

Original Research Article

# Curcumin Ameliorative Efficacy on Type 1 Diabetes Mellitus Coexisted with Rheumatoid Arthritis in Wistar Rats

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## Abstract

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This study was designed to evaluate the effect of curcumin on type 1 diabetes mellitus and/or rheumatoid arthritis in male Wistar rats. Type 1 diabetes mellitus was induced by multiple-low dose intraperitoneal injection of streptozotocin at dose of 20 mg/kg b.wt/day for 5 consecutive days. Rheumatoid arthritis was induced in normal and diabetic rats by a single subcutaneous injection of 0.1 ml Freund's complete adjuvant into a footpad of the right hind leg of male rats. Diabetic, arthritic and diabetic/arthritic rats were orally treated with curcumin at dose level of 50 mg /kg b.wt /day for 21 days. The treatment of diabetic and diabetic/arthritic rats with curcumin significantly improved the impaired glucose tolerance, the lowered insulin level, the  $\beta$ -cell function and the deteriorated serum lipid profile. The disrupted pancreatic islets architecture and integrity were amended by curcumin administration. The increased ankle joint circumference, deleteriously affected ankle joint histological architecture, articular inflammatory cell infiltration, pannus formation, cartilage erosion and synovial hyperplasia in arthritic and diabetic/arthritic rats were counteracted by curcumin treatment. The elevated pro-inflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ) levels and the lowered anti-inflammatory cytokine (IL-10) level in diabetic, arthritic and diabetic/arthritic rats, reflecting the dominance of T helper 1 over T helper 2, were significantly improved as a result of curcumin administration. In conclusion, curcumin could have both antidiabetic and antiarthritic potentials which may be mediated *via* its anti-inflammatory and immunomodulatory potentials.

**Key words:** Curcumin, Immunomodulatory effects, Islets of Langerhans, Joints, Rheumatoid arthritis, Type 1 diabetes mellitus

## INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease characterized by autoimmune joint inflammation, pain, swelling and eventually joint deformity (Breitenberger, 2008; Majeed and Borole, 2015). It is more common in women than men (Borashan *et al.*, 2009). It affects 1% of the population and its incidence increases with increasing age (Majithia and Geraci, 2007; Mohamed *et al.*, 2014).

Diabetes mellitus type 1, insulin-dependent diabetes or juvenile diabetes, is another chronic inflammatory

disease that results from the autoimmune destruction of the insulin producing  $\beta$ -cells in the pancreas (Amirshahrokhi *et al.*, 2008). It accounts for 5-10% of diabetes mellitus and its prevalence is increasing throughout the world (American Diabetes Association, 2015).

The mechanisms, involved in the joint degenerative effects in rheumatoid arthritis and the destruction of  $\beta$ -cells in diabetes mellitus type 1, have been the subject of

intensive research. These mechanisms include direct damage of cells by CD8<sup>+</sup> T-cells or other lytic cells, the damaging effects of cytokines that are produced by effector CD4<sup>+</sup> T-cells that recognize their antigenic targets, or even by non-T-cells that release innate inflammatory mediators, such as interleukin (IL)-1 $\beta$  (Akirav *et al.*, 2008). In addition, a CD8<sup>+</sup> and CD4<sup>+</sup> Th1/Th2 cytokine imbalance with a predominance of Th1 cytokines has been suggested by many publications to be of pathogenetic importance in both disease (Berner *et al.*, 2000; Amirshahrokhi *et al.*, 2008; Ahmed, 2009).

Because of side effects and toxicity of the conventionally used anti-diabetic and anti-arthritic drugs, there are greater needs for alternative, safer and more effective natural product based drugs (Rao *et al.*, 1999; Borashan *et al.*, 2009). There is scant number of publications in the literature investigating the anti-arthritic efficacy of turmeric and its active constituent, curcumin, in animal or human studies (Funk *et al.*, 2006; Mobasheri *et al.*, 2012). On the other hand, many publications, revealing the ameliorative effects of curcumin on diabetes mellitus, were previously reported (Ahmed, 2005; Ahmed and Abdel-Reheem, 2005; Meng *et al.*, 2013; Castro *et al.*, 2014). No available publications that assess the effect of curcumin on the coexistent diabetes mellitus type 1 and rheumatoid arthritis were previously reported.

Thus, this study aimed to assess the effect of turmeric active ingredient, curcumin, on oral glucose tolerance, serum insulin level, Th1 and Th2 cytokines, serum lipid profile and pancreatic islets and ankle joint histological integrity of arthritic and/or type 1 diabetic rats.

## **MATERIALS AND METHODS**

### **Experimental animals**

Male Wistar albino rats aging 8-10 weeks and weighing 190  $\pm$  10 g were used as experimental animals in the present investigation. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22 $\pm$ 2  $^{\circ}$ C) and humidity (55 $\pm$ 5%) with 12 h light and 12 h dark cycle and were fed rat chow diet and were given drinking water *ad libitum*. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care (CCAC) (Ernest *et al.*, 1993). All efforts were done to reduce the number of animals to the minimum and to decrease the suffering of animals.

### **Induction of Type 1 diabetes mellitus:**

Type 1 diabetes mellitus was experimentally induced in animals fasted for 12 hours by intraperitoneal injection of

20 mg/kg b. wt/day streptozotocin (Sigma Chemical Company, USA) dissolved in 1 ml citrate buffer (pH 4.5) for 5 consecutive days (Amirshahrokhi *et al.*, 2008). Ten days after streptozotocin injection, rats were screened for blood glucose levels. Overnight fasted (10-12 hours) animals were given glucose (3g/kg b. wt) by intragastric tube. After 2 hours of oral administration, blood samples were taken from lateral tail vein, left to coagulate and centrifuged then serum glucose concentration was measured. Rats having serum glucose level higher than 180 mg/dl, after 2 hours of glucose intake, were included in the experiment, while the others were excluded.

### **Induction of adjuvant-induced arthritis**

Adjuvant arthritis was induced as previously described by Whitehouse (2007). Arthritis was induced by a single subcutaneous injection of 0.1 ml Freund's complete adjuvant (FCA), suspension of heat-killed *Mycobacterium tuberculosis* in mineral oil, into a footpad of the right hind of male rats. FCA was obtained from Sigma Chemical Company, USA.

### **Curcumin Preparation**

Curcumin was obtained from Hedel-De Han AG, Germany. Curcumin dissolved in 1% CMC (Carboxymethyl cellulose) at 1% concentration (50 mg/5ml) and was orally administration at dose level of 50 mg /kg b.wt /day (Ahmed, 2005) for 21 days.

Curcumin (diferuloylmethane), the major yellow pigment from the root of turmeric (*Curcuma longa*), is common food additive used as a spice and a colouring agent (Aggarwal and Harikumar, 2009).

### **Animal grouping**

The considered rats were divided into seven groups designated as follow:

#### **Normal control group**

It consists of normal rats that received the equivalent volume of 1% carboxymethyl cellulose (CMC) by oral administration for 21 days.

#### **Arthritic control group**

It consists of arthritic rats and it received the equivalent volume of 1% carboxymethyl cellulose (CMC) by oral administration for 21 days.

### **Arthritic treated group**

The rats of this group were treated with 50 mg/kg b.wt/day curcumin (dissolved in 1%CMC) by oral administration for 21 days.

### **Diabetic control**

It consists of diabetic rats and it received the equivalent volume of 1% CMC by oral administration for 21 days.

### **Diabetic group treated**

The rats of this group were treated with 50 mg /kg b.wt/day (dissolved in 1% CMC) by oral administration for 21 days.

### **Diabetic/arthritic control group**

It consists of diabetic and arthritic rats and it received the equivalent volume of 1% CMC by oral administration for 21 days.

### **Diabetic/arthritic treated group**

This group was treated with curcumin (dissolved in CMC 1%) at dose level of 50 mg/kg b.wt/day for 21 days.

All treatments were daily performed at 10:12 hours AM by oral gavage for 21 days received 50 mg/kg b.wt curcumin dissolved in 1% CMC.

At the end of experiment, the ankle circumference of right hind leg was measured and rats were anesthetized by ether inhalation and blood was collected. Serum was separated by centrifugation of blood at 3000 rpm for 15 minutes and the clear non-haemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at -20°C till used. Pancreata and hind ankle region and paw were removed, fixed in 10% neutral buffered formalin for histopathological analysis.

### **Oral glucose tolerance test**

At day before sacrifice, blood samples were obtained from lateral tail vein of overnight fasted rats (10-12 hours). Successive blood samples were then taken at 30, 60, 90 and 120 minutes following the administration of glucose solution (3g/kg b.wt.) through oral gavage. Blood samples were centrifuged and sera were obtained quickly for determination of glucose concentration according to

the method of Trinder (1969) using reagent kit purchased from Spinreact Company (Spain).

### **Detection of serum insulin and HOMA (Homeostasis Model) $\beta$ -cell function**

Serum rat insulin was determined using ELISA kit that was obtained from DRG international, Inc. USA. Homeostasis model assessment (HOMA) of  $\beta$ -cell function (HOMA- $\beta$  cell function) was calculated from fasting glucose and insulin concentrations according to Hsing *et al.* (2003) and Aref (2013) using the formula  $HOMA \beta\text{-cell function} = (20 \times I_0) / (G_0 - 3.5)$  where  $I_0$  is fasting insulin in  $\mu$ IU/ml and  $G_0$  is the fasting glucose in mg/dl.

### **Detection of serum lipid profile**

Serum triglycerides concentration was determined according to the method of Fossati and Prencipe (1982) using reagent kit purchased from Reactivos Spinreact Company (Spain). Serum cholesterol concentration was estimated according to the method of Allain *et al.* (1974) using reagent kit purchased from Spinreact Company (Spain). Serum HDL-cholesterol concentration, after precipitation of LDL- and vLDL-cholesterol, was measured according to the method of Allain *et al.* (1974) using reagent kit purchased from Spinreact Company (Spain). Serum LDL- cholesterol concentration was determined according to Friedewald *et al.* (1972) formula:  $LDL\text{-cholesterol} = Total\text{-cholesterol} - Triglycerides/5 - HDL\text{-cholesterol}$ . Serum vLDL-cholesterol was calculated according to Norbert (1995) formula:  $vLDL\text{-cholesterol conc.} = Triglycerides / 5$ .

### **Detection of serum cytokines**

The levels of serum TNF- $\alpha$  and IL-10 of control and experimental groups were determined using specific ELISA kits purchased from R and A systems, USA. Serum IL-1 $\beta$  was assayed by ELISA kit that was purchased from Thermo Scientific (USA) and Invetrogen (Canada). Serum TNF- $\alpha$ , IL-10 and IL-1 $\beta$  concentrations were determined using spectrophotometer at 450 nm according to the manufactures instructions.

### **Histopathological investigation**

After sacrifice and dissection, pancreata, hind paws and ankle regions were removed, washed in saline and fixed in 10% neutral buffered formalin. The ankle regions were decalcified for 10 days with ethylenediamine tetraacetic acid and embedded in paraffin for histological

**Table 1.** Effect of curcumin administration on oral glucose tolerance of arthritic and/or diabetic rats.

Time Groups	0 min	30 min	60 min	90 min	120 min
Normal control	78.60 ± 4.23 <sup>m</sup>	109.43 ± 4.04 <sup>ijklm</sup>	115.80 ± 2.02 <sup>ijklm</sup>	107.13 ± 5.85 <sup>ijklm</sup>	86.63 ± 4.44 <sup>lm</sup>
Arthritic control	103.23 ± 8.11 <sup>ijklm</sup>	133.73 ± 3.71 <sup>ijkl</sup>	135.43 ± 2.14 <sup>ijkl</sup>	140.23 ± 13.26 <sup>hijk</sup>	120.05 ± 1.42 <sup>ijklm</sup>
Arthritic rats treated with curcumin	89.13 ± 2.19 <sup>klm</sup>	105.83 ± 16.98 <sup>ijklm</sup>	145.03 ± 5.11 <sup>ijk</sup>	100.03 ± 7.45 <sup>ijklm</sup>	95.86 ± 6.50 <sup>klm</sup>
Diabetic control	243.43 ± 7.31 <sup>de</sup>	285.37 ± 12.81 <sup>cd</sup>	408.20 ± 15.46 <sup>a</sup>	296.79 ± 14.97 <sup>c</sup>	272.57 ± 13.74 <sup>cd</sup>
Diabetic rats treated with curcumin	103.90 ± 8.67 <sup>ijklm</sup>	141.5 ± 2.60 <sup>ijk</sup>	187.21 ± 12.14 <sup>fgh</sup>	164.67 ± 7.85 <sup>ghi</sup>	134.13 ± 16.83 <sup>ijkl</sup>
Diabetic/Arthritic control	226.69 ± 7.93 <sup>ef</sup>	314.97 ± 10.57 <sup>c</sup>	359.33 ± 11.76 <sup>b</sup>	240.71 ± 7.45 <sup>de</sup>	261.00 ± 12.27 <sup>cd</sup>
Diabetic/Arthritic rats treated with Curcumin	114.23 ± 9.35 <sup>ijklm</sup>	131.5 ± 12.43 <sup>ijkl</sup>	209.26 ± 16.04 <sup>efg</sup>	226.27 ± 6.94 <sup>ef</sup>	219.17 ± 2.88 <sup>f</sup>

- Data are expressed as Mean ± SE. Number of animals in each group is six.
- Means which share the same superscript symbol(s) are not significantly different.
- F- prob.: P < 0.001; LSD at the 5% level is: 49.41; LSD at the 1% level is: 66.54.

analysis in Histopathology Department, Faculty of Veterinary Medicine, Beni-Suef University. The sections were stained with hematoxylin and eosin, and then examined.

### Statistical analysis

Results were expressed as mean ± standard error (SE). The data are analyzed by one-way analysis of variance (ANOVA) using PC-STAT, University of Georgia, followed by LSD analysis to discern the main effects and to compare various groups with each other (Roa *et al.*, 1985). Values of P > 0.05 were considered statistically non-significantly different, while values of P < 0.05 and P < 0.01 were significantly and highly significantly different respectively.

### RESULTS

The serum glucose level of normal rats was gradually increased as the time after oral glucose loading extended from 0 to 60 minutes, and then it was decreased to reach a value near the fasting level. In the arthritic control rats, serum glucose concentration was non-significantly (p > 0.05) increased at all tested points of oral glucose tolerance test as compared to the normal control. In diabetic and arthritic/diabetic rats, the serum glucose level was significantly elevated (p < 0.01) as compared to that of normal rats at the corresponding times after oral glucose administration. The treatment of diabetic and arthritic/diabetic rats with curcumin significantly ameliorated the impaired oral glucose tolerance. With the exception of the effect at 30 minutes after glucose loading, curcumin seemed to be more efficient in improving glucose tolerance in diabetic rats than in

arthritic/diabetic rats (Table 1).

Serum insulin concentration was significantly (p < 0.01) decreased in arthritic and/or diabetic rats. This deterioration was more pronounced in arthritic/diabetic rats than in diabetic and arthritic rats. The treatment with curcumin induced a significant increase (p < 0.05) in serum insulin concentration in diabetic and arthritic/diabetic rats while it produced no significant (p > 0.05) effect in arthritic rats as compared to the corresponding control (Table 2). The calculated β-cell function was significantly decreased in diabetic (p < 0.05) and arthritic/diabetic (p < 0.01) rats. The treatment of these diabetic animals with curcumin significantly increased (p < 0.01) the β-cell function (Table 2).

Serum lipid profile represented in table 3 revealed that serum total cholesterol, triglycerides, LDL-cholesterol and vLDL-cholesterol levels were significantly elevated (p < 0.01) in diabetic and arthritic/diabetic rats. These elevated levels were significantly ameliorated (p < 0.01) in diabetic and arthritic/diabetic rats treated with curcumin in comparison with the corresponding controls. Serum HDL-cholesterol level, on the other hand, was significantly decreased in arthritic, diabetic and arthritic/diabetic rats. It was more deteriorated in arthritic/diabetic group than in arthritic or diabetic groups. These lowered serum HDL-cholesterol level was significantly increased as a result of treatment of arthritic/diabetic rats with curcumin (Table 3).

The ankle joint circumference of right hind leg was significantly increased (p < 0.01) in arthritic and arthritic/diabetic rats. The treatment of these arthritic animals with curcumin significantly (p < 0.01) decreased the ankle joint circumference (Table 4).

Serum TNF-α and IL-1β levels were significantly elevated in arthritic, diabetic and arthritic/diabetic groups as compared to those of normal rats. Their levels were more deleteriously elevated in arthritic/diabetic group than in either arthritic or diabetic rats as compared to

**Table 2.** Effect of curcumin administration on serum insulin and beta cell function in of arthritic and/or diabetic rats

Group	Parameter	Insulin ( $\mu$ U/ml)	HOMA $\beta$ -cell function
Normal control		27.53 $\pm$ 2.20 <sup>a</sup>	2.48 $\pm$ 0.16 <sup>a</sup>
Arthritic control		19.36 $\pm$ 1.61 <sup>bc</sup>	2.34 $\pm$ 0.32 <sup>a</sup>
Arthritic rats treated with curcumin		21.53 $\pm$ 1.09 <sup>b</sup>	2.85 $\pm$ 0.11 <sup>a</sup>
Diabetic control		13.20 $\pm$ 1.38 <sup>de</sup>	1.67 $\pm$ 0.04 <sup>b</sup>
Diabetic rats treated with curcumin		18.21 $\pm$ 1.39 <sup>bc</sup>	2.92 $\pm$ 0.45 <sup>a</sup>
Diabetic/Arthritic control		11.13 $\pm$ 1.31 <sup>e</sup>	1.37 $\pm$ 0.04 <sup>b</sup>
Diabetic/Arthritic rats treated with Curcumin		15.53 $\pm$ 0.84 <sup>cd</sup>	2.98 $\pm$ 0.08 <sup>a</sup>
F- Probability		P < 0.001	P < 0.001
LSD at the 5% level		4.40	0.65
LSD at the 1% level		6.11	0.87

- Data are expressed as Mean  $\pm$  SE. Number of animals in each group is six.
- Means which share the same superscript symbol (s) are not significantly different.
- HOMA- $\beta$  cell function is homeostasis model assessment (HOMA) of  $\beta$ -cell function = (20  $\times$  I<sub>0</sub>)/(G<sub>0</sub>-3.5). I<sub>0</sub> is fasting serum insulin level while G<sub>0</sub> is fasting serum glucose level

**Table 3.** Effect of curcumin administration on serum lipid profile of arthritic and /or diabetic rats.

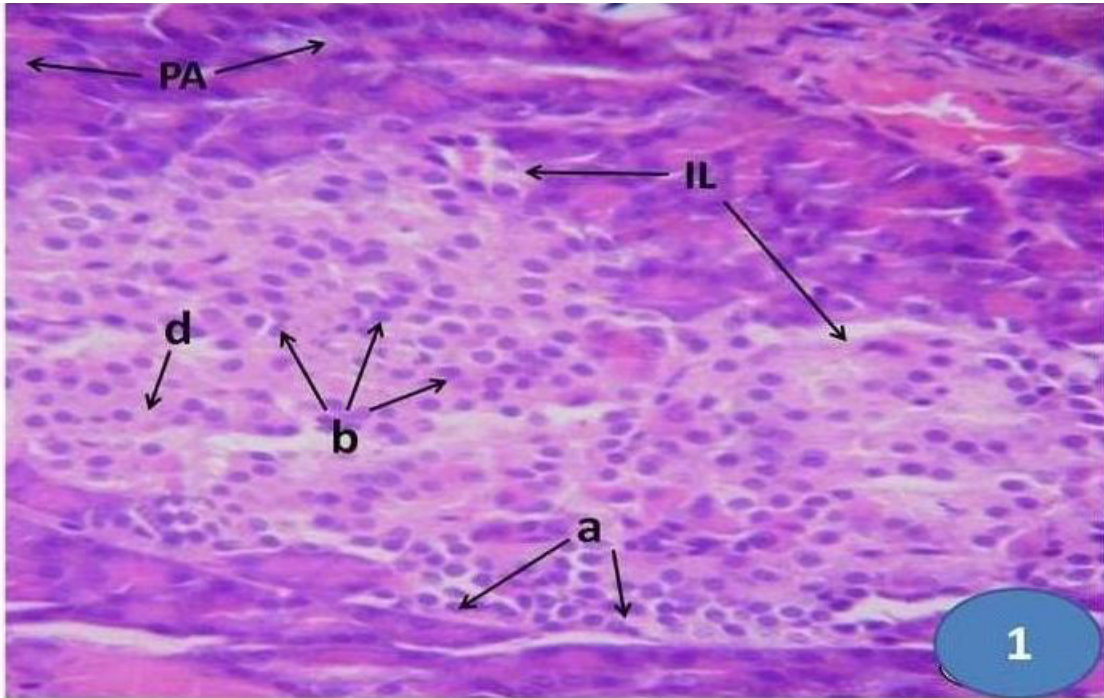
Group	Parameter	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	vLDL-cholesterol (mg/dl)
Normal control		68.56 $\pm$ 2.17 <sup>c</sup>	51.00 $\pm$ 1.78 <sup>d</sup>	37.63 $\pm$ 1.31 <sup>a</sup>	22.65 $\pm$ 1.15 <sup>d</sup>	10.20 $\pm$ 0.36 <sup>d</sup>
Arthritic control		78.98 $\pm$ 3.92 <sup>c</sup>	57.28 $\pm$ 4.01 <sup>c</sup>	30.56 $\pm$ 2.87 <sup>b</sup>	45.71 $\pm$ 1.02 <sup>c</sup>	11.45 $\pm$ 0.80 <sup>cd</sup>
Arthritic rats treated with curcumin		77.78 $\pm$ 6.11 <sup>c</sup>	68.01 $\pm$ 5.73 <sup>c</sup>	29.02 $\pm$ 2.63 <sup>b</sup>	32.41 $\pm$ 4.12 <sup>d</sup>	13.00 $\pm$ 0.67 <sup>c</sup>
Diabetic control		137.86 $\pm$ 3.68 <sup>a</sup>	134.73 $\pm$ 4.16 <sup>a</sup>	27.56 $\pm$ 1.58 <sup>b</sup>	75.23 $\pm$ 3.75 <sup>b</sup>	26.94 $\pm$ 0.83 <sup>a</sup>
Diabetic rats treated with curcumin		99.46 $\pm$ 2.24 <sup>b</sup>	97.26 $\pm$ 7.62 <sup>b</sup>	31.23 $\pm$ 0.73 <sup>b</sup>	47.19 $\pm$ 1.27 <sup>c</sup>	19.11 $\pm$ 1.27 <sup>b</sup>
Diabetic/Arthritic control		140.30 $\pm$ 10.55 <sup>a</sup>	134.65 $\pm$ 5.88 <sup>a</sup>	21.07 $\pm$ 1.41 <sup>c</sup>	96.71 $\pm$ 6.23 <sup>a</sup>	26.93 $\pm$ 1.17 <sup>a</sup>
Diabetic/Arthritic rats treated with Curcumin		99.15 $\pm$ 1.44 <sup>b</sup>	90.26 $\pm$ 1.34 <sup>b</sup>	30.38 $\pm$ 0.58 <sup>b</sup>	51.04 $\pm$ 1.30 <sup>c</sup>	18.05 $\pm$ 0.27 <sup>b</sup>
F- probability		P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
LSD at the 5% level		15.01	13.97	5.15	13.05	2.43
LSD at the 1% level		20.22	18.81	6.93	17.58	3.28

- Data are expressed as Mean  $\pm$  SE. Number of animals in each group is six.
- Means which share the same superscript symbol(s) are not significantly different.

**Table 4.** Effect of curcumin administration on ankle joint circumference of right hind leg and serum TNF-  $\alpha$ , IL-1 $\beta$  and IL-10 levels of arthritic and/or diabetic rats.

Group	Parameter	Ankle joint Circumference (mm)	TNF- $\alpha$ (pg/ml)	IL-1 $\beta$ (pg/ml)	IL-10 (pg/ml)
Normal control		20.66 $\pm$ 2.16 <sup>c</sup>	55.63 $\pm$ 2.32 <sup>d</sup>	67.17 $\pm$ 2.95 <sup>c</sup>	109.40 $\pm$ 4.65 <sup>a</sup>
Arthritic control		32.17 $\pm$ 1.60 <sup>a</sup>	103.87 $\pm$ 10.79 <sup>ab</sup>	89.26 $\pm$ 3.32 <sup>b</sup>	53.23 $\pm$ 6.34 <sup>c</sup>
Arthritic rats treated with curcumin		27.50 $\pm$ 0.84 <sup>b</sup>	77.89 $\pm$ 3.68 <sup>c</sup>	65.4 $\pm$ 2.51 <sup>c</sup>	96.87 $\pm$ 3.88 <sup>a</sup>
Diabetic control		20.67 $\pm$ 1.63 <sup>c</sup>	105.6 $\pm$ 7.13 <sup>ab</sup>	97.63 $\pm$ 7.84 <sup>ab</sup>	61.40 $\pm$ 6.93 <sup>c</sup>
Diabetic rats treated with curcumin		20.83 $\pm$ 0.41 <sup>c</sup>	78.97 $\pm$ 2.64 <sup>c</sup>	69.33 $\pm$ 4.13 <sup>c</sup>	79.73 $\pm$ 1.71 <sup>b</sup>
Diabetic/Arthritic control		30.66 $\pm$ 2.50 <sup>a</sup>	118.67 $\pm$ 5.20 <sup>a</sup>	112.03 $\pm$ 8.87 <sup>a</sup>	50.33 $\pm$ 1.34 <sup>c</sup>
Diabetic/Arthritic rats treated with Curcumin		26.67 $\pm$ 2.73 <sup>b</sup>	88.43 $\pm$ 3.92 <sup>bc</sup>	88.19 $\pm$ 5.31 <sup>b</sup>	79.07 $\pm$ 2.35 <sup>b</sup>
F- Probability		P < 0.001	P < 0.001	P < 0.001	P < 0.001
LSD at the 5% level		2.205	17.57	16.65	13.32
LSD at the 1% level		2.970	24.39	23.12	18.48

- Data are expressed as Mean  $\pm$  SE. Number of animals in each group is six.
- Means which share the same superscript symbol (s) are not significantly different



**Figure 1.** Pancreatic section of normal rats. The exocrine portion of the pancreas consists of pancreatic acini (PA) while endocrine portion consists of the islets of Langerhans (IL) which are scattered throughout the pancreas, and consists of  $\alpha$ -cells (a) at the periphery of islet,  $\beta$ -cells (b) in the core and  $\delta$ -cells (d) which have larger size than  $\alpha$ -cells and  $\beta$ -cells.

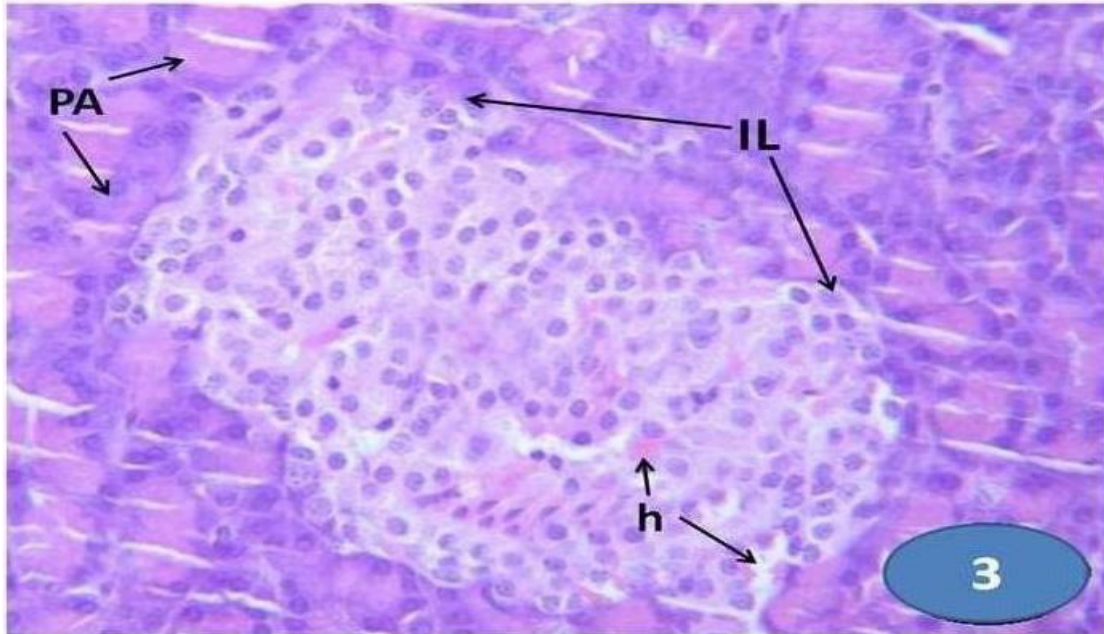


**Figure 2.** Pancreas of arthritic rats showing nearly normal histological structure of the islets. Haemorrhagic lesions (h) and eosinophilic deposits are found in the islet (IL): Islets of Langerhans; (a)  $\alpha$ -cells (b)  $\beta$ -cells.

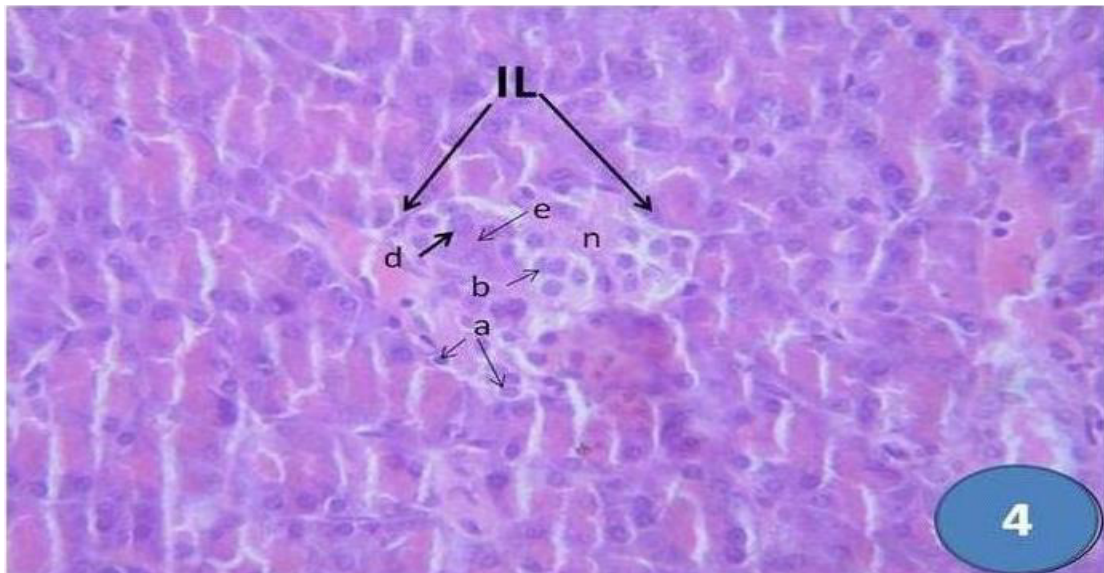
their normal levels. The treatment of the arthritic, diabetic and arthritic/diabetic groups with curcumin significantly improved the deleteriously elevated serum TNF- $\alpha$  and IL-1 $\beta$  levels as compared to the arthritic and/or diabetic controls (Table 4).

As indicated in figure 1, the pancreas of rat is compo-

sed of exocrine portion that consists of pancreatic acini and endocrine portion represented by islets of Langerhans. The latter are scattered throughout the pancreas, and consists of  $\alpha$ -cells at the periphery of islet,  $\beta$ -cells (b) in the core and  $\delta$ -cells (d) which have larger size than  $\alpha$ -cells and  $\beta$ -cells.



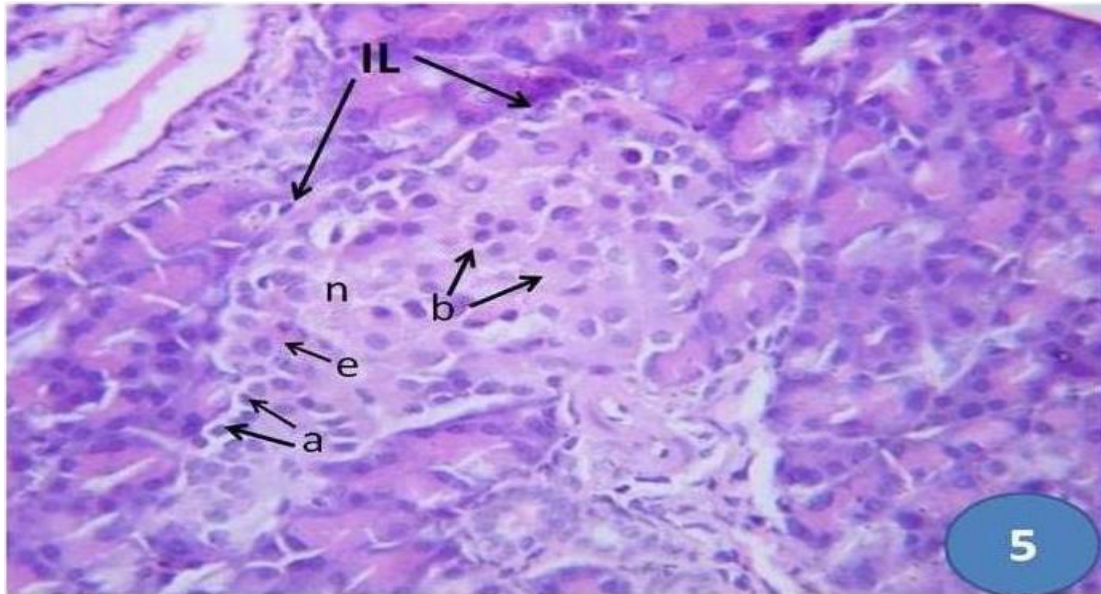
**Figure 3.** Pancreas of arthritic rats treated with curcumin. The pancreatic acinar cells (PA) are seen to be normal. The islets of Langerhans (IL) are present with a large proportion of islet cells. There is no inflammatory cell infiltration and no eosinophilic deposits were seen. Haemorrhagic lesions (h) are markedly reduced.



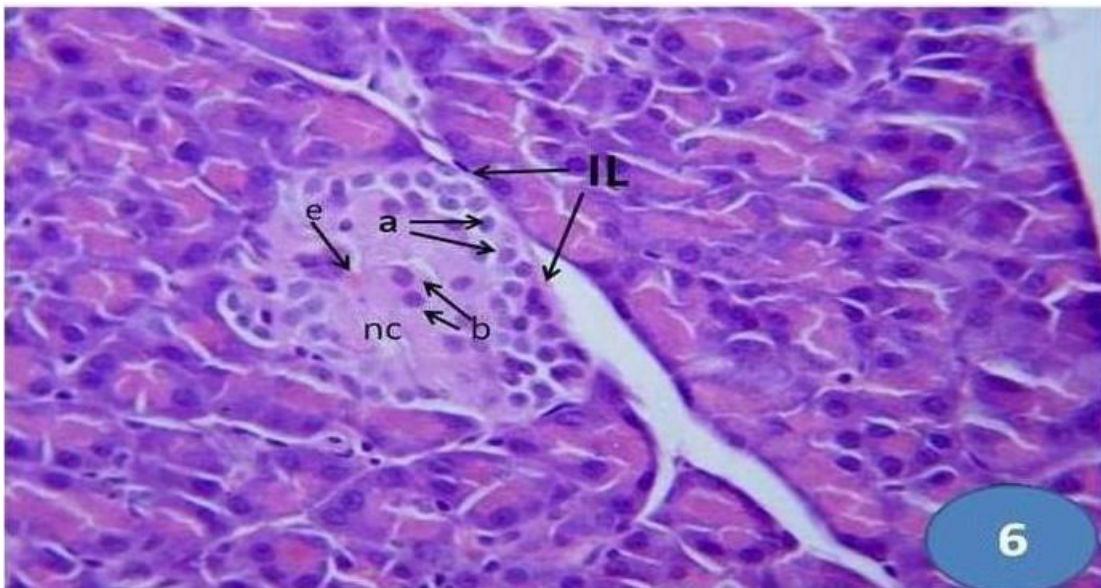
**Figure 4.** Pancreas of diabetic control rats. Normal architecture of the islets is disrupted. Islets showed extreme necrosis and degeneration. The islets attained a small size with vigorous depletion in the islet cells (a, b and d) number. Necrosis (n) and eosinophilic deposits (e) are clearly observed.

The pancreas of arthritic rats exhibited nearly normal histological structure of the islets. Mild haemorrhagic lesions and eosinophilic deposits are found in the islet (Figure 2). In the arthritic rats treated with curcumin (Figure 3), the hemorrhagic lesions were greatly reduced, eosinophilic deposits disappeared and the islets retained their normal structure and integrity.

The normal architecture of islets of Langerhans is disrupted in diabetic rats. The pancreatic islets were markedly reduced in size and number of islets' cells was vigorously reduced. Both necrosis and eosinophilic deposits were observed in the islets of diabetic rats (Figure 4). The treatment of diabetic rats with curcumin for 21 days remarkably improved the islets architecture



**Figure 5.** Pancreas of diabetic rats treated with curcumin. The acinar cells are seen to be normal. The islets are present with relatively large proportion of alpha cells (a) and beta cells (b) though mild degenerative changes and necrosis (n) are present. There is very scanty inflammatory cell infiltration and little eosinophilic deposits (e) were seen.

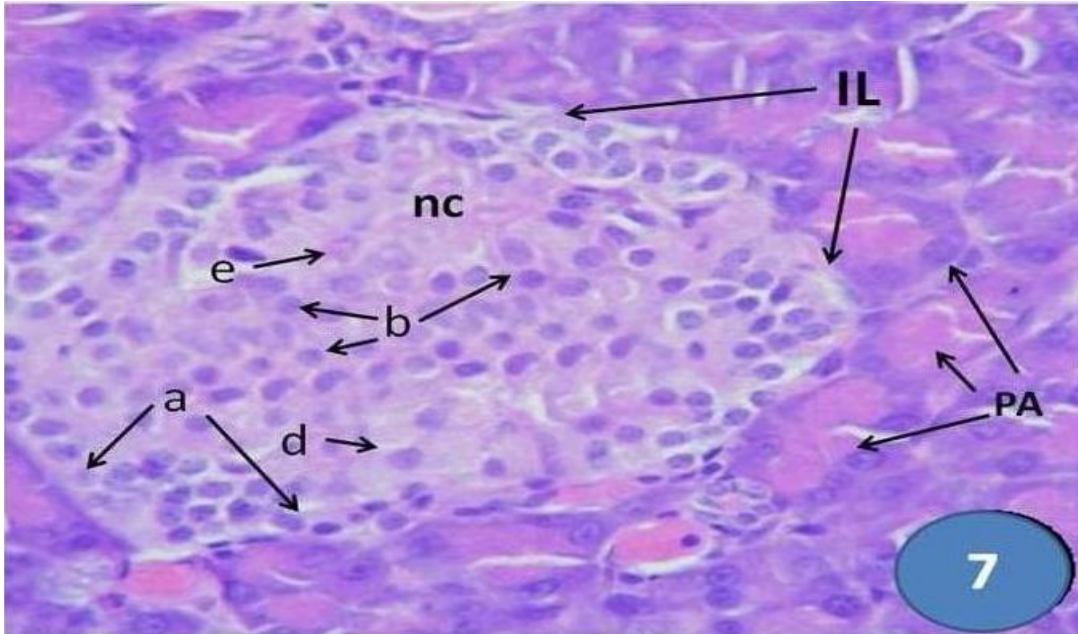


**Figure 6.** Pancreas of diabetic/arthritis control rats. The acinar cells around the islets seem to be in normal proportion though they do not look classical. The islets are largely occupied by a uniform eosinophilic material (e) and atrophic cells and necrosis (n). Eosinophilic materials also surround the blood vessel. The beta cells (b) are greatly decreased in number while alpha cells (a) are nearly unaffected.

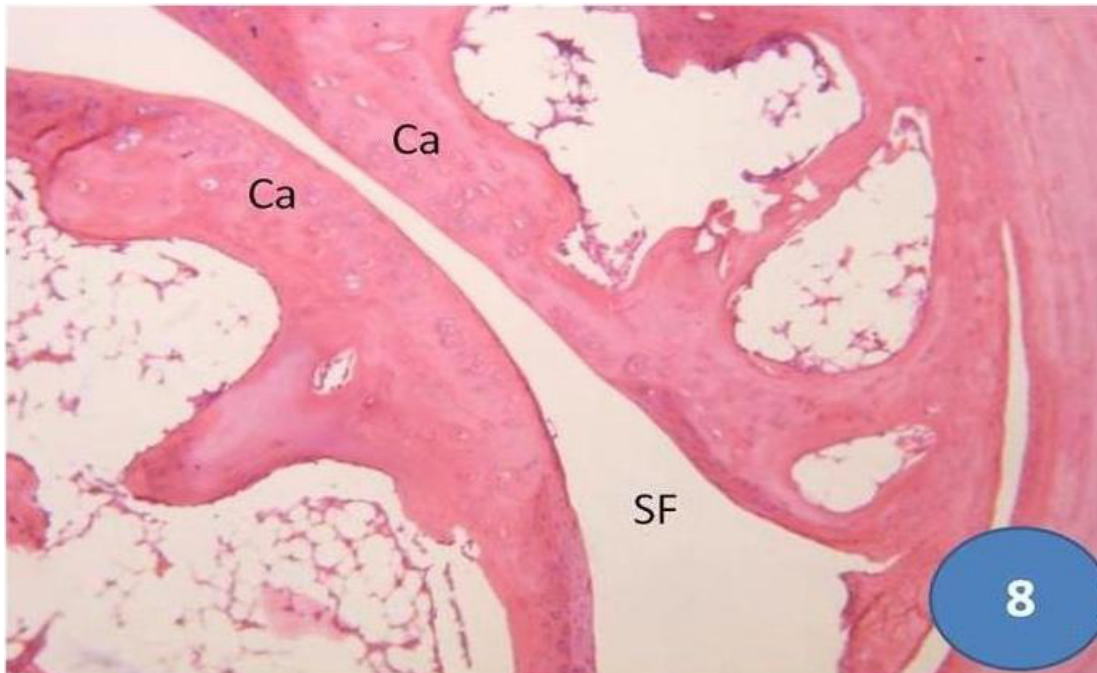
and integrity. The number of alpha cells and  $\beta$ -cells were increased (Figure 5). Instead of this amelioration, very scanty inflammatory cell infiltration and little eosinophilic deposits (e) were observed.

The pancreatic islets of diabetic/arthritis control rats exhibited atrophic cells and necrosis. Eosinophilic

materials also surround the blood vessel. The  $\beta$ -cells are greatly decreased in number while alpha cells are nearly unaffected. The  $\beta$ -cells were more deleteriously affected in diabetic/arthritis rats than in diabetic non-arthritis ones (Figure 6). The histological architecture and integrity of pancreatic islets of diabetic/arthritis rats treated with



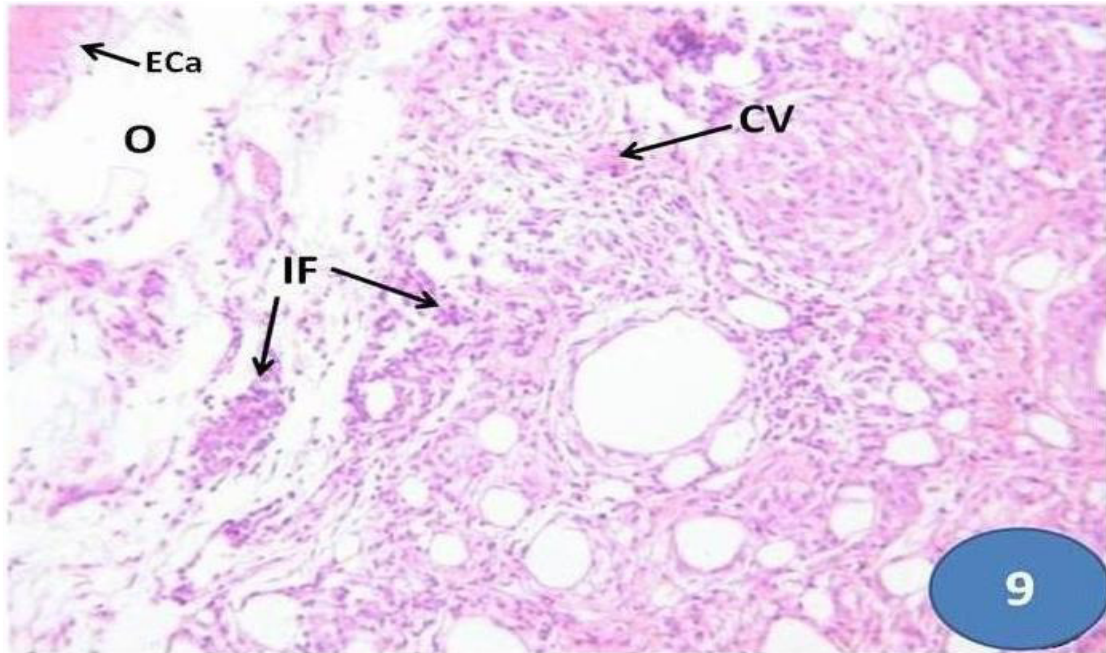
**Figure 7.** Pancreas of diabetic/arthritis rats treated with curcumin. The pancreatic acinar cells (PA) are seen to be normal. The islets are present with a large proportion of islet cells though there are still necrotic foci (n). Very scanty inflammatory cell infiltration and eosinophilic deposits (e) were seen. The symbols a, b and d respectively refer to alpha, beta and delta cells.



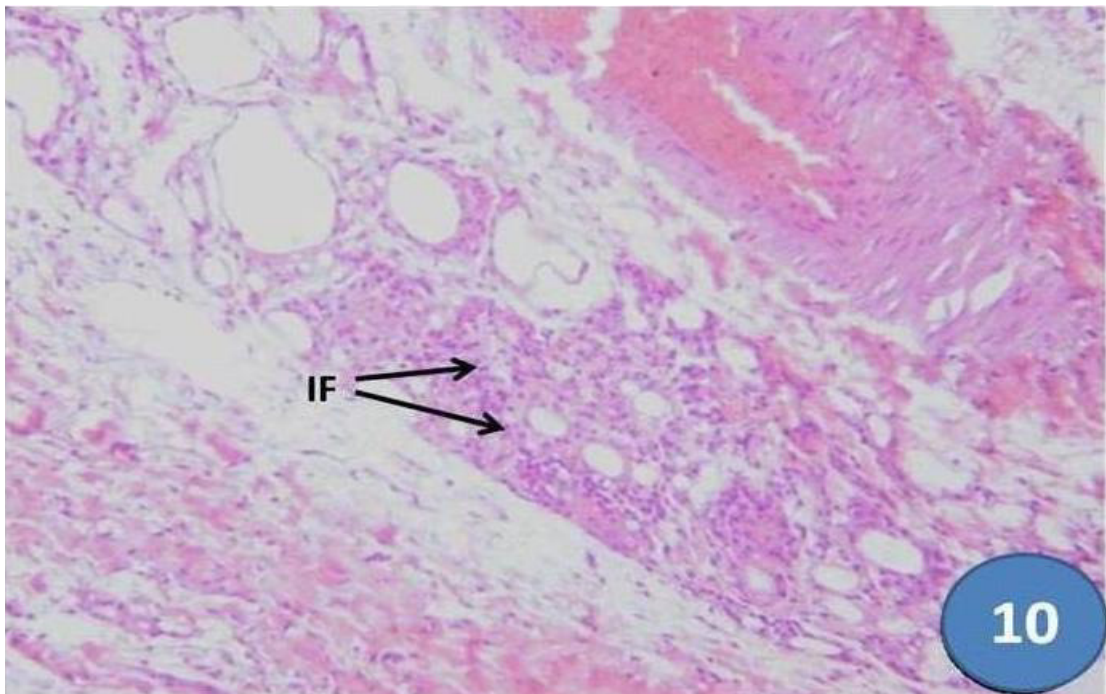
**Figure 8.** Joint of normal control rats showed normal joint structure. The symbols Ca and SF respectively refer to cartilage and synovial fluid.

curcumin were potentially alleviated (Figure 7). The islets attained larger size and the number of  $\beta$ -cells was increased. Very scanty inflammatory cell infiltration and eosinophilic deposits were noticed.

The normal architecture of articular ankle joint of right hind leg (Figure 8) was disrupted in arthritic rats (Figure 9) which exhibited hyperplasia in the synovial tissue, congested blood vessels in the peri-articular tissue,



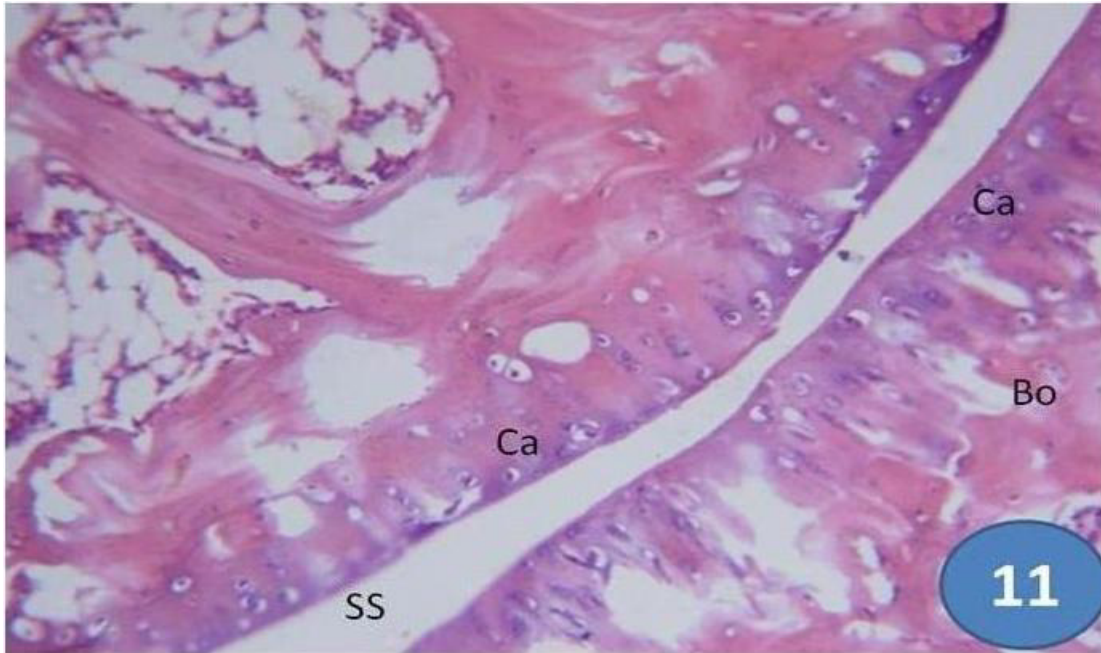
**Figure 9.** Joint of arthritic control rats. There were congested blood vessels (CV) in the peri-articular tissue, erosion of articular cartilage, heavy inflammatory cell infiltration (IF), oedema (O) hyperplasia in the synovial tissue.



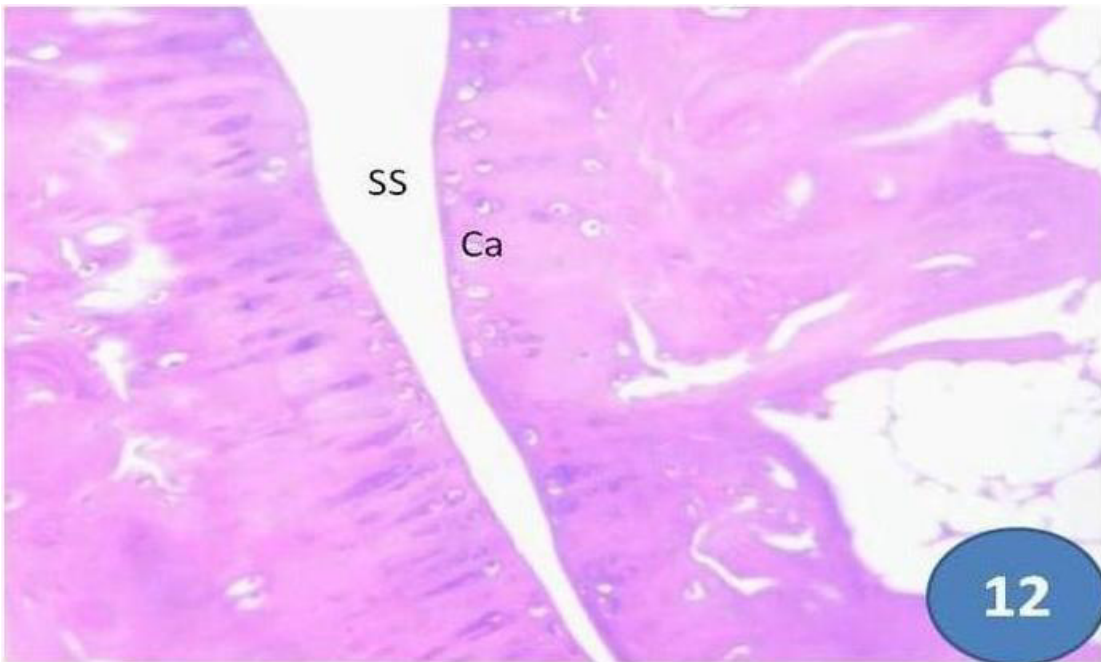
**Figure 10.** Joint of arthritic rats treated with curcumin. The peri-articular tissue showed moderate inflammatory cell infiltration (IF). The articular cartilage showed some erosion with hyperplasia in the synovium.

heavy inflammatory cell infiltration and oedema. The articular joint of arthritic rats treated with curcumin (Figure 10) showed moderate inflammatory cell infiltration in the

peri-articular tissue, articular cartilage erosion and hyperplasia of the synovial tissue. The severity of these lesions is less in arthritic rats treated with curcumin than



**Figure 11.** Joint of diabetic control rats revealed nearly normal articular surface. The symbols Ca, Bo and SS respectively refer to cartilage, bone and synovial space (SS) filled with synovial fluid.



**Figure 12.** Joint of diabetic rats treated with curcumin with nearly normal articular surface. The symbols Ca and SS respectively refer to cartilage and synovial space (SS) filled with synovial fluid.

in arthritic controls.

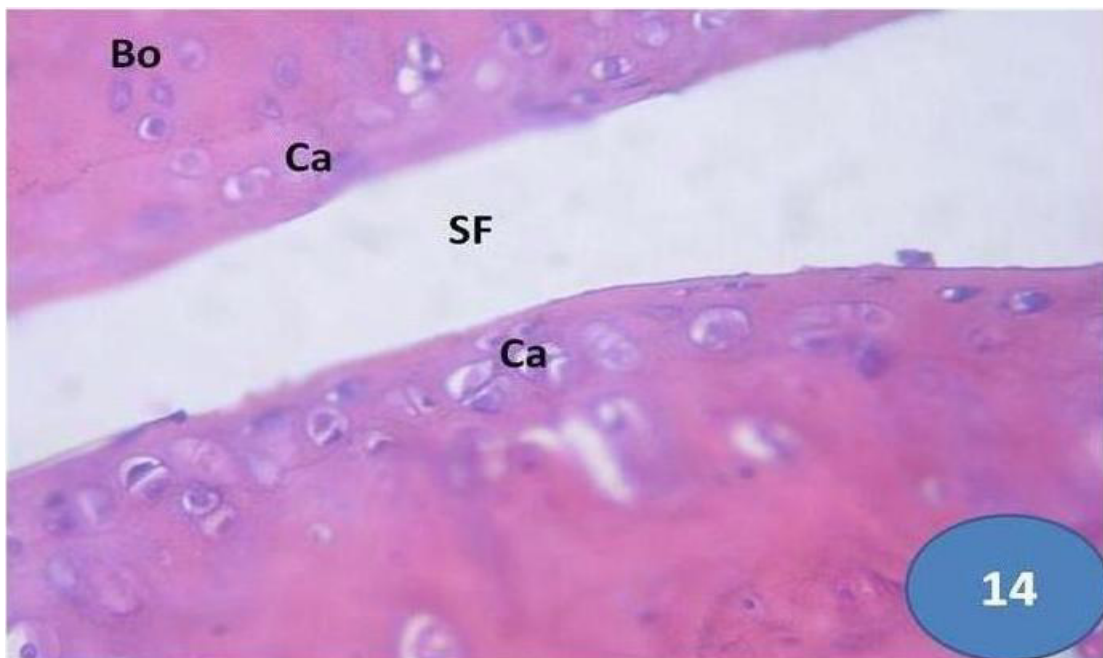
The joint of diabetic control (Figure 11) and diabetic rats treated with curcumin (Figure 12) exhibited normal histological architecture and integrity.

The arthritic/diabetic rats exhibited formation of pann-

us around articular cartilage in addition to marked erosion in some areas of articular cartilage (Figure 13). The treatment of arthritic/diabetic rats with Curcumin led to absence of these lesions; the articular cartilage was intact and the pannus was not found (Figure 14).



**Figure 13.** Joint of diabetic/arthritis control rats. Articular cartilage show some eroded areas of cartilage (ECa) and pannus formation.



**Figure 14.** Joint of diabetic/arthritis rats treated with curcumin. There was marked reduction in the erosion of articular cartilage (Ca). Synovial fluid (SF) and peri-articular bone (Bo) seemed normal.

## DISCUSSION

Both rheumatoid arthritis and type 1 diabetes mellitus are autoimmune inflammatory chronic disorders and are

characterized by preponderance of Th1 lymphocytes over Th2 cells. The interaction of the two diseases was scarcely investigated by the previous publications. Moreover, although curcumin was demonstrated to have

improvement effects on rheumatoid arthritis and on type 1 diabetes mellitus by previous publications (Ahmed, 2005; Ahmed and Abdel-Reheem, 2005; Kamarudin *et al.*, 2012), its efficacy on the concomitance of both diseases is not previously reported. Hence, this study is designed to evaluate the efficiency of curcumin on the experimentally induced - arthritic/diabetic rats.

In the present study, the intraperitoneal injection of streptozotocin at dose level of 20mg/kg b. wt/day for 5 consecutive days into male Wistar rats successfully produces hyperglycemia, insulin deficiency and impaired  $\beta$ -cell function which are the characteristics of type 1 diabetes mellitus. These results are in concordance with Amirshahrokhi *et al.* (2008) who found that intraperitoneal daily injection of low dose streptozotocin for 5 consecutive days in mice results in type 1 diabetes mellitus that matches in its mechanism type 1 diabetes in humans.

In the present study, the serum glucose level was significantly elevated in diabetic and arthritic/diabetic rats at all points of oral glucose tolerance test as compared to the corresponding values of normal rats. The treatment of diabetic and arthritic/diabetic rats with curcumin significantly improved the impaired glucose tolerance. These results are in accordance with many authors who stated that curcumin had anti-hyperglycemic effects in alloxan-induced diabetic rats (Abdul-Hamid and Mostafa, 2013), streptozotocin- (STZ-) induced diabetic rats models (Ahmed, 2005), STZ-nicotinamide-induced rats models (Murugan *et al.*, 2008) and high fat diet-fed mice (He *et al.*, 2012; Zhang *et al.*, 2013). The amelioration of the glucose tolerance as a result of treatment with curcumin could be attributed to the increase of lowered insulin level in the diabetic and arthritic/diabetic rats. This increase in serum insulin level in diabetic and arthritic/diabetic rats treated with curcumin was concomitant with the amendment of the pancreatic islets architecture and integrity and the increase in the number of  $\beta$ -cells as indicated in the current study. The regenerated  $\beta$ -cells is believed to be the consequence of either multiplication of  $\beta$ -cells that survived after the streptozotocin poisoning or a new formation of  $\beta$ -cells from duct epithelium of the exocrine portion of pancreas (Abdel Moneim *et al.*, 2001; Ahmed *et al.*, 2007).

With regards to the serum lipid profile, the serum total cholesterol, triglycerides, LDL-cholesterol and vLDL-cholesterol levels were elevated while HDL-cholesterol level was decreased in diabetic and arthritic/diabetic rats. Serum HDL-cholesterol and LDL-cholesterol levels were more deleteriously affected in arthritic/diabetic than in the diabetic rats. In the arthritic non-diabetic rats, serum triglycerides and LDL-cholesterol were significantly elevated, but serum HDL-cholesterol level was significantly decreased. The treatment of the diabetic and arthritic/diabetic rats with curcumin successfully improved the deteriorated serum lipid profile. These results go parallel with those of Ahmed and Abdel Raheim (2005)

who found that curcumin treatment improved the deteriorated effect of streptozotocin diabetes on serum lipid profile in rats. The amelioration of the lipid profile in serum may be secondary to the alleviation of the glycemic state, serum insulin level and  $\beta$ -cell function.

Complete Freund's Adjuvant-induced Arthritis in Wistar rat model is used in this study because it is characterized by infiltration of inflammatory cells, hyperplasia of synovial membrane in association with destruction of joints looking like rheumatoid arthritis in humans. Most of the investigators have reported that inhibition of adjuvant-induced arthritis in rats is one of the most suitable test procedures to screen anti-arthritic agents since it closely resembles human arthritis (Mishra *et al.*, 2011; Gandomani and Malati, 2014).

In the present study, the increased ankle joint circumference of right hind leg of arthritic and arthritic/diabetic rats was significantly decreased as a result of treatment with curcumin. The decrease in ankle joint circumference as a result of curcumin treatment reflects the decrease in swelling rate which may be attributed to the reduction in oedema, attenuation of inflammatory process and the reduction of synovial tissue hyperplasia as indicated by the histological results of joint in the present study and reported by previous publication (Kamarudin *et al.*, 2012).

Concerning the histological results of arthritic ankle joint, there were congested blood vessels in the peri-articular tissue, heavy inflammatory cell infiltration, oedema, hyperplasia in the synovial tissue, pannus formation around articular cartilage and erosion of articular cartilage in the arthritic and arthritic/ diabetic rats. As reported by previous publications, the articular and peri-articular infiltration of inflammatory cells, that act as the mother cells for osteoclasts/chondroblasts, in the cartilage and bone, play a substantial role in cartilage and bone erosion in experimentally-induced arthritis (Joe *et al.*, 2004; Schett, 2009; Knowles *et al.*, 2012). In addition, the pannus formation is also one of the causative factors that initiate cartilage erosion (Kamarudin *et al.*, 2012). The pannus formation occurred as a result from the aggregation of the inflammatory cells such as lymphocytes and macrophages as well as the immune complexes within the synovial space that contained the synovial fluid, on the articular cartilage surface of an arthritic joint (Romas *et al.*, 2002; Arend, 2001). Curcumin treatment of the arthritic and arthritic/diabetic rats led to the improvement of these lesions to a great extent *via* its anti-inflammatory action. Suppression of cell infiltration by curcumin in the present study is similar to earlier research findings which reported that curcumin could decrease the expression of adhesion molecules on the surface of macrophages (monocytes) (Seemayer *et al.*, 2005). This prevents the adhesion of macrophage activating factor and the macrophage inflammatory effects in the joints. Furthermore, these inactive macrophages may not produce pro-inflammatory

cytokines or other chemokines that could attract other inflammatory cells into the joint areas. Additionally, curcumin was also reported to lower the pro-inflammatory cytokines expression produced by cells such as activated synovial fibroblasts, macrophages and neutrophils in the joints of arthritic rats (Shakibaei *et al.*, 2007). This effect may cause lesser recruitment of leucocytes to the joint areas. It is worth mentioning that normal synovial tissue consists of an acellular structure with synoviocytes within it (Funk *et al.*, 2006). Synovial hyperplasia occurred due to an increase in the proliferation and activation of synoviocytes that mimics the fibroblasts and macrophages. This further increased the production of cytokines, such as IL-1 $\beta$ , TNF- $\alpha$ , IL-8 and many others in the synovium (Funk *et al.*, 2006). Decreased synoviocytes apoptotic rate was also reported to cause synovial hyperplasia, even without the increase in cell proliferation (Sweeney and Firestein, 2004).

To investigate the possible anti-inflammatory mechanisms of action of curcumin in the present study, the serum concentrations of TNF- $\alpha$ , IL-1 $\beta$  and IL-10 were determined. The serum pro-inflammatory cytokines, TNF- $\alpha$  and IL-1 $\beta$ , levels were significantly elevated in arthritic, diabetic and arthritic/diabetic control rats; the effect was more deteriorated in arthritic/diabetic rats than in arthritic or diabetic rats. The serum level of anti-inflammatory cytokine, IL-10, was depleted in arthritic, diabetic and arthritic/diabetic control rats; it was also more deteriorated in arthritic/diabetic rats than in arthritic or diabetic rats. These cytokine changes ensure that in both experimental CFA-induced arthritis and/or streptozotocin-induced diabetes, there is a dominance of Th1 cytokines over Th2. This evidence was supported by many previous authors (Amirshahrokhi *et al.*, 2008; Zhu *et al.*, 2013; Zhang *et al.*, 2013). TNF- $\alpha$  plays a crucial role in the pathological change in the process of rheumatoid arthritis (Matsuno *et al.*, 2002). In the process of cartilage and bone erosion, TNF- $\alpha$ , secreted from macrophages, triggers the production of other cytokines and endothelial adhesion molecules, and induces chondroclast and osteoclast differentiation (Filter *et al.*, 1996; Knowles *et al.*, 2012). Furthermore, TNF- $\alpha$  exerts its arthritogenic potency through the induction of IL-1 $\beta$ . Therefore, TNF- $\alpha$  has been shown to be the dominant player in the induction of inflammatory process and cartilage and bone erosion in rheumatoid arthritis (Bonecchi *et al.* 1998; Joosten *et al.*, 1999; Zhu *et al.*, 2013). Both TNF- $\alpha$  and IL-1 $\beta$  induce the recruitment of monocytes and lymphocytes from blood into articular joints. Curcumin treatment significantly decreased the elevated serum pro-inflammatory cytokines, TNF- $\alpha$  and IL-1 $\beta$ , levels and increased the lowered serum anti-inflammatory cytokine, IL-10 level in arthritic, diabetic and arthritic/diabetic rats. Thus, curcumin has potent anti-inflammatory potentials which in turn suppress the joint deleterious changes represented by pannus formation, articular cartilage erosion and hyperplasia of the

synovium in arthritic and arthritic/diabetic rats. The decrease in TNF- $\alpha$  and IL-1 $\beta$  and the increase in IL-10 levels in diabetic and arthritic/diabetic rats also play a substantial role in the improvement of islet histological architecture and integrity leading to the amelioration of  $\beta$ -cell function and insulin secretory response. These results go parallel with Soetikno *et al.* (2011) and El-Azab *et al.* (2011) reported that the improvement effect of curcumin on the glycemic state in diabetes models may be explained its attenuation effects on TNF- $\alpha$  and nuclear factor-kappa B (NF- $\kappa$ B).

In conclusion, curcumin has potent anti-inflammatory effects in arthritic, diabetic and arthritic/diabetic rats as well as it also has anti-hyperglycemic and anti-hyperlipidemic in diabetic and arthritic/diabetic rats. Thus, curcumin may have beneficial effects in the therapy of the concomitant rheumatoid arthritis and type 1 diabetes mellitus. However, further studies are required to assess the efficacy of curcumin in arthritic and diabetic human beings.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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