

The Effect of Aqueous Extract of *Curcuma longa* on Some Parameters of Cytogenetic, Immunity and Fertility in Female mice

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Abstract

The research work was conducted to investigate the effect of oral administration of aqueous extract of turmeric at doses of (5, 10) mg/kg body weight for two weeks daily by determining the genotoxic effect (mitotic index), evaluation of immunological effect (IgG, IgM, IgA, C₃, C₄) and measuring fertility hormones (follicles stimulation hormone/FSH, lutenising hormone/LH) levels with histological examinations of female albino swiss mice ovaries in comparison with control (normal saline). A clear effect in increasing mitotic activity was revealed for both doses in comparison with control. Results also showed a significant increase in the value of all immunological parameters at both doses, in comparison with control. Also obvious raise was seen in the levels of FSH and LH hormones for both doses when compared with normal saline treated mice with no significant damage seen in female ovaries tissue, there were certain changes in mice ovaries tissue which were represented by increasing in the numbers of primary and secondary follicles and numbers of corpus luteum at both doses also.

Key words: turmeric, curcumin, mitotic index, immunity, fertility

Introduction

Turmeric, a spice obtained from the rhizome of *Curcuma longa* Linn (Zingiberaceae), has been regularly used for its coloring, flavoring and medicinal properties (1). *Curcuma longa* rhizomes contain approximately volatile oil; composed mainly of monoterpenes, curcuminoids; composed mainly of curcumin, minerals, carotene and vitamin C (2). Curcumin, the active principle of turmeric, is commonly used as a coloring agent in foods, drugs and cosmetics, and has a wide range of effects (3). The continuing research indicates that turmeric and its active compound 'curcumin' are unique antioxidants,

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antimutagenic, antitumorigenic, anticarcinogenic, anti-inflammatory, antiarthritics, antimicrobial and immunomodulatory properties as reviewed recently (٤, ٥).

The immunoglobulins, also known as antibodies, are a family of proteins that exist in the plasma. The immunoglobulin family includes immunoglobulin "A" (IgA), immunoglobulin "G" (IgG), immunoglobulin "M" (IgM), immunoglobulin "D" (IgD), and immunoglobulin "E" (IgE). All of the immunoglobulins play a role in the immune system's defense mechanisms. The immune system manufactures the immunoglobulins in response to exposure to a foreign invader. After exposure to a foreign invader, such as a specific virus, bacteria, or toxin produced by an organism, a certain type of lymphocyte (a type of white blood cell) produces the immunoglobulins. then the immunoglobulins level can be measured in the blood (٦) .

Complement analysis in the clinic is usually associated with the quantification of C٣ and C٤ and screening for complement activity together with complement activation products. These analyses have been available in routine diagnostic laboratories for decades. In recent years, however, the field of complement analysis has expanded considerably, with the introduction of novel assays to detect complement activation products, and spreading still further towards genetic analysis to reveal the basis of complement deficiencies and identify mutations and polymorphisms associated with some diseases (٧).

Fertility hormones, FSH (Follicle Stimulating Hormone) is a hormone secreted by the pituitary gland in the brain. It stimulates the follicles in the ovaries to ripen several eggs. FSH also readies the mammary glands for milk production. In men, the FSH initiates sperm production. While LH (Lutenising Hormone) is secreted by the pituitary gland to stimulate ovulation that is, the release of the egg or ovum from the follicles. LH secretion signals the remnants of the follicle to change into the corpus luteum. The corpus luteum then begins producing progesterone and estrogens (٨).

The aim of the present study was to investigate the effect of aqueous extract from turmeric on some parameters of cytogenetic, immunity and fertility in female mice.

Materials and Methods All the chemicals were obtained from Sigma Chemical Co. (USA) and BDH (England).

Experimental Animals: Three groups of female albino Swiss BALB/c mice, which were obtained from the Biotechnology Research Center / AL-Nahrain University, were used in this study. Their ages were ranged between (٨-١٢) weeks and weighting (٢٥-٣٠) gm. They were divided into subgroups, and each group was putted in a separate plastic cage. The cages were kept in a room with

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(٢٣-٢٥) C° temperature. The animals were fed with a suitable quantity of water and complete diet.

Administration of Experimental Animals: The animals in this experiment were treated with a cumulative dose of turmeric for ١٤ days. The main aim of this experiment was to evaluate the acute treatment effect of turmeric in normal mice. The mice were divided into three experimental groups, each group consisted of (٥) mice and to which the turmeric was administered orally.

Group I: Negative control (٥mice), treated with (٠,١ ml) of normal saline.

Group II: turmeric treatment (٥mice) treated with (٠,١ ml) of (١mg /Kg)

Group III: turmeric treatment (٥mice), treated with (٠,١ ml) of (٥mg /Kg).

The animals were monitored for apparent signs of toxicity for ١٤ days. Then they were sacrificed on the ١٥th day after administration and the blood was separated to measure the levels of (IgG, IgM, IgA, C٣, C٤) and (FSH, LH) fertility hormones by using a specific measuring kits. After that the ovaries were collected and fixed in ١٠٪ buffered formaldehyde solution.

Preparation of aqueous extract: water extraction of turmeric was prepared by boiling (١٠٠)gm in (١٠٠٠)ml distilled water over low flame for (١٥)minutes. The flask was then plugged and removed from the heat and allowed to cool. After cooling the content of the flask was filtered and dried to prepare the required concentrations (٩).

Chromosomal preparation from somatic cells of the mouse bone marrow: This experiment was done according to (١٠). Each animal was injected with ٠,٢٥ml of colchicine with a concentration of (١mg/ml) intraperitoneally (I.P) ٢hr before sacrificing the animal, Then the animal was sacrificed by cervical dislocation and fixed on its ventral side on the anatomy plate and the abdominal side of the animal and its thigh region were swabbed with ٧٠٪ ethanol. The femur bone was then taken and cleaned from the other tissues and muscles and gapped from the middle with a forceps in a vertical position over the edge of the test tube, and by sterile syringe ٥ml of PBS were injected so as to wash and drop the bone marrow in the test tube. The test tube was taken and centrifuged at speed of ٢٠٠٠ rpm for ١٠min. After that the supernatant was removed and ٥ml of potassium chloride (٠,٠٧٥) M was added as a hypotonic solution, then the test tubes were left for ٣٠min in the water bath at ٣٧C° and shaken from time to time. The tubes were then centrifuged at ٢٠٠٠ rpm for ١٠min and the supernatant was removed and the fixative solution was added (as drops) on the inside wall of the test tube with the continuous shaking, the volume was fixed to ٥ml and the content shaken well. The tube was kept at ٤C° for ٣٠min to fix the cells. After that the tubes were centrifuged at ٢٠٠٠ rpm for ١٠min. The process was repeated three times and the cells were suspended in ٢ml of the fixative solution. By a pasture pipette, few drops from the tube were

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dropped vertically on the chilled slides from a height of 3 feet at a rate of (2-3) drops to give the chance for the chromosomes to spread well. Later the slides were kept to dry at room temperature, and then stained with Giemsa stain and left for 10 min and washed with D.W.

Assay measurements of hormones: Serum hormones (FSH, LH) concentrations were evaluated with a Bio merieux Italia S.P. a vidia campigliano, 58 50010-point A EMA (F) Italia miniVIDAS, following the manufacturer's recommendations.

Assay measurements of immunoglobulins and complements: serum levels of (IgG, IgM, IgA, C3, and C4) were evaluated by using radial immunodiffusion plate, following the kit manufacturer's method.

Histological Examinations: At the time of death, mouse organs ovaries were taken for histopathological examination. The perfuse-fixed ovaries placed in Bouin fluid overnight, and processed for routine paraffin embedding. The ovaries were cut into 5-µm sections. Three serial sections per ovaries were mounted on slides, deparaffinized, rehydrated, and stained with hematoxyline - eosin stain. Sections of the ovaries were examined by light microscopy; primary and secondary follicles and corpus luteum diameters were assessed in each ovary using a previously calibrated micrometer eyepiece. This was performed by using method of (11).

Statistical Analysis: Data were analyzed by 1-way analysis of variance with ANOVA- test. Data are presented as means ± SE. The level of significance was $P < .05$. (12).

Results and Discussion

After the mice have been orally given two doses (5, 10) mg/kg of the water extract from the dried rhizome of *Curcuma longa*, neither signs of toxicity nor death of mice were observed during the 14 days of the experimental period, similar results were also obtained by many authors (13, 14, 15), in which they showed that, no toxic effects due to feeding turmeric or curcumin in rat, guinea pig or monkey were recorded. Significant difference in mitotic percentage between treated and untreated (control) animals were occurring as shown in Table 1. A clear effect in increasing mitotic activity was revealed for both concentrations (11, 16, 17, 18)% respectively in comparison with control (5, 16)%. The turmeric (*Curcuma longa* Linn (Zingiberaceae)) has traditionally been used as both spice and medicine. It contains small quantities of chemopreventive compounds such as B-carotene, curcumin, volatile oils. The antimutagenic activity of turmeric could be related to the large number of these potent chemopreventive compounds and especially curcumin which were shown to be a promising antimutagenic compound (16, 17).

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However, similar results were also obtained in which powdered turmeric were given in combination with genotoxic agents and it was shown to be so effective in reducing the mutational events induced by these agents either by suppression of metabolic activation or interaction with the active groups of mutagens and this suggest to be the mechanism by which the turmeric exert its antimagnetic property (١٨, ١٩).

Table (١): Cytogenetic effects of *Curcuma longa* in comparison with control (normal saline) on mouse bone marrow cell.

Groups	Mitotic index (mean±SE)
Control	A ٥١,١٦±٣,٧٦
Curcuma ٥mg	BC ٦١,٦٦±٢,٥١
Curcuma ١٠mg	C ٧٠,٠٠±١,٧٣

Differences A, B, C are significant ($P<٠,٠٥$) to compared rows.

To determine the immunomodulatory effect in mice treated with the extract, immunological parameters were examined as presented in Tables (٢). The concentration of immunoglobulins and complements (IgG, IgM, IgA, C٣, C٤) in the treated mice with (٥ and ١٠)mg/kg of the extract was higher than that of the control group. Results show a significant raise in the levels of them

Groups	Immunoglobulin mg/dl (mean±SE)				
	IgG	IgM	IgA	C٣	C٤
Control	A ١٠٢,٠±٨٤,٦١	A ١٣٠,٦±٢٦,٩	A ٢١٣,٦٢±١٧,٥١	A ٢٢٤,٣٢±٢٠,٨	A ٣٢٠,٩±٤٠,٢
Turmeric ٥mg	C ١١٠,٥±٩٦,٣٧	C ١٥١,٤±٣٠,٢	B ٢٢٩,٣±١٨,٧٢	C ٢٥٢,٨±١٧,٦	B ٣٤٢,٧±٣٩,٠
Turmeric ١٠mg	C ١١٢,٦±٧٨,٧٦	C ١٥٨,٤±٢٢,٣٢	C ٢٤٠,٨±٢٣,٢٦	D ٢٦٣,٩±١٩,٤٨	C ٣٥٨,٩±٤١,٣

(١١٠,٥, ١٥١,٤, ٢٢٩,٣, ٢٥٢,٨, ٣٤٢,٧ and ١١٢,٦, ١٥٨,٤١, ٢٤٠,٨, ٢٦٣,٩, ٣٥٨,٩)mg/dl respectively at both doses in comparison with control (١٠٢,٠, ١٣٠,٦, ٢١٣,٦٢, ٢٢٤,٣٢, ٣٢٠,٩)mg/dl.

Table (٢): Immunomodulatory activity of *Curcuma longa* in comparison with control (normal saline) in mice.

Differences A, B, C, D are significant ($P<٠,٠٥$) to compared rows.

These results were come in agreement with (٢٠, ٢١, ٢٢, ٢٣) in which the researchers show that both turmeric or curcumin alone is a potent immunomodulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells. The spice were seen to poses an anti-carcinogenic and anti-inflammatory activity and may act as immunorestor agent through altering the immune system by increasing the number of immune cells and bone marrow cellularity and this in turn lead to increase in the level of immunoglobulins and complements in additions to other

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types of immune system cells. Thus the turmeric had no immunotoxic effect and may be considered as immunologically safe compound.

However, with fertility aspect, turmeric shows promising results. A significant raise in the levels of fertility hormones (FSH and LH) was seen after treatment with the two doses of the extract when compared with control treated mice as shown in table (٣). The levels of FSH were (٢,٠١, ٢,٦٣) mIU/m while LH (١,٩٩, ٢,٦٢) mIU/m at the two doses respectively when compared with normal saline treatment (١,٤٦, ١,٣٢) mIU/m.

Table (٣): Fertility activity of *Curcuma longa* in comparison with control (normal saline) in mice.

Groups	FSH mIU/m (mean±SE)	LH mIU/m (mean±SE)
Control	A ١,٤٦±٠,٠٦	A ١,٣٢±٠,٦٣
Turmeric ٠mg	B ٢,٠١±٠,٠٨	B ١,٩٩±٠,٣٠
Turmeric ١٠mg	C ٢,٦٣±٠,٦٢	C ٢,٦٢±٠,٦١

Differences A, B, C are significant ($P < 0.05$) to compared rows.

Moreover, these observations were further investigated by the histopathological assessment of the female ovaries tissues. The results showed that the water extract of *C. longa* did not produce a significant damage in these tissues, however, significant changes were seen in the number of primary and secondary follicles and corpus luteum in the turmeric treated mice when compared with the control treated mice as shown in table (٤). After oral administration of the two extract doses, the number of the primary follicles was (٥,٢٠, ٦,٠٠) and secondary follicles (٧,٠٠, ٨,٠٢) respectively, while control was (٤,٣٦, ٦,٤٣). On the other hand, the number of corpus luteum was (٥,٢٠, ٥,٨٦) at both doses respectively, while normal saline treated mice showed (٤,٤٠).

Table (٤): Effect of *Curcuma longa* on primary and secondary follicles and corpus luteum in comparison with control (normal saline) in mice.

Differences A, B, C are significant ($P < 0.05$) to compared rows.

Groups	No. of primary follicles (m±SE)	No. of secondary follicles (m±SE)	No. of corpus luteum (m±SE)
Control	A ٤,٣٦±٠,٨٢	A ٦,٤٣±١,٢٢	A ٤,٤٠±٠,٨٦
Turmeric ٠mg	C ٥,٢٠±١,٠٨	B ٧,٠٠±٢,١٢	B ٥,٢٠±٠,٩٦
Turmeric ١٠mg	D ٦,٠٠±١,٣٢	C ٨,٠٢±١,٩٤	C ٥,٨٦±١,٣٠

Turmeric was shown to have powerful antioxidant (٥, ٢٤), and since many studies show a strong relation between antioxidants and fertility inductions (٢٥,

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٢٦, ٢٧) and between increased free radicals and reduced fertility (٢٨). These studies showed that the imbalance between antioxidant defense and free radical activity is more evident in the infertility condition, thus turmeric extract shows significant effect in increasing fertility in mice. Moreover, similar studies were also conducted to investigate the effect of powdered turmeric on fertility and reproduction in rats and they proven that; there were no adverse toxicological effects on the reproductive capacity of rats that received dietary concentrations of curcumin up to ٥٠٠ mg/kg for two successive generations but in fact, turmeric had enhance their fertility (٢٩, ٣٠).

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تأثير المستخلص المائي لنبات الكركم على بعض معاملات الوراثة الخلوية

والمناعة والخصوبة في اناث الفئران

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الخلاصة

تم اجراء هذا العمل البحثي بهدف التحري عن تأثير المستخلص المائي لنبات الكركم المعطى عن طريق الفم وبجرعتين (١٠، ٥) ملغم/كغم من وزن الجسم ولمدة اسبوعان يوميا من خلال حساب التأثير السمي الوراثي (معامل الانقسام الخيطي)، وتقييم التأثير المناعي بقياس معدل المعاملات المناعية (IgG, IgM, IgA, C٣)، وقياس معدل الهرمونات الانثوية (FSH, LH) مصحوبة بفحص التغيرات المرضية في مبايض الاناث المعاملة بالمستخلص ومقارنتها مع السيطرة (الداري الملحي). أظهرت النتائج وجود تأثير واضح في زيادة نشاط معدل الانقسام الخيطي ولكلا الجرعتين بالمقارنة مع السيطرة. كذلك اظهرت النتائج حدوث زيادة معنوية في جميع قيم معاملات المناعة لكلا الجرعتين بالمقارنة مع السيطرة. لوحظ ايضا حدوث ارتفاع ملموس في معدل هرموني FSH و LH وايضا عند كلا الجرعتين بالمقارنة مع الفئران المعاملة بالداري الملحي وفي الوقت نفسه لم يتم مشاهدة حدوث اي تلف في نسيج مبايض الاناث ولكن كان هنالك بعض التغيرات والتي تمثلت بحدوث زيادة في عدد الجريبات الاولى والثانوية والاجسام الصفراء.