco, Italy**)** (19).

Platelets aggregation assay:

The blood samples in sodium citrate-containing eppendorffs were used for platelets aggregation assay. From all blood samples, platelet-rich plasma was collected by centrifuging the blood at 230 × g for 10 min at room temperature. Platelets sediments were prepared and washed. Platelet aggregation was induced by addition of collagen 10 µl/ml. The maximum degree of aggregation was determined by measuring the maximum height of the aggregation wave over a 4-min period beginning at the onset of platelet aggregation and it was expressed as a percentage, using aggregometer (CLOT2; SEAC, Italy)(14,20)**.**

Statistical analysis:

All the data was expressed as mean ± standard error of mean (SEM) and analyzed using Statistical Package for Social Sciences (SPSS) program (IBM SPSS Statistics, Version 20). All the comparisons among groups were carried out using one way Analysis of Variance (ANOVA)

followed by Bonferroni post hoc test to test the significance difference among group means. Data were considered statistically significant with $P \leq 0.05$.

Table 1: Red blood cells count and hematological indices in the studied groups

	RBC (x 10^6 /µl)	Hb (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	RDW (%)
Control adult group	8.73±0.24	14.1±0.37	39.8±1.4	45.75±0.75	16.18±0.23	35.58±0.38	12.7±0.12
Curcumin-treated adult group	8.03±0.17	12.7±0.24	34.3±1.1[*c]	42.67±0.61	15.82±0.06	37.1±0.48[*e]	13.5±0.19
Control aged group	8.73±0.23	13.8±0.45	39.5±1	44.75±1.18	15.73±0.45	35.02±0.35	12.98±0.1
Curcumin-treated aged group	6.84±0.37[*a]	10.5±0.46[*b]	30.2±1.5[*d]	43.8±1.16	15.4±0.39	35.24±0.53	14.42±0.44[*f]

[*a] Significant decrease in RBC count in curcumin-treated aged group vs. control adult, control aged and curcumin-treated adult groups (p = 0.001,0.001 and 0.023 respectively.)[*b] Significant decrease in Hb concentrations in curcumin-treated aged group vs. control adult, control aged and curcumin-treated adult groups (p = 0.000, 0.000 and 0.003 respectively.)[*c] Significant decrease in HCT in curcumin-treated adult group vs. control adult groups (p = 0.049.)[*d] Significant decrease in HCT in curcumin-treated aged group vs. control adult and control aged groups. (p = 0.001 in both comparisons.)[*e] Significant increase in MCHC in curcumin-treated adult group vs. control aged group (p = 0.04.)[*f] Significant increase in RDW in curcumin-treated aged group vs. control adult and control aged groups. (p = 0.004 and 0.016 respectively.)

Table 2: Platelets count and platelets indices in the studied groups

	Platelets count X 10^3 /µL	MPV (fL)	PCT (%)	PDW (%)
Control adult group	581.25 ± 43.95	9.9±0.23	0.58±0.06	10.93±0.22
Curcumin-treated adult group	1014.50±124.26	10.87±0.42	2.24±0.48[*b]	16.82±2.77
Control aged group	758.75±87.76	9.9±0.20	0.76±0.1	13.88±3.14
Curcumin-treated aged group	1255.60±102.56[*a]	10.74±0.36	3±0[*c]	18.08±3.14

[*a] significant increase in platelets count in curcumin-treated aged group vs. control adult and control aged groups. (p = 0.003 and0.034 respectively.)[*b] significant increase in PCT in curcumin-treated adult group vs. control adult and control aged groups. (p = 0.012 and 0.027 respectively.)[*c] significant increase in PCT in curcumin-treated aged group vs. control adult and control aged groups. (p = 0.001 in both comparisons.)

Results

Blood indices

Curcumin administration caused a significant decrease in RBC count and Hb concentrations in curcumin-treated aged group versus control adult, curcumin-treated adult and control aged groups. Also curcumin administration significantly decreased HCT in curcumin-treated adult group versus control adult group and in curcumin-treated aged group versus control adult and control aged groups. Curcumin administration in adult group caused a significant increase in MCHC versus control aged group. Also curcumin significantly increased RDW in aged group versus control adult and control aged groups (table 1.)

Platelets indices

Curcumin administration increased platelets count in adult group but this increase was insignificant. In aged group curcumin administration significantly increased the platelets count versus control adult and control aged groups. Also curcumin administration caused a significant

increase in PCT in adult and aged groups versus both control adult and control aged groups(table 2)

Total and differential WBCs counts

There was no significant change in total WBCs count or differential WBCs count among the studied groups after curcumin administration. But there was an observed tendency to an increase in the total WBCs count in curcumin adult group. Also there was a tendency to increase the percentage of neutrophils and a tendency to decrease the percentage of lymphocytes in curcumin adult and aged groups (table 3)

Tail bleeding time

There was statistically insignificant tendency of tail bleeding time to increase in adult group and aged groups after curcumin administration versus their control groups. Curcumin administration to aged group caused a significant increase in the tail bleeding time versus control adult group (p = 0.039) (Figure 1)

Platelet aggregation assay

Curcumin caused no significant effect on platelets aggregation induced by collagen in adult or aged groups when they were compared to their respective control group. On comparison to control adult group, curcumin-treated aged group had a significant increase in the percentage of platelets aggregation (Figure 2)

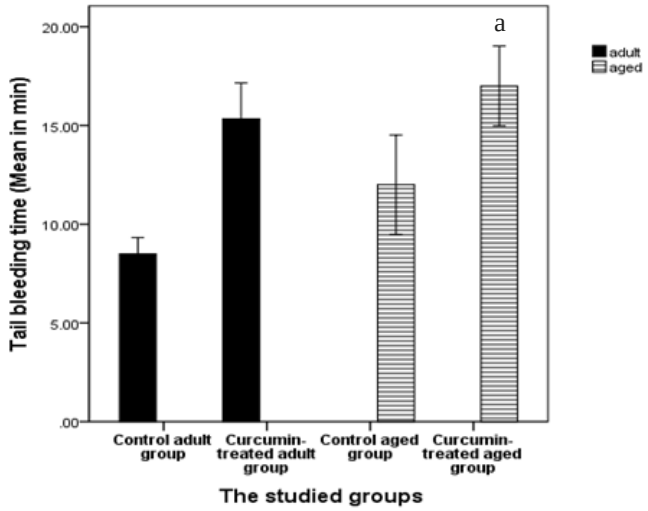

Fig. 1: Rat tail bleeding time in the studied groups, *a Significant increase in tail bleeding time in curcumin-treated aged group vs. control adult group (p = 0.039)

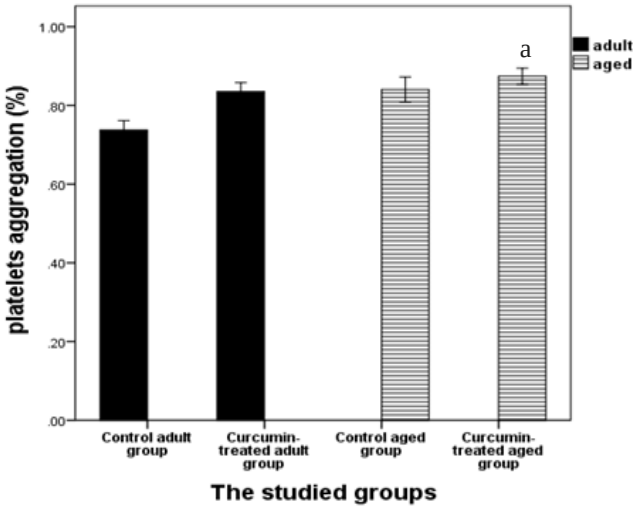

Fig. 2: The percentages of platelets aggregation induced by collagen in the studied groups *a Significant increase in the percentage of platelets aggregation in curcumin-treated aged group vs. control adult group (p = 0.009).

Table 3: Total and differential white blood cells count in the studied groups

	WBC count x 10^3/µL	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
Control adult group	5.55±0.96	88.8 ± 4.1	1.8±0.2	8.3±4.1	1.3±0.3	0±0
Curcumin-treated adult group	10.33±1.99	80±5.7	2±0.5	16.8±4.9	1.2±0.5	0±0
Control aged group	9.83±1.33	76.3±9.9	4±1.2	17.5±7.8	1.8±0.8	0.5±0.29
Curcumin-treated aged group	8.7±1.34	73.2±4.3	3.6±.4	21±4.2	2.2±0.2	0±0

Discussion:

In the current study, two important observations were made. First observation was that curcumin administration in aged rats caused anemia manifested by significant decrease in RBCs count, Hb concentration and HCT and significant increase in RDW. In adult rats curcumin significantly decreased HCT only. The mechanism of this finding may be attributed to the iron chelating effect of curcumin leading to impaired iron absorption, as Jiao et al., found that curcumin was able to disturb all parameters of iron metabolism and they concluded that curcumin may have the potential to contribute to the development of anemia in patients with marginal iron status(11).Also another study showed that long-term oral supplementation with curcumin may promote iron deficiency even under conditions of sufficient iron intake in middle aged mice (21). In the elderly, the serum iron level and the iron-binding capacity fall (4) and this change may make elderly more vulnerable to the effect of curcumin. RDW is a parameter describes the size variation of RBC. It is calculated by dividing the standard deviation of the MCV by the respective actual MCV, and is expressed as percentage. A high RDW represents a large variation of the RBC volume, called anisocytosis, which is found in conditions with an increased number of small or large RBC. RDW is increased in iron deficiency anemia (22). Lower HCT with curcumin administration was also reported in Alzheimer's patients (23). MCHC was significantly increased in curcumin adult rats this result may be explained by that MCHC is computed by dividing hemoblogin (g/dL) by hematocrit(11).Curcumin administration in adult group caused a significant decrease in hematocrit only without a significant decrease in Hb concentration and this may result in this significant increase in MCHC in adult group.

Second observation was that curcumin increased platelets count in adult and aged rats but this increase was statistically significant in aged rats only. In agreement with current finding, Nemmar et al., found that curcumin administration increased platelets count but not significantly in normal mice and curcumin significantly prevented the decrease in platelets count in mice repeatedly exposed to diesel exhaust particles (24).The increased platelets count may be secondary to iron deficiency anemia which is a cause of reactive thrombocytosis by unknown mechanism (25). Iron was suggested as an important regulator of thrombopoiesis as normal iron concentrations are required to prevent thrombocytosis by inhibiting thrombopoiesis and in the same time a minimum amount of iron is required to maintain platelet production. So thrombocytosis is usually associated with a mild iron deficiency which results in a lack of inhibition of thrombopoiesis while severe defect of iron may be accompanied by thrombocytopenia (26).PCT represents the volume of blood on a percentage that comprises platelets and is a good indicator of the circulating mass of platelets(27). In the current study PCT was significantly increased in adult and aged rats after curcumin administration. This finding may be due to the observed increase in platelets count as PCT is a

measurement derived from the platelet count and the mean platelet volume (28).

In the current study, on comparison to respective control group, curcumin administration did not affect tail bleeding time or in vitro platelets aggregation induced by collagen. However it was observed that both tail bleeding time and in vitro platelets aggregation induced by collagen had a tendency to increase in control aged rats versus control adult rats and this increase became significant only after curcumin administration. The significant increase in tail bleeding time in aged rats after curcumin treatment may be attributed to that, the antiplatelets effect of curcumin which was demonstrated in several previous studies (29,30, 31) augmented the observed tendency of aged rats to have prolonged bleeding time versus adult rats. The significant increase in platelets aggregation induced by collagen in aged rats may be explained by the enhanced in vitro reactivity to platelet-aggregating agents as collagen that has been observed with aging (32). In addition, the observed significant increase in platelets count in aged rats after curcumin administration which may represent reactive thrombocytosis had been associated with excessive aggregation (33)

In the current study, curcumin administration was found to have no significant change in total or differential WBCs in adult and aged rats. This result was in agreement with El-Habibi et al., result concerning WBCs as they found that curcumin administration into normal rats did not produce any significant changes in all tested parameters(34). The current results may be questioning the use of curcumin in elderly without taking in consideration the risk of its hematological consequences.

Conclusion:

Curcumin administration in aged rats caused anemia, the cause of anemia may be the iron deficiency. Curcumin also caused an increase in platelets count and this may represents reactive thrombocytosis to the iron deficiency anemia and it prolonged the bleeding time. So these hematological sequelae of curcumin administration must be seriously taken in consideration with practical implications of curcumin in aging. It should be noted that, to my knowledge, no other studies have reported the hematological effect of curcumin administration in elderly which represented a challenge for discussing the current study findings so further researches are required to address these effects and their possible mechanisms.

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