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# THE OSTEOPROTECTIVE EFFECT OF *Azadirachta excelsa* LEAVES EXTRACT ON BONE OF STREPTOZOTOCIN-INDUCED DIABETIC RATS

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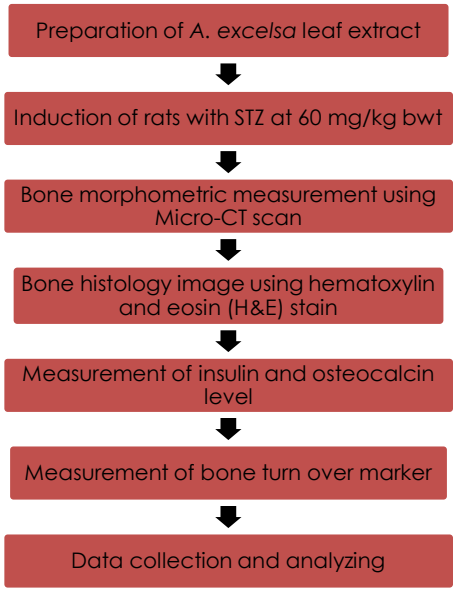
## Graphical abstract




## Abstract

Insulin signalling in bone favours whole-body glucose homeostasis by activating osteocalcin, which is important for bone remodeling. However, diabetes causes deficient production of insulin which consequently affects the osteocalcin and bone turnover marker. The aim of this study was to explore the potential of *A. excelsa* to improve insulin and osteocalcin secretion, resulting in improved bone histomorphometric and bone turnover marker in STZ- induced diabetic rats. The experimental rats were divided into normal control (NC), diabetic control (DC), Metformin-treated diabetic (DMET) (positive control) and *A. excelsa*-treated diabetic (DAE) rats with the treatment period of eight weeks. After the treatment, the femoral bones were removed and bone morphometrical parameters were defined using Micro-CT scan. The bones were analysed for mineral density (BMD) and trabecular parameters. The bones samples were decalcified for histological preparation and images of the was hematoxylin and eosin (H&E) stained bones were captured and analysed. The concentrations of serum insulin, osteocalcin and bone turnover marker were evaluated using specific ELISA kits. The study showed that *A. excelsa* caused a significant increase in insulin and osteocalcin levels. *A. excelsa* also represents ameliorative effects on trabecular bone of the diabetic rat. The data also demonstrated that with *A. excelsa* treatment, it moderately restored the balance between bone formation and bone resorption markers. These data confirmed that *A. excelsa* extract could attenuate the STZ-induced bone loss and reverses the deterioration of bone microarchitecture in diabetic rats. This finding indicates the osteoprotective effects presence in the *A. excelsa* extract.

*Keywords*: *Azadirachta excelsa*, streptozotocin-induced diabetes, bone morphometric, Micro-CT, insulin, osteocalcin

## Abstrak

Penisyaratan insulin dalam tulang menyokong homeostasis glukosa seluruh badan, adalah penting untuk pembentukan semula tulang. Walau bagaimanapun, diabetes menyebabkan kurang pengeluaran insulin yang menjejaskan osteokalsin dan pusingan ganti marker tulang. Kajian ini bertujuan untuk meneroka potensi ekstrak *A. excelsa* untuk meningkatkan pengeluaran insulin dan osteokalsin, yang hasilnya ialah pembaikkan pada histomorfometri dan pusingan ganti marker tulang tikus diabetes teraruh menggunakan streptozotosin (STZ). Kumpulan tikus yang diujikaji dibahagikan kepada kawalan normal (NC), kawalan diabetes (DC), tikus

diabetes yang dirawat dengan metformin (DMET) (kawalan positif) dan tikus diabetes yang dirawat dengan ekstrak *A. excelsa* (DAE), dalam tempoh perawatan selama lapan minggu. Selepas tempoh perawatan, tulang femur dikeluarkan dan parameter morfometri tulang ditakrif menggunakan imbasan tomografi mikro-komputer (mikro-CT). Sampel tulang dinyah-kalsium untuk penyediaan histologi dan imej tulang yang diwarnakan dengan hematoksilin-eosin (H&E) diambil dan dianalisa. Kepekatan insulin, osteokalsin dan pusingan ganti marker tulang dalam serum dinilai menggunakan kit ELISA yang khusus. Kajian menunjukkan ekstrak *A. excelsa* menyebabkan peningkatkan yang signifikan pada aras insulin dan osteokalsin. Ekstrak *A. excelsa* juga menunjukkan kesan ameliorasi tulang trabekula pada tikus diabetes. Data juga menunjukkan perawatan ekstrak *A. excelsa* secara sederhana memulihkan keseimbangan marker pembentukan dan serapan semula tulang. Data ini mengesahkan ekstrak *A. excelsa* dapat mengurangkan kehilangan tulang akibat aruhan STZ dan memulihkan mikroarkitektur tulang pada tikus diabetes. Penemuan ini menunjukkan ekstrak *A. excelsa* mampu memberi pelindungan pada struktur tulang.

Kata kunci: *Azadirachta excelsa*, diabetes aruhan streptozotosin, morfometri tulang, Mikro-CT, insulin, osteokalsin



## 1.0 INTRODUCTION

Diabetes mellitus (DM) is a silent pandemic with substantial morbidity and death rate. It currently inflicts approximately 347 million people worldwide. Therefore, 1.2 million people have been diagnosed in Malaysia [1]. Long term complication of uncontrolled diabetes adversely affect different parts of the body, which include bones [2]. Like DM, osteoporosis (OP) has become an alarming health problem and approximately 200 million people in the world are under the threats and it is common among DM patients [3]. It is noteworthy, patients with DM have increased rate of osteoporosis and bone fractures [4].

Insulin signaling in bone favours whole-body glucose homeostasis by activating osteocalcin, which is initiated by osteoclasts to destroy old bone and stimulate more insulin secretion in the pancreas [5]. For that reason, high glucose due to diabetes alters the insulin and osteocalcin secretion and at least alters the osteoblast differentiation, bone formation and mineralisation [6]. Recent evidences had verified that chronic hyperglyceamia induces bone fracture by delaying the bone healing process [7, 8]. The DM also appears to be a major contributing factor in bone degeneration osteopenia and osteoporosis [9]. In spite of many observations, the exact mechanism of how DM induces osteoporosis remains elusive.

Some diabetes medicines caused bone loss [10]. Hence, conventional treatment of osteoporosis usually involved anti-resorption drugs which can cause osteonecrosis (death of bone cells or tissue) of the jaw (ONJ) as much as a kidney problem [11]. Previously, a report stated that Tongkat ali, Kacip Fatimah and Kaduk consist of flavonoid and phenolic compounds which can prevent overiectomy-induced osteopenia and strengthened the bones [12].

*A. excelsa* plants belong to the family Meliaceace and genus Azadirachta. It is a typical wild plant found in Malaysia and locally known as marrango tree or sentang [13,14]. *A. excelsa* mainly contains azadirachtin which has a promising effect as an antioxidant, antimicrobial, antimalarial, antiseptic agent and antidiabetic properties tested in various animal models [15]. It is noteworthy that *A. excelsa* has been proven to reduce fasting blood glucose [16]. Currently Zin *et al.* [17] revealed that, *A. excelsa* consists of high concentration of flavonoid compound. In accordance to the bio-property effect of medicinal plants, this study was conducted to investigate the potential of *A. excelsa* as a traditional medicinal therapy for bone pathology due to diabetes.

## 2.0 METHODOLOGY

### 2.1 Preparation of Leaf Ethanolic Extract of *Azadirachta excelsa*

The leaves of *A. excelsa* plant (UKMB40314) were obtained from Forest Research Institute of Malaysia (FRIM), Kepong, Kuala Lumpur. The leaves were cut, dried and ground to form powder. It was then soaked in 70% ethanol at 1:10 ratio (100 g powder: 1000 ml ethanol) for three days at room temperature (27°C). The ethanolic extract was collected in a conical flask. The mixture was filtered by using a vacuum pump, Buchner funnel and filter papers. The filtrate obtained was evaporated by using a rotary evaporator at 40°C [18]. A dark semi-solid paste obtained was stored at 4°C for further use.

### 2.2 Experimental Animal and Animal Management

Twenty-four male Sprague-Dawley rats weighed between 200-250 g (Chenur Supplier, Selangor, Malaysia) were used in this study. The rats were acclimatised for a week and exposed to 12-h dark/light cycle at 22°C. They were housed in groups of six, and fed with a standard commercial rodent diet (Gold Coin Feedmills, Gold Coin Holdings, Malaysia) and plain water *ad libitum*. The care and maintenance of the experimental animals were closely observed and followed the standard guideline approved by the Committee of Animal Research Ethics, Universiti Tekonologi MARA (UiTM Care) (Ref No: 112/2015).

Induction of diabetes was conducted through an intraperitoneal injection of 0.5 ml Streptozotoxin (STZ) at 60 mg/kg bwt. STZ was freshly prepared in saline (9% sodium chloride) at 4°C. After a week, animals with fasting blood glucose levels >11 mmol/L were considered diabetic. The rats were divided into four groups of six rats each (Table 1).

**Table 1** Treatment group

| Group | Treatment | Dosage |
|---|---|---|
| Normal control (NC) | Saline | - |
| Diabetic control (DC) | Saline | - |
| Diabetic rats + Metformin (DMET) | Metformin | 1000 mg/kg b.wt |
| Diabetic rat + *A. excelsa* (DAE) | *A. excelsa* Extract | 250 mg/kg b.wt |

Metformin and ethanolic extract of *A. excelsa* were dissolved in saline. A single dose of treatment was given daily by forced feeding for eight weeks. At the end of the treatment, the rats were fasted overnight and under slight anesthesia using diethyl ether. Then the blood samples were collected through cardiac puncture into plain sterilised centrifuge tubes. Serum was separated and stored at -80°C until analysis.

### 2.3 Insulin and Osteocalcin Level Assessment

The levels of serum insulin were determined by Rat Enzyme Linked Immunosorbent Assay kit for insulin (Cloud-Clone Incorporation, USA). The osteocalcin level was determined by using Rat-Mid Osteocalcin ELISA kit (IDS, UK). All assays involved were provided by the manufactures.

### 2.4 Bone Collection and *Ex-Vivo* Micro-Computed Tomography (µCT) Analysis

The femoral bones collected from euthanised rats were cleansed off soft tissue, fixed in formalin for 24 hours, and stored in 70% alcohol at -20°C until scanning which followed the methods described by Verdelis *et al*. [19]. The fixed femurs were thawed at 37°C and analyzed by SkyScan 1176 micro-CT scanner (Bruker, Kontich, Belgium) with 12 µm spatial resolutions and 1 mm aluminum filter at a voltage of 41 kV and a current of 232 µA. Trabecular bone parameters such as trabecular numbers (Tb.N), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th) and bone volume ratio (BV/TV) were quantified the metaphysis. All µCT parameters were reported according to international guidelines [20].

### 2.5 Bone Histological Assessment

Bone samples were decalcified in 0.5 mmol·L−1 EDTA phosphate buffered saline (pH 7.4) at 4°C for 2 months [21]. The EDTA solution was changed every week as followed by the dehydration and embedding in paraffin and the bone specimens were sectioned at 4 µm, and stained with H&E. All slides were examined using light microscope (Motic BA410, Wetzlar, Germany) equipped with a digital camera (Moticam Pro 285A, Wetzlar, Germany) under 200x and 400x magnifications.

### 2.6 Preparation of Bone Homogenate

The pieces of the femoral bones were ground using mortar and pestle. Hundred milligram of bone tissues were homogenised in a homogenising buffer (50 mM Tris- HCl, 1.15% KCl pH 7.4) using a Teflon pestle (Glass-Col, USA). The homogenates were centrifuged at 3000 rpm for 15 minutes at 4°C (Centrifuge 5417R; Eppendorf, Germany). The supernatants were then collected and stored at -80°C until used.

### 2.7 Bone Turnover Marker Assessment

The bone formation and resorption marker in the bone homogenate samples were measured. The rat bone alkaline phosphatase (BALP) ELISA kit (Qayee, Shanghai) was used to examine the levels of BALP and Rat deoxypyridinoline (DPD) was used to measured bone resorption by using ELISA Kit (Qayee, Shanghai).

### 2.8 Statistical Analysis

All data were expressed as means ± standard error of the means (mean ± SEM) and dissected using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM Corporation, USA). The differences among the means of each group were tested using One-way analysis of variance (ANOVA) followed by Duncan's multiple range test. All analysis was performed at 95% confidence level.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Serum Insulin and Osteoocalcin

Over a few decades, insulin has been established as an important hormone for reducing blood glucose [22]. Table 2 showed the concentration of insulinl in the DC group, whixch significantly decreased ($p$<0.05) by 62.02% to 1.58 µIU/ml as compared to the NC group (4.16 µIU/ml). The DMET group indicated a slightly improved insulin level into 1.77 µIU/ml, as compared to the DC group. Meanwhile, treatment with *A. excelsa* significantly had 44.68% increased of insulin level up to 2.10 µIU/mL, as compared with the DC group.

STZ-induction caused a significant reduction of serum insulin level among the DC group (Table 2). However, the DAE group showed a significant increase in serum insulin level. These findings were aligned with previous studies where, STZ-induced animals showed disrupted insulin-producing and insulin secretion in pancreatic-cells [23,24]. This result indicated that the treatment with *A. excelsa* extract may have reduced the action of STZ and improved the insulin levels. Even, finding by Nurliyani [25] stated that increased insulin secretion might be due to improvement of pancreatic β-cells in diabetic rats.

Table 2 also shows serum osteocalcin (OC) concentration decreased significantly to 14.35 mg/ml (89.58%, $p$<0.05) in the DC group with respect to the NC group, (137.78 mg/ml). In contrast, treatment with metformin had caused a significant increase of serum OC, (57.42 mg/ml, 300.14%, $p$<0.05). Meanwhile, the DAE group indicated a significant increase serum OC concentration to 25.76 mg/ml, 79.51% higher, $p$<0.05 as compared to DC group.

**Table 2** The serum insulin and osteocalcin level in the experimental groups

| Group | Insulin(µIU/ml) | Osteocalcin (mg/ml) |
|---|---|---|
| NC | 4.16 ± 0.10$^{c}$ | 137.78 ± 6.92$^{d}$ |
| DC | 1.58 ± 0.11$^{a}$ | 14.35 ± 0.97$^{a}$ |
| DMET | 1.77 ± 0.24$^{a}$ | 57.42 ± 8.24$^{c}$ |
| DAE | 2.10 ± 0.31$^{b}$ | 25.76 ± 1.04$^{b}$ |

Values are presented as means ± SEM. Superscripts $^{a,b,c,d}$ in a column differ significantly at $p$<0.05. NC= Normal rats, DC= Diabetic rats, DMET= Diabetic rats treated with metformin, DAE= Diabetic rats treated with *A. excelsa*.

In this study, the DC group also showed a reduction of serum OC as compared the to the NC group (Table 2). Osteocalcin is a hormone produced only by osteoblasts to enhance bone remodeling by accelerating insulin delivery to bone [26]. Other than that, *in vitro* study stated that insulin treatment induced a 3-fold increase in osteocalcin promoter activity [27]. It is suggested that indirect deformity of insulin and OC consequently diverts the normal homeostasis differentiation in osteoblasts and may affect the bone mineralisation. Previous study stated that, the lack of osteocalcin level was demonstrated to cause hyperglycemia and insulin resistance in the animal models [28].

The *A. excelsa* treatment group had significantly increased in serum OC together with increased insulin level (Table 2). Similar finding emphasized by another study showed that, the serum OC level increased after treatment of diabetes [29]. In another study, it showed that osteocalcin increased the expression of insulin in pancreatic β cells [30]. This finding suggests the existence of a bone-pancreas, endocrine loop through which insulin signaling in the bone osteoblast stimulates osteocalcin production, which in turn regulates insulin sensitivity and pancreatic insulin secretion. The study identified that the treatment of *A. excelsa* may increase the insulin expression and secretion throughout the improves of OC secretion.

### 3.2 Bone BALP and DPD Levels

Biochemical markers can be used as indicators of bone quality [31]. The STZ-induced diabetic rats had significantly altered bone marker activity. The BALP and DPD levels were tabulated in Table 3. The BALP activity was significantly reduced 67.06 ng/ml, $p$<0.05, (16.62% lower) in the DC group compared with the NC group, (101.49 ng/ml). The DMET group showed enhanced BALP activity with 81.38 ng/ml, which was 17.35% significant higher, ($p$<0.05) with respect to the DC group.

Meanwhile, the DAE group had 14.23% increase of the BALP activity in the bone with value 73.39 ng/ml as compared to the DC group. The STZ-induced diabetic rats also showed significantly enhanced bone resorption activity. The DPD level was significantly increased 6.38% higher with value 167.10 ng/ml ($p$<0.05) in the DC group as compared to the NC group, (157.08 ng/ml). The metformin treatment group showed a significant reduction ($p$<0.05) of DPD activity in serum, (152.16 ng/ml) with respect to the DC group. Noteworthy, treatment with *A. excelsa* reduced the DPD activity in serum by 51.43% the highest reduction with value 130.02 ng/ml as compared to DC group.

**Table 3** The BALP and DPD levels in the bone of the experimental groups

| Group | BALP(ng/ml) | DPD (ng/ml) |
|---|---|---|
| NC | 101.49 ± 7.59$^{b}$ | 157.08 ± 5.33$^{b}$ |
| DC | 67.06 ± 4.70$^{a}$ | 167.10 ± 0.21$^{d}$ |
| DMET | 81.38 ± 0.45$^{a}$ | 152.16 ± 4.08$^{b}$ |
| DAE | 73.39 ± 8.31$^{a}$ | 130.02 ± 0.41$^{a}$ |

Values are presented as means ± SEM. Superscripts $^{a,b,c,d}$ in a column differ significantly at $p$<0.05. NC= Normal rats, DC= Diabetic rats, DMET= Diabetic rats treated with metformin, DAE= Diabetic rats treated with *A. excelsa*.

The result showed that the DC group had significantly decreased in BALP levels, but a significant increase in DPD level (Table 3). The inhibition of bone formation in the DC group was confirmed through the finding of decreased BALP levels [32]. Previous findings indicated that a decrease in osteoblastic bone formation is a major contributor to diabetic osteoporosis, while higher levels of resorption markers were associated with increased fracture risk [33]. These findings are in agreement with Zhukouskaya *et al*. [34], who showed that reduced bone turnover is the key characteristic of DM-associated bone disorder.

In contrast to the DC group, the DAE group had significantly decreased DPD level (Table 3). It is important to mention that the decrease in the DPD level indicates low bone resorption [35]. In fact, there is evidence which emphasized that markers of bone resorption is a better potent predictor of future bone loss than markers of bone formation [36]. Besides, A. excelsa extract, numerous polyphenolic compounds such as flavonoids were reported to exert a positive effect on osteoporosis [37], prohibit osteoclast differentiation [38] and stimulate osteoblast formation *in vitro* and *in vivo* [39]. Reduction of bone resorption activity is particularly important in delaying the bone loss and maintains bone strength. The presence of polyphenolic compounds in *A. excelsa* may be involved in ameliorating bone complication caused by diabetes. This finding lends further support on the role of *A. excelsa* in improving bone remodeling under diabetic condition.

### 3.3 Histomorphometric Measurement of Bones

Prolonged hyperglycemia is known to cause deformity to bone microstructure [40]. Histomorphometrical changes of the trabecular bones are shown in Table 4. The BMD values were found lower in the DC and treated diabetic groups. Therefore, a significant increased femoral trabecular separation (Tb.Sp, mm) and decreased femoral trabecular thickness (Tb. Th, mm) and trabecular bone volume (BV/TV, %) were observed in the DC group as compared to the NC group. The micro-CT results showed a dramatic deficit in trabecular bone at the proximal femur metaphysis of diabetic rats compared to that found in the NC group. It was recorded that the mean values of BV/TV were significantly decreased in all of the diabetic rats. However, DMET and DAE groups marked a significant increase in Tb.N and Tb.Sp. In addition, the DAE group showed a significant increased in BV/TV compared to the DC and DMET groups.

**Table 4** Bone histomorphometric measurement of the experimental groups

| Group | BMD ($g/cm^3$) | Tb.N (1/mm) | Tb.Sp (mm) | Tb.Th (mm) | BV/TV (%) |
|---|---|---|---|---|---|
| NC | 5.78 ± 0.36$^{c}$ | 2.28 ± 0.07$^{c}$ | 0.28 ± 0.01$^{a}$ | 0.25 ± 0.01 | 56.66 ± 0.30$^{c}$ |
| DC | 5.47 ± 0.45$^{bc}$ | 1.13 ± 0.01$^{a}$ | 1.12 ± 0.00$^{c}$ | 0.25 ± 0.01 | 28.33 ± 0.44$^{a}$ |
| DMET | 5.59 ± 0.31$^{c}$ | 1.66 ± 0.21$^{b}$ | 0.79 ± 0.27$^{b}$ | 0.18 ± 0.04 | 29.39 ± 4.34$^{a}$ |
| DAE | 5.04 ± 0.03$^{ab}$ | 1.71 ± 0.29$^{b}$ | 0.41 ± 0.02$^{a}$ | 0.26 ± 0.05 | 36.31± 0.59$^{b}$ |

Values are presented as means ± SEM. Superscripts $^{a,b,c,d}$ in a column differ significantly at $p<0.05$. NC= Normal rats, DC= Diabetic rats, DMET= Diabetic rats treated with metformin, DAE= Diabetic rats treated with *A. excelsa*.

The quantitative data from micro-CT scans showed that the DC group had a significant decrease in BMD, BV/TV and Tb.N, but an increased in the Tb.Sp value (Table 4). BMD is known as an important component in determining the bone strength [41]. Therefore, BMD values might relate to the risk of osteoporosis. However, the loss in trabecular bone structure also indicates the reduction of bone strength in the STZ-induced diabetic rats [42]. Similar finding was also reported by another study [43]. Therefore, it is plausible to propose that the loss of trabeculae bone is associated with lower BMD, which may increase the risk of osteoporosis.

The valuable finding in this study was that treatment with *A. excelsa* had significantly increased BV/TV, Tb.Th, Tb.N but a decrease in Tb.Sp value. The trabecular bone loss in diabetic animals was presumably delayed by *A. excelsa* treatment. Nevertheless, the BMD value remained unchanged with *A. excelsa* treatment. Likewise, can be emphasized that treatment with *A. excelsa* has positively ameliorates trabecular bone rather than BMD. However, an elevated trabecular bone appears to represent strong fracture-resistant microarchitecture [44]. This is supported by another study that highlighted the decrease of the fracture risk through improvement of trabecular bone is more important than improving BMD value [45]. Hence, this finding suggested that treatment with *A. excelsa* may help in reducing the progression of osteoporosis in rats.

### 3.4 Histological Analysis of Bone

To further confirm the effect of STZ on trabecular micro-structure, the histomorphometry in the distal femoral bone was also examined by H&E staining (Figure 1). The histological sections of the bone in NC group showed normal articular cartilage (AC) (Figure 1-1a). Light microscopic examination also showed healthy networks of bone trabeculae separated with bone marrow spaces at the distal femoral metaphysics (Figure 1-1b). As presented in Figure 1-1c, the well-

developed growth plate was observed in the NC group with regular chondrocyte column arrangement. The normal arrangement on the NC group epiphyseal growth plate was accompanied with four distinct zones of resting, proliferate, prominent hypertrophic and calcification zones. Then, the calcified cartilage layer was flanked by an undulating tidemark (Figure 1-1d).

In contrast, the histological analysis revealed the thinner layer of AC (Figure 1-2a), with disconnected trabeculae and wider bone marrow spaces filled with many fat cells (Figure 1-2b) in bone of the DC group. On epiphyseal growth plate sections, the DC group displayed disorganised growth of plate zones and disorder of chondrocyte column arrangement (Figure 1-2c), as well as the loss of cortical layer thickness (Figure 1-2d).

In the meantime, the stained sections of the proximal metaphysis of the femoral bone of the DMET group revealed the eroded AC (Figure 1-3a). The thinning and widely separated trabeculae were shown in the proximal metaphysis of the DMET group (Figure 1-3b). The epiphyseal plate sections of the rats revealed disorganised column arrangement of shrunken and atrophied chondrocytes (Figure 1-3c). The DAE group was associated with a thicker calcified cartilage (Figure 1-4a) and the bone trabeculae were orderly arranged and increased bone matrix density (Figure 1-4b). As illustrated in Figure 1-4c, the epiphyseal plate was arranged in layered array with less cortical erosion found in the DAE group (Figure 1-4d).

The results conveyed the parallel outcomes which support the findings of the bone histomorphometric measurement. Both results indicated a destruction of bone architecture in the DC group. The bone histology of the DC group showed degradation of articular cartilage thickness (Figure 1-2a), disconnected of trabeculae (Figure 1-2b) and disorganised chondrocyte column(Figure 1-2c). In line with bone histomorphometric measurement result, the deterioration of trabecular bone is expected to increase the incidence of fracture in the DC group as mention in the study of Ma *et al.* [46]. It is worth to mention that articular cartilages are highly specialised tissues that protect the bone ends and degradation of its thickness could lead to osteoarthritis symptoms [47].

Treatment with *A. excelsa* resulted in the thickening of the articular cartilages and trabecular bone (Figure 1-4a). This finding provides a support that thickening in articular cartilages could reduce the risk of osteoarthritis. Indeed, the result from this treatment corresponded with report on another plant that has similar anti-osteoporotic activity with healing features of bone [48]. The epiphyseal plate and chondrocytes arrangement with the DAE group were almost identical to those observed in the NC group. Previous studies had highlighted that the epiphyseal plate is the most important site for mineralisation, a cartilage where new bone growth takes place [49]. It is very important to note that, the DAE treatment might inhibit the progression of trabecular bone loss while restoring mineralisation. It is plausible to propose that *A. excelsa* treatment may also be useful to delay the progression of osteoarthritis, and bone loss in STZ-induced diabetic rats.

## 4.0 CONCLUSIONS

The study revealed the link between diabetes, lack of insulin and OC level due to the incidence of osteoporosis. This work also showed the protective property of *A. excelsa* extract on the femoral bone architecture among the STZ induced diabetic rat models as proven by the bone morphometric, histology and bone turnover marker as a results of the improvement of insulin and OC levels. Hence, it is possible to further explore the potential of *A. excelsa* as the agent for the improvement of bone quality in aging, osteoporosis and diabetes diseases.

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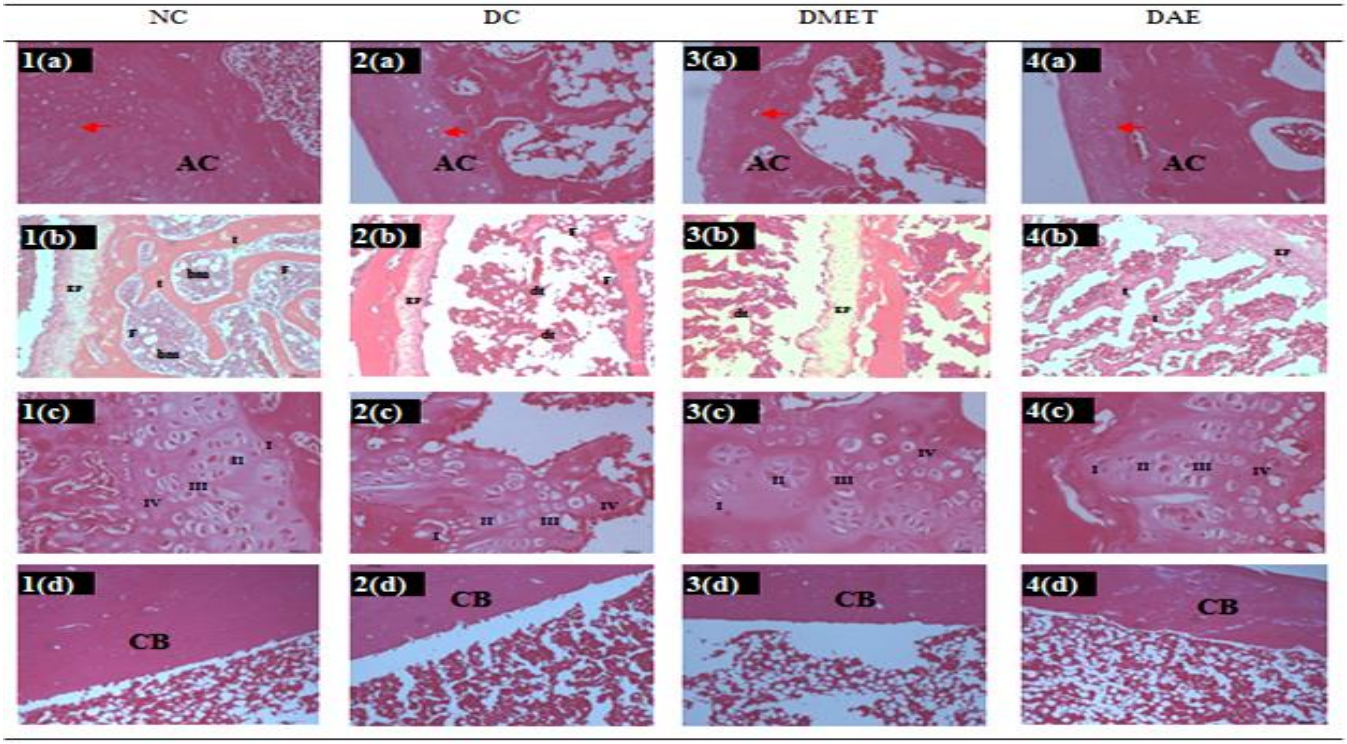


**Figure 1** Light photomicrographs of epiphysis, metaphysis and cortical of rats femoral bones. (1) NC group, (2) DC group, (3) DMET group, and (4) DAE group. a, b and c, d represent different parts of femoral bone: (a) Articular cartilage (AC); (b) Metaphysis trabeculae, (c) Epiphyseal plate (EP); resting (I), proliferative (II), hypertrophied (III) and calcified (IV) (d) Corticol bone (CB), NC= Normal rats, DC= Diabetic rats, DMET= Diabetic rats treated with metformin, DAE= Diabetic rats treated with *A. excelsa*. Osteocytes (arrows) surrounded by their lacunae are seen in the bone matrix; The proximal metaphysis separated by bone marrow spaces (bm); Thin widely separated disconnected trabeculae of proximal metaphysis (dt); Fat cells in marrow spaces (F). [H&E stain; a,b= magnification 200x and c= magnification 400x]

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