Structural and functional changes of adrenal cortex and pancreas in mature male rats due to (*Curcuma Longa*)

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Abstract

Thirty mature male rats were randomly divided into three equal groups and treated for 6 weeks as follows: Rats in the first group were received 3 ml ordinary tap water and served as control group (group C), animals of the second and third groups were received 150, 300 mg\kg B.W. of *Curcuma Longa* respectively.

Serum concentration of cortisol and glucose as an index of adrenal cortex function and pancreas function. Sections from adrenal cortex and pancreas were assessed for histological studies.

The results revealed that treatment with two doses caused significant ($P<0.05$) increase in serum cortisol and glucose concentration.

Furthermore, water suspension of *Curcuma Longa* caused significant ($p<0.05$) decrease in serum glucose concentration in the treated groups as compared with control group at the same two treated periods, besides, significant increase was observed at day 42.

Histological studies of adrenal cortex of treated rats (T1 and T2 groups) indicated a case of hyperplasia of zona fasciculata demonstrated by increasing which type of cells clear vacculation of cytoplasm. On other hand, histological hyperplasia of secretary cells with mild filtration of lymphocytes and neutrophils. It was concluded that treatment of male rats with two doses of *Curcuma Longa* suspension had positive significant functional and structural effects on adrenal cortex and pancreas.

التغيرات التركيبية والوظيفية لقشرة الغدة الكظرية والبنكرياس في ذكور الجرذان البالغة المعاملة بعشبة الكركم *Curcuma Longa*

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الخلاصة

تم استخدام ذكور الجرذان البالغة وبأعمار تتراوح بين (8–10) أسبوع وقسمت عشوائياً إلى ثلاث مجاميع بواقع (عشرة حيوانات/مجموعة) وعوملت كالآتي لمدة 6 أسابيع، حيوانات مجموعة السيطرة(م) المعالجة عن طريق الفم وأعطيت 3 مل من الماء العادي يومياً في حين أعطيت حيوانات المجموعة الثانية (م1) 3 مل من المعلق المائي للكركم وبجرعة مقدارها 150 ملغم/كغم من وزن الجسم يومياً وأما المجموعة الثالثة (م2) فقد أعطيت 3 مل من المعلق المائي للكركم وبجرعة مقدارها 300ملغم/كغم من وزن الجسم يومياً ولمدة 6 أسابيع.

تم سحب عينات الدم في الأيام 0، 14، 28 و 42 من فترة المعاملة وتم فصل المصل في جهاز الطرد المركزي لغرض دراسة المعايير التالية:

تركيز هورمون الكورتيزول في مصل الدم كمؤشر على وظيفة قشرة الغدة الكظرية، تركيز كلوكوز مصل الدم كمؤشر على وظيفة البنكرياس. وتم اخذ قطع نسيجية من قشرة الغدة الكظرية ومن البنكرياس لغرض دراسة المقاطع النسيجية لقشرة الغدة الكظرية والبنكرياس. إضافة إلى حساب وزن الجسم كل أسبوعين من فترة المعاملة.

أظهرت النتائج ان معاملة ذكور الجرذان البالغة بجرعتين من المعلق المائي لعشبة الكركم (150 و300) ملغم/كغم من وزن الجسم أدى إلى حصول زيادة معنوية في تركيز هورمون الكورتيزول في مصل الدم للمجموعتين المعاملتين مقارنةً مع مجموعة السيطرة في الأسبوع الثاني والرابع إضافة إلى حصول انخفاض معنوي ($P<0.01$) في تركيز كلوكوز مصل الدم في هاتين المجموعتين مقارنةً بمجموعة السيطرة وخلال نفس الفترة أعلاه.

أشارت الدراسة النسيجية لقشرة الغدة الكظرية للمجموعتين المعاملتين بعشبة الكركم إلى حدوث حالة فرط تنسج في النطاق الحزيمي وحدوث زيادة واضحة في إعداد تلك الخلايا وتفجي هيولي هذه الخلايا، من جهة أخرى أظهر الفحص الفحص النسيجي للبنكرياس في المجموعتين المعاملتين ووجود حالة فرط تنسج للخلايا الإفرازية (جزر لانجرهانس) مع حدوث ارتشاح خفيف لخلايا الدم البيضاء العدلة واللمفاوية.

يستدل من نتائج هذه التجربة ان معاملة ذكور الجرذان البالغة بجرعتين من المعلق المائي لعشبة الكركم ولمدة 6 أسابيع قد تتسبب بحدوث تأثيرات نسيجية واضحة في قشرة الغدة الكظرية والبنكرياس تمثلت بحدوث زيادة في النشاط الوظيفي لهاتين الغدتين.

Introduction

Turmeric (*Curcuma longa*) is a medicinal plant extensively used in ayurvedia and Greek a home remedy for various diseases (1). Turmeric is used as a food additive preservative and coloring agent in Asian countries, in old Indian medicine; it was extensively used for treatment of sprains and swelling caused by injury (2). In recent time, traditional Indian medicine uses *Curcuma longa* powder for the treatment of billiary disorders, anorexia, cough, hepatic disorders, rheumatism and sinusitis (3). *Curcuma longa* is used for diseases associated with abdominal pains (4). and patients with respiratory diseases (5).

Curcumin is the active constituent of *Curcuma longa* which first isolated by Vogel and Pelletier 1815(6). Extensive work has been done to establish the biological activities and pharmacological action of *Curcuma longa*: it has antitumor (7, 8) antioxidant (9), antidiabetic (10) and anti-inflammatory properties (11).

However, there is a few information about the effect of Curcuma on adrenal cortex and pancreas function and its mechanism of action in regulation of blood glucose, therefore, this study was designed to know the role of *Curcuma longa* on adrenal cortex and pancreas function through measuring the body weight, serum cortical concentration, Serum blood glucose and Histological sections study of adrenal cortex and pancreas.

Materials and Methods

1. **The rhizomes of *Curcuma longa*** were purchased from the local market in Kut and certified at the Iraqi National iterbarium in AboGhraib. The rhizomes were cleaned and ground in grinder to prepare suspension.

2. **Experimental design:** Thirty male rats were divided randomly into 3 groups, 10 animals per group and treated as follows:

Control group: Animals of this group received 3 ml of ordinary tap water by oral intubations using gavages' needle.

(T1) group: Animals of this group received by oral intubation, a 3ml of ***Curcuma longa*** suspension (150mg/kg B.W.) daily.

(T2) group: Animals of this group received by oral intubation, a 3ml of ***Curcuma longa*** suspension (300mg/kg B.W.) daily.

3. **Blood Sampling:** Fasting blood samples (3ml) were collected at zero, 14, 28, 42 days of experiment via cardiac puncture technique after general anesthesia.

Blood samples were kept into tube and held for not to more than four hours before serum was collected by centrifugation (2500rpm) for 15 minutes, liquated and frozen at -20C until analysis.

4. Parameters studied:

a. Body weight: body weight was measured through treatment period (zero, 14, 28 and 42) days by electrical balance.

b. Cortisol hormone (nmol/L): Cortisol determined by using VIDAS cortisol (Vietic Immuno Diagnostic Analysis System) which is an enzyme immunosorbent assay for the detection of total cortisol in serum using ELFA technique (12).

c. blood glucose concentration (mg/dl): glucose concentration was enzymatically measured by using enzymatic oxidation method (13).

d. Histological study: for histological studies, the adrenal gland and pancreas were excised and preserved in 10 % natural formalin buffer solution till the preparation of histological sections.

Tissues were embedded in paraffin and several tissues sections of adrenal cortex and pancreas were stained with hematoxylin-Eosin stain (14) for histological study.

Statistical analysis of data was performed on the basis of two way analysis of variance (ANOVA) depending on experimental design at each time. Specific group differences were determined using least significant differences LSD (15).

Results

Body weight (gm):

Table (1) illustrated the mean values of body weight in control and treated groups. The results showed non significant differences in mean values of body weight between control and two treated group at the pretreatment period. Statistical analysis indicated that the mean values of body weight of control and two treated groups of male rats tended to increase but not significantly along the periods of treatment. The range of mean values (221.60 ±4.96, 264.16 ±7.55), (222.30±12.06, 269.00±11.36) for T1 and T2 respectively compared with (218.83±2.66 -248.66±12.27)for control group.

On the other hand there were no significant differences ($P<0.05$) in body weight within group T1 and T2 at all periods of experiment.

Table (1) The effect of two different doses of *Curcuma longa* on body weight (gram) of adult male rats for 24 days

Group / Time	Control group N=10	T1 group N=10 150mg/kg.B.W.	T2 group N=10 300mg/kg.B.W.
Day zero	218.83± 2.66	221.60 ±4.96	222.30 ±12.06
Day (14)	221.66 ±4.94	249.60 ±14.62	243.16 ±9.98
Day (28)	242.16± 11.31	261.16 ±12.30	262.00 ± 13.85
Day (42)	248.66± 12.27	264.16± 7.55	269.00± 11.36

- **Mean ± SE.**

Blood glucose (mg/dl):

The results showed of significantly decrease ($p< 0.05$) of glucose in T1 and T2 compared with control group at 14 and 28 day, the mean values (126.83 ± 6.43) ، (125.83± 5.15), (125.66 ±3.81), (126.83± 15.25) for T1 and T2 respectively compared with (142.00 ± 7.63), (143.50 ± 7.42)for control group, while at 42 day there was no significant differences between them (145.33 ± 5.98), (144.83±7.87) with (144.50± 7.18) for T1 and T2 and control group respectively.

Furthermore, there were a significant differences ($P<0.05$) between T1 and T2 groups at $2^{nd,}$ 4^{th} weeks as compared with 6^{th} week of treatment.

Table (2) The effect of two different doses of Curcuma longa on glucose concentration (mg/dl) of adult male rats for 42 days

Group / Time	Control group N=10	T1 group N=10 150mg/kg.B.W.	T2 group N=10 300mg/kg.B.W.
Day zero	144.00 ±6.75 A a	142.16± 6.79 A a	143.83± 7.85 A a
Day (14)	142.00± 7.63 A a	126.83± 6.43 B b	125.66± 3.81 B b
Day (28)	143.50± 7.42 A a	125.83 ±5.15 B b	126.83± 15.25 B b
Day (42)	144.50± 7.18 A a	145.33± 5.98 A a	144.83 ±7.87 A a

- **Capital letters denote between groups differences ($p< 0.05$).**
- **Small letters denote within group differences ($p< 0.05$)**
- **Mean ± SE.**

Serum cortisol concentration (nmol\L):

The effects of two doses of *Curcuma longa* on mean values of serum cortisol concentration were shown in table (3).

The level of cortisol showed significant increase($p< 0.05$) in two treated group (T1, T2) after exposure of male rats to *Curcuma longa* at 14 and 28 day of treatment period while at 42 day of treatment there were non significant differences between T1 and T2 group as compared with control group.

On the other hand there were no significant differences in cortisol concentration within group T1 at all three periods of treatment and the mean values of cortisol were significantly increase within T2 group at 14 day of treatment period as compared with 28 and 42 days.

Furthermore, there were a significant increase ($p<0.05$) in cortisol level in T1 at all three periods of treatment as comparing to pretreatment period.

Table (3) The effect of two different doses of *Curcuma longa* on cortisol concentration (nmol/ L) of adult male rats for 42days

Group / Time	Control group N=10	T1 group N=10 150mg/kg.B.W.	T2 group N=10 300mg/kg.B.W.
Day zero	23.38± 3.48 A a	25.38± 3.04 A a	24.43 ±4.30 A a
Day (14)	26.36± 2.74 A a	40.48± 3.24 B b	48.81± 3.77 B b
Day (28)	27.±1.68 A a	36.83± 4.61 B b	38.65 ±2.87 B c
Day (42)	28.46± 4.61 A a	31.86 ±4.76 A b	34.45± 4.04 A c

- **Capital letters denote between groups differences (p< 0.05).**
- **Small letters denote within group differences (p< 0.05).**
- **Mean ± SE.**

Histological status of adrenal cortex and pancreas:

The histological structure of adrenal cortex of untreated rats (control group) was shown in figure (1), the adrenal cortex consist of three zones (zona glomerulosa, zona fasciculata and zona reticularis) in normal structure.

In treated rats (T1 and T2) illustrated in (figure 2 and 3) indicate a case of moderate hyperplasia manifested by proliferation of fasciculata and reticularis cells with vacculation of cytoplasm. On the other hand these changes were mild in T1 group as compared with T2 group.

The histological section of pancreas of control group was demonstrated in figure (4) there was a normal structure of pancreatic cells especially secretary cells of Islet of Langerhans.

The histological changes in group T1 showed hyperplasia and vacculation of pancreatic cells (secretary cells) figure (5) the T2 group showed the same histological changes but there were little in filtration of monocyte and lymphocyte cells adjacent to islet of Langerhans especially around blood vessels figure (6).

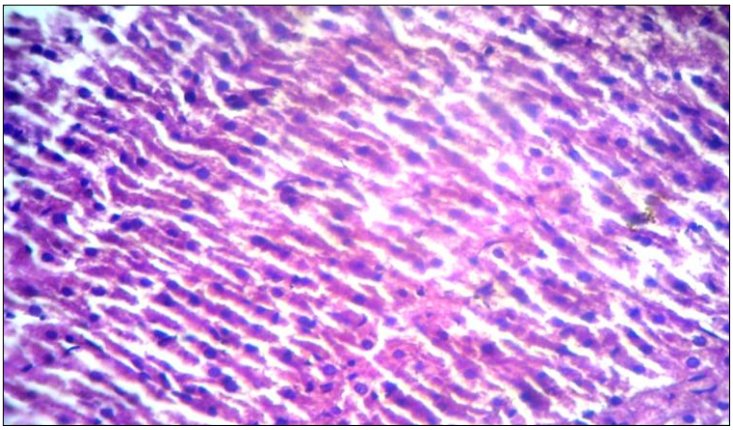

Figure (1) section in adrenal cortex of untreated rat (control group) note normal structure of cortex (H &E 40 X)

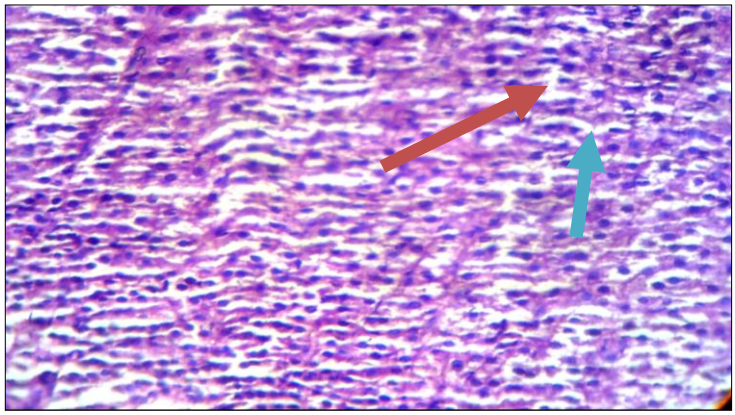

Figure (2) Section in adrenal cortex from *Curcuma longa* treated rats (group T1) note hyperplasia of zona fasciculate ( ) with vacculation of cytoplasm ( ) of it's cells (H&E 40X)

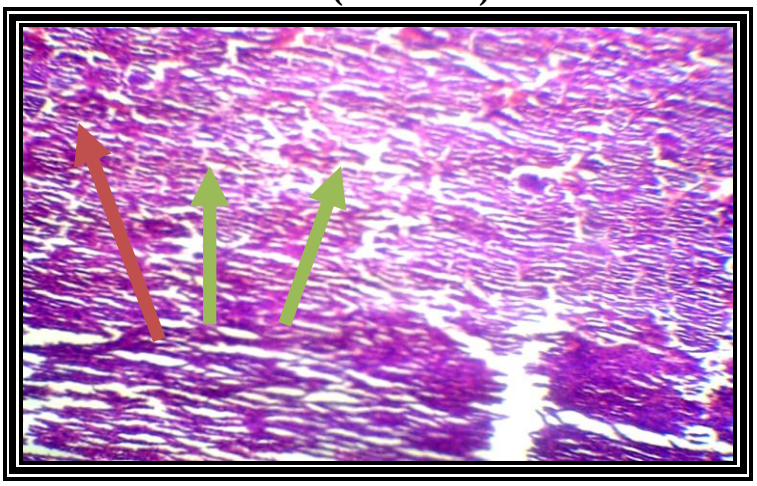

Figure (3) section in adrenal cortex from *Curcuma longa* treated rat (group T2) note mild hyperplasia of zona reticularis and zona fasciculata ( ) with congestion of blood vessels around zona reticularis ( ) (H&E 40X)

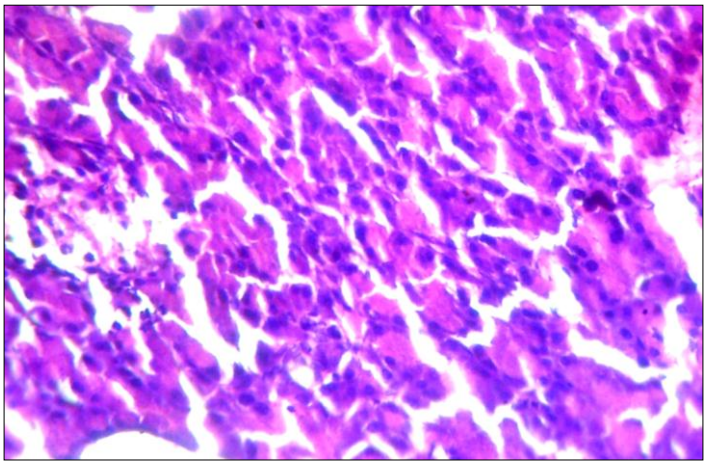

Figure (4) Section in pancreas of untreated rat (control group)note normal histological structure(H&E 40X)

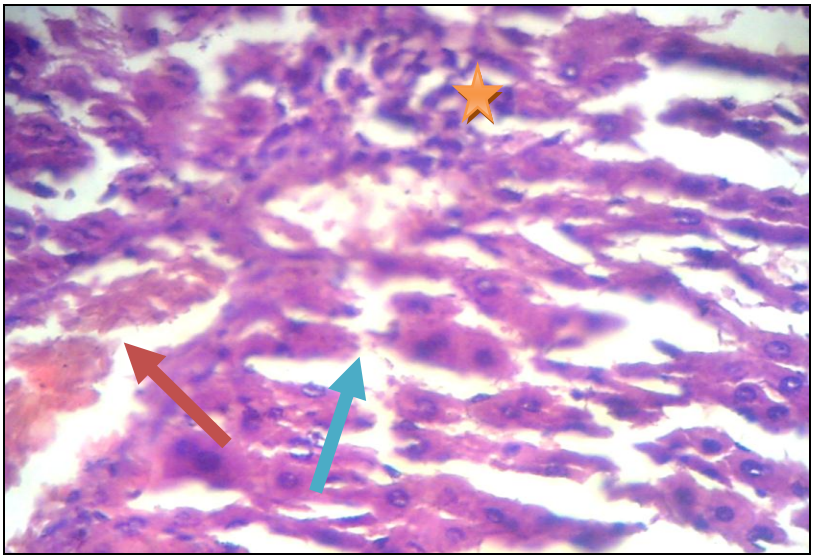

Figure (5) Section in pancreas from *Curcuma longa* treated rat (group T1) note hyperplasia of islet of Langerhans cells ( ) with blood vessels congestion( ) and accumulation of monocyte around pancreatic blood vessels ( ) (H&E40X)

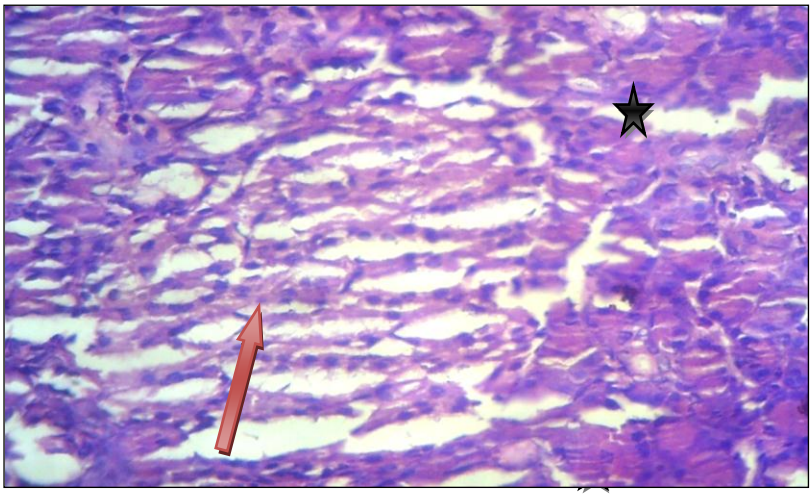

Figure (6) Section in pancreas from *Curcuma longa* treated rat (group T2) note mild hyperplasia of islet of Langerhans cells ( ) with accumulation of monocyte and lymphocyte around blood vessels ( ) (H&E 40X)

Discussion

The animals in this study showed normal activities and viabilities, during the periods of study which indicates natural healthy state and the safety of daily oral administration of *Curcuma longa* at dose (150 and 300 mg \kg B.W).

Table (1) showed that different oral doses of *Curcuma longa* for two, four and six weeks in male rats resulted in non significant increase of body weight .The increment in body weight may be due to the role of *Curcuma longa* in regulation of digestion and metabolism, this regulation discussed according to the importance of *Curcuma longa* and it

derivative as a digestive stimulant through stimulate secretion of some digestive enzyme especially pancreatic enzyme trypsin, chemotrypsin, lipase, α-amylase and also gastric enzyme like maltase, sucrase and lipase (16,17). *Curcuma longa* also may increase the release of bile which plays important role in digestion and absorption of lipid and fat soluble vitamins (18).

On the other hand, curcumin contain high level of essential nutrients such as sugar, carbohydrate and minerals(19). Also *Curcuma longa* and its derivatives which has antioxidant properties may be protected the body tissues from free radical and help in increment of animals health (20,21,22).

The decrease mean value of glucose concentration might be attributed to the effect of *Curcuma longa* as antidiabetic due to their stimulation β-cell of pancreas to increase secretion of insulin and decrease the glucose level (23).

At the last period of treatment (42 days) glucose concentration tended to increase in the two treated groups as compared with it's concentration in 14 and 28 days of treatment periods (table 2), this increment in glucose concentration may be due to increase of cortisol hormone in the two treated groups in 2^{nd} and 4^{th} week of treatment (table 3) ,thus cortisol causes increase of glycogenolysis and decrease the utilization of glucose by tissues and also decrease the insulin secretion(anti-insulin effect) (13, 24).

The adrenal cortex hormones (glucocorticoids) especially cortisol play an important role in the regulation and metabolism of carbohydrates, protein and lipids (25, 26) and because *Curcuma longa* acts to increase basal metabolic rate due increase thyroid hormones and regulate of metabolites, so that cortisol may be increase in treated animals with *Curcuma longa* water suspension due to increase metabolism(27).

On other hand cortisol may be increase in treated animals as a result of action of Curcumin on pancreas as antidiabetic in order to elevate the level of glucose which was decrease by *Curcuma longa* treatment (10).

Furthermore *Curcuma longa* may be affected the pituitary gland and cause stimulation of ACTH release so that cortisol level will be increase (28, 29) or directly Curcumin act on adrenal cortex resulting in stimulation of synthesis and release of cortisol (30).

At the last period of treatment (42 days) cortisol concentration tended to decrease in the two treated groups as compared with it's concentration in 14 and 28 days of treatment period (table 3), this findings may be due to the stress or exhausted of adrenal cortex cells as a result of length of treatment period or may be due to high level of glucose in the same period (42 days) (table 2) which decrease the requirement of cortisol for gluconeogensis.

Microscopic study of adrenal cortex and pancreas reflected many histological changes of treated rats (figure2, 3, 5 and 6) these change could be explained by several ways: increase of cortisol secretion in treated rats may be occur as a result of direct stimulation of *Curcuma longa* on adrenal gland or to the hypothalamic-adrenal axis, so that the function at ctivity increase especially to zona fasciculata and reticularis and these cells undergo hyperplasia on expence of other zones (12, 31).

Furthermore, *Curcuma longa* which caused some histological changes of adrenal cortex manifested by hyperplasia of reticularis and fasciculate cell and vacculation of its cytoplasm (figure 2 and 3) may be occur in order to increase secretion of cortisol hormone in high levels to regulate and maintain the glucose and cholesterol levels which were decreased by *Curcuma longa* (32, 10).

Also increase of adrenal cortex activity may occur due to effect of Curcumin which lead to decrement of glucose by increase secretion of insulin, glucocorticoids play important role to increase gluconeogenesis and decrease insulin hormone secretion (33).

On the other hand the histological changes of pancreas in treated rats (figure 5 and 6) indicate increase of activity of pancreatic secretory cells (Islet of Langerhans) manifested by hyperplasia of these cells with blood vessels congestion. These changes may be reflect the important role of *Curcuma longa* to increase secretion of insulin due to the effect of Curcumin as antidiabetic (10).

Furthermore the increase of insulin secretion from β-cells of Islet of Langerhans may be occur in order to decrement the expected increase of glucose as a result of high levels of cortisol (cortisol causes increase gluconeogensis) (27).

The mild effect of *Curcuma longa* on adrenal cortex and pancreatic tissues in T1 compared with T2 group may be explained due to dosage effect of treatment.

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