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Experimental Model of Type II Diabetes-induced Osteoarthritis in Rats of Wistar Strain

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ABSTRACT: Type II diabetes mellitus (T2DM) has been suggested to predispose to osteoarthritis (OA) leading to T2DM-induced OA. Hence this study aims to develop an experimental model for T2DM-induced OA in order to advance research on diabetes-osteoarthritis conditions. Male Wistar rats (N=40) were divided into control and experimental T2DM group. T2DM was induced by a single intraperitoneal injection of nicotinamide (110 mg/kg) and streptozotocin (65 mg/kg). Rats with fasting blood glucose levels >200 mg/dL on day 4 post-induction were considered diabetic. Glucose levels were subsequently monitored on days 7, 14, 21, 28 post-induction. Feasible osteoarthritis markers; joint swelling diameter, gait test (Stride length and paw dimension), arthritis index, histological evaluations and MANKIN osteoarthritis scores were done; Data were analyzed using Student's t-test and Bonferroni post-hoc test, $p < 0.05$. Data obtained from T2DM group showed significantly higher blood glucose levels on days 4, 7, 14, 21, and 28 compared with controls. T2DM group experienced increased joint swelling, arthritis index, and decreased stride length, paw width, and paw area. Histological analysis revealed significant knee joint deterioration, including synovium derangement, cartilage abnormalities, and higher MANKIN osteoarthritis scores in the T2DM group compared to controls. This study shows an experimental T2DM-induced OA model in Wistar rats with signs and symptoms consistent with diabetes-induced osteoarthritis in human.

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Osteoarthritis (OA) refers to chronic disorder associated with degeneration of joint cartilage, synovium and the underlying bone. As a consequence of wear and tear of the joint due to overtime use, it leads to pain, stiffness, swelling and inflammation. It has been reported to be a most common manifestation from middle age onward, and it occurs mainly on the hip, knee, feet, thumb and spine joints (Kim, 2022). Factors like aging, obesity, injuries as well as diabetes mellitus have been associated with osteoarthritis (Berenbaum, 2012; Jiménez *et al.*, 2018). Diabetes mellitus (DM) is a glucose toxicity condition that result from impaired glucose homeostasis either due to damage to pancreatic islets and/or insulin insensitivity at the tissue level resulting to increase in blood glucose concentration (hyperglycemia) (Sarkar *et al.*, 2019). It is usually either Type I (Insulin-dependent) which is associated with impaired insulin production or Type II (non-insulin-dependent) which results from insulin resistance (Kumar *et al.*, 2020). DM and OA have been reported to co-exist due to shared risk factors such as age, sex, race and metabolic syndromes such as obesity, hypertension, and dyslipidemia (Alenazi *et al.*, 2019). Studies have also identified a link between

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diabetes mellitus, especially type II and osteoarthritis (Louati *et al.,* 2015). Type II diabetics have been reported to have an increased susceptibility to develop arthritis compared to those without it (Piva *et al.,* 2015). The complications arising from the coexistence of OA and T2DM underscore the need to elucidate the mechanisms underlying OA development in diabetes and to develop comprehensive therapeutics specific for the T2DM-induced OA phenotype. Given the paucity of appropriate experimental models exhibiting this phenotype, this study was therefore designed to develop an experimental model of type II diabetes-induced osteoarthritis so as to provide an experimental model for research into mechanisms, pharmacological interventions and amelioration of type II diabetes-induced osteoarthritis condition.

MATERIALS AND METHODS

Study design: Forty (40) male Wistar rats (average weight 141.00 ± 2.45 g) were housed in well-aerated cages and acclimatized to laboratory conditions for 14 days. They were maintained on standard rat chow with free access to drinking water *ad libitum* and exposed to natural alternating 12 hours day and 12 hours night cycles. The animals were evenly divided in control and diabetic group. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA and as approved by University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/059-0723/10).

Induction of Type II Diabetes Mellitus: Type II diabetes was induced using streptozotocin-nicotinamide model (Masiello *et al.,* 1998; Adeyemi and Olayaki, 2018), which mimics moderate and stable hyperglycemia featured in humans with type II diabetes mellitus. Briefly, Animals were fasted overnight and thereafter, nicotinamide (110mg/kg) in normal saline was administered intraperitoneally. This was followed after 15 minutes with an intraperitoneal administration of freshly prepared streptozotocin (65 mg/kg) ((Masiello *et al.,* 1998) in cold citrate buffer solution (0.1M, pH 4.5). The development of experimental type II diabetes was confirmed 4 days post-induction using blood samples collected by the tail tipping method (Ponnulakshmi *et al.,* 2019). Animals with fasting blood glucose concentration greater than 200mg/dl were considered as diabetic (Adewoye *et al.,* 2007).

Development and Confirmation of Type II Diabetes-induced Osteoarthritis: Development of osteoarthritis was confirmed using feasible osteoarthritis biomarkers on day 0 before induction of experimental type II diabetes mellitus and on days 4, 7, 14, 21 and 28 post type II diabetes mellitus induction, respectively. Osteoarthritis biomarkers evaluated were joint swelling, stride length, paw dimension (width and area) using gait test (Sahin *et al.,* 2021) and arthritis index (Brand *et al.,* 2007). Histological evaluation and MANKIN Osteoarthritis score was also performed.

Joint swelling: Joint swelling (diameter in mm) was measured using electronic digital vernier caliper (Aerospace, L. S. Starrett Company, USA) as described by Sahin *et al.* (2021) and expressed in cm.

Gait test: Stride length and paw dimension: Stride length, paw width and paw area were evaluated using gait test method described by Sahin *et al.* (2021) with slight modification. Briefly, gait test path (Fig. 1) with inside dimension (length = 60cm, width = 7cm and height = 9cm) was constructed with white cardboard paper (dimension: 65cm long, width 10cm) was placed under the path. Thereafter, the hind paws of the rats were stained with different colors (left hind paw = blue, right hind paw = black) of tempera paint (Marie's Baby brand, Shanghai SIIC Marie Painting Materials Co., Ltd., China) and were then allowed to walk on the gait test path, one at a time. Stride length and paw width were measured on the paw print displayed on the cardboard paper (Fig. 2) using meter rule while paw area (cm^2) was calculated as paw length (cm) multiply by paw width (cm).

Arthritis index: Feasible arthritis incidence was evaluated using the method described by Brand *et al.* (2021) on Day 0 (before diabetes induction), 4, 7, 14, 21 and 28. The arthritis index scale (Table 1) was scored based on the following severity score.

Histological evaluations and MANKIN osteoarthritis score: After arthritis index scoring on each, animals were euthanized using ketamine (75 mg/kg) + xylazine (10 mg/kg) and the knee joints were dissected out for histological scoring for osteoarthritis using Mankin score as described by Sahin *et al.* (2021) and structural-morphological changes using Haematoxylin and Eosin stain. The MANKIN score assesses cartilage structure, cellularity, Safranin O staining, and tidemark integrity (Mantripragada *et al.,* 2017). The MANKIN osteoarthritis score of the knee joint (Table 2) using the histological finding as described by Sahin *et al.* (2021) was used in this study.

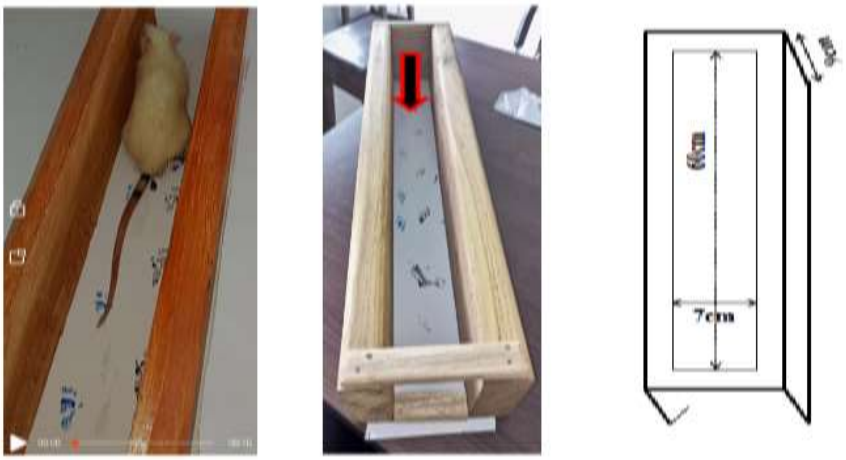
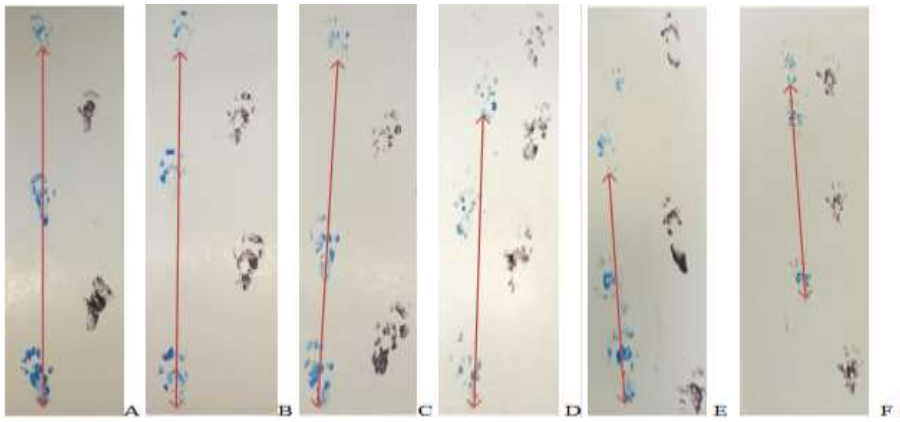


Fig. 1: Gait test path: inside dimension (length = 60cm, width = 7cm, height = 9cm)



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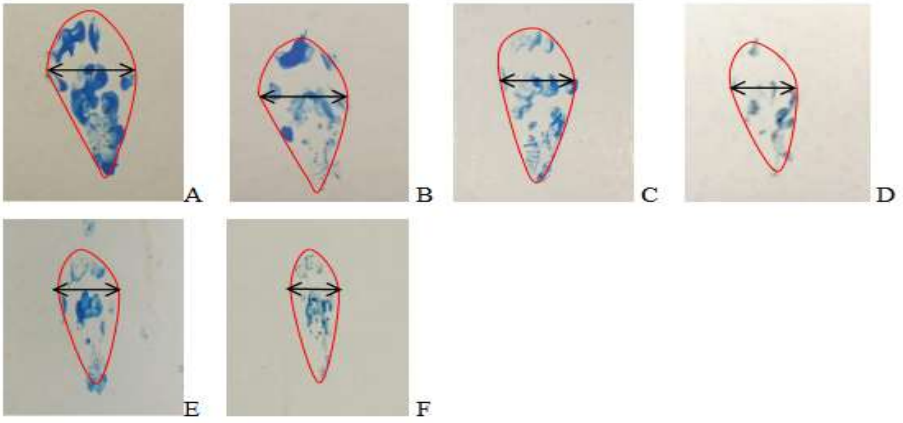

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Fig. 2: Photograph of stride length [E] and paw dimension (width and area) [F] respectively: A= Control; B=Day 4 post-T2DM (Confirmation of T2DM); C=Day 7 post-T2DM induction; D=Day 14 post-T2DM; E=Day 21 post-T2DM; F=Day 28 post-T2DM; Blue=left hind paw, Black= right hind paw, Red=stride length of left hind paw.

Table 1: The arthritis index scaled

Score	Description
0	No evidence of erythema and swelling
1	Erythema and mild swelling confined to the tarsals or ankle joint
2	Erythema and mild swelling extending from the ankle to the tarsals
3	Erythema and moderate swelling extending from the ankle to metatarsal joints
4	Erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb

Table 2: MANKIN osteoarthritis score

A	Structure	Score	Description
		0	Smooth intact surface
		1	Slight surface irregularities
		2	Pannus/surface fibrillation
		3	Clefts into the transitional zone
		4	Clefts into the radial zone
		5	Clefts into the calcified zone
		6	Total disorganization
B	Cells	0	Uniform cell distribution
		1	Diffuse cell proliferation
		2	Cell clustering
		3	Cell loss
C	Tidemark integrity	1	Vascularity

Statistical Analysis: Data obtained are expressed as mean ± standard error of mean (SEM). Multiple comparison within and between experimental groups was done using student T and Bonferroni post-hoc test respectively. Statistical significance was taken at $p < 0.05$.

RESULTS AND DISCUSSION

Body weight: Body weight (g) reduced significantly in T2DM group on days 4, 7, 14, 21 and 28 of post-T2DM induction ($P < 0.05$) compared with control (Fig. 3); this model when used has been reported to result in a non-obese model of T2DM (Masiello *et al.*, 1998) and may be accompanied with a reduction in body weight, which was observed in this study and is in agreement with the reports of Yang *et al.* (2016) and Bao *et al.* (2019) who described this occurrence as a common feature in both DM and OA. This decline in body weight has been associated with increased muscle breakdown due to reduced or altered glucose uptake (Merz and Thurmond 2020).

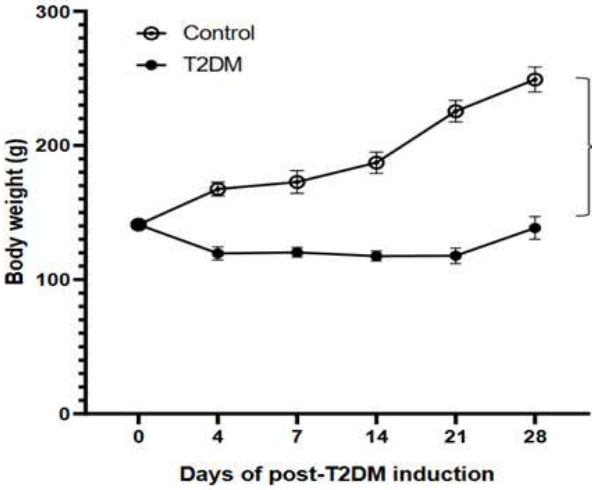

Fig. 3: Body weight (g) changes in control and diabetes group. Values are mean±SEM, n=5, P<0.05, *indicates significant differences between control and T2DM group.

Blood glucose level: Blood glucose level (mg/dl) increased significantly in T2DM group compared with control on day 4, 7, 14, 21 and 28 of post-T2DM induction (Fig. 4). Diabetes mellitus (DM) is characterized by hyperglycemia resulting from either reduced insulin secretion or activity (Okerulu *et al.*, 2023). In this study, the diabetes model used led to sustained hyperglycemia from day 4 to 28 post induction of T2DM which has been attributed to the moderating action of nicotinamide on the toxic effect of streptozotocin on pancreatic β cells (Yan, 2022) resulting in hyperglycemia and partially viable β cell activity.

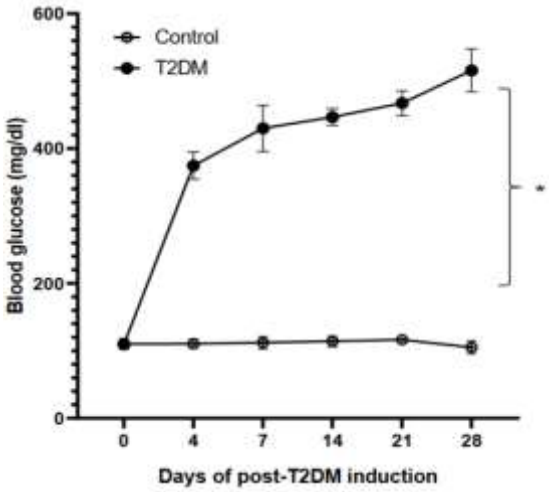

Fig. 4: Blood glucose level (mg/dl) in control and diabetes group. Values are mean±SEM, n=5, P<0.05, *indicates significant differences between control and T2DM group.

Feasible osteoarthritis markers: Joint swelling (joint diameter in cm) increased significantly on days 7 ($P < 0.05$), 14, 21, 28 ($P < 0.05$) post-T2DM induction, while stride length (cm), paw width (cm) and paw area (cm^2) decreased significantly days 4, 7, 14, 21 and 28 in T2DM group compared with control and within T2DM group compared with day 0 values (Fig. 5). Osteoarthritis, a degenerative condition associated with any joint in the body, causes joint swelling and stiffness (Sahin *et al.*, 2021). In this study, joint swelling, a response to inflammation in joint tissues, significantly increased in T2DM group compared to control. Differences in joint swelling were observed on day 4 and from day 14 onwards, marked differences were observed between control and experimental T2DM animals suggesting the persistent presence of OA symptoms from day 14 onwards. According to Adeyemi and Olayaki (2018), joint swelling in OA is accompanied by increased joint diameter. The development of joint swelling, a characteristic feature of OA and corresponding increase in joint diameter in this study suggests that OA can result as a

complication of T2DM. T2DM is characterized by hyperglycemia-induced down-regulation of antioxidant system which leads to increased activation of inflammatory pathways (Lin *et al.,* 2005; Tsalamandris *et al.,* 2019). The response of tissue-resident macrophages and mediators of inflammation to insulin resistance and hyperglycemia in T2DM has been reported to influence the recruitment of circulating monocytes to tissues, which then differentiate to more pro-inflammatory macrophages (Kraakman *et al.,* 2014; Nedosugova *et al.,* 2022). This T2DM-induced increase in the recruitment, differentiation of circulating monocytes to macrophages, and hence increased production of pro-inflammatory cytokines may have contributed to inflammation of joint tissues resulting in diabetes-induced osteoarthritis phenotype seen persistently from day 14 onwards in this study.

Gait test (gait analysis) is a diagnostic tool used to assess the way a person walks or runs. It involves the systematic observation and measurement of an individual's walking pattern to identify any abnormalities or issues with mobility, balance, or coordination (Naili *et al.,* 2019). Essential to gait test are stride length, paw width and paw area. In a complete gait cycle, stride length refers to the distance covered in one full stride. It provides valuable information on individual's walking or running pattern. Paw width along with paw area evaluates an animal's walking or running pattern (Chen *et al.,* 2017). Deviations from the norm may indicate musculoskeletal issues, pain, or other health concerns (Broström *et al.,* 2012). Decreases in stride length, paw width and paw area has been associated with OA (Sahin *et al.,* 2021). The reductions in stride length, paw width and size as well as the observed increase in joint swelling from day 14 onwards in the experimental group compared to controls, further buttresses the presence of osteoarthritis in T2DM.

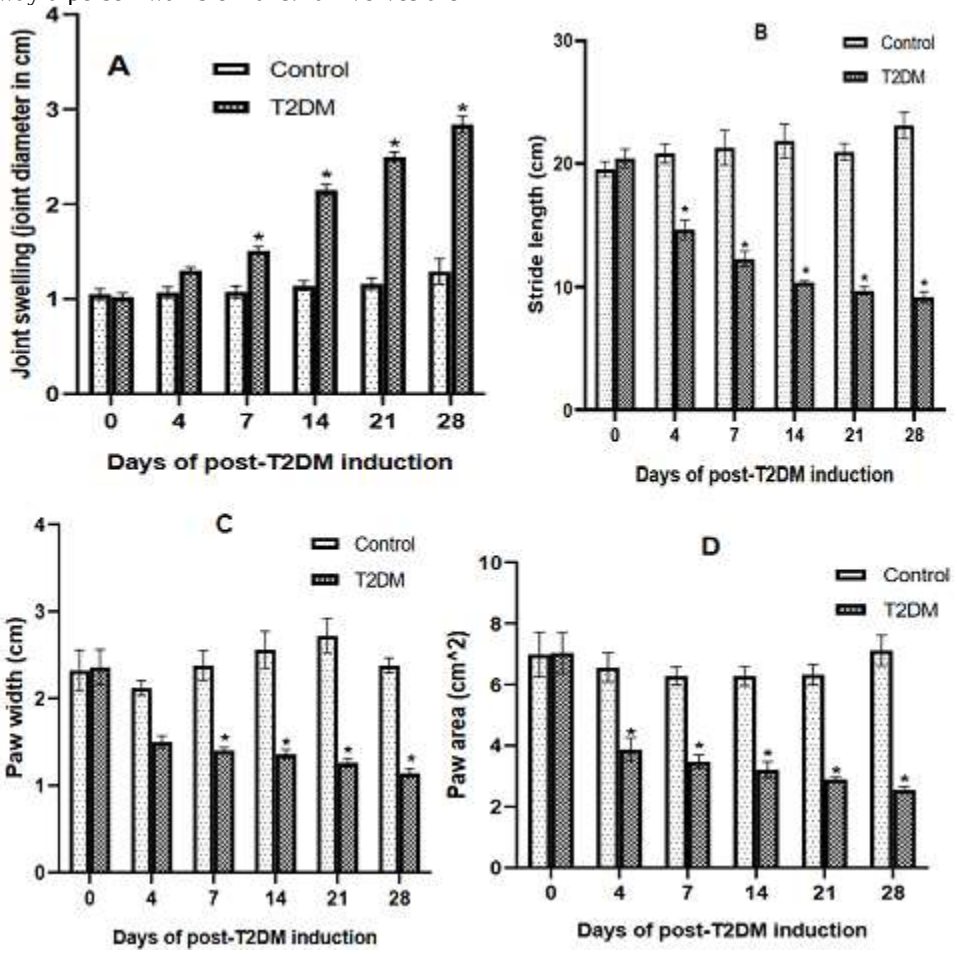

Fig. 5: Joint swellings (A), stride length (B), paw width (C) and paw area (D) in control and diabetes group. Values are mean±SEM, n=5, P<0.05, *indicates significant differences between control and T2DM group

Arthritis index increased significantly in T2DM group compared with control on day 4, 7, 14, 21 and 28 of post-T2DM induction (Fig. 6). According to Brand *et al.* (2007), arthritis index is used to mark arthritis, and increase in this index value suggest predisposition to arthritis. In this study, arthritis index increased following establishment of experimental T2DM on day 4, and further increased from day 14 onwards indicative of occurrence of osteoarthritis in diabetes with increased and or persistent severity in this experimental model.

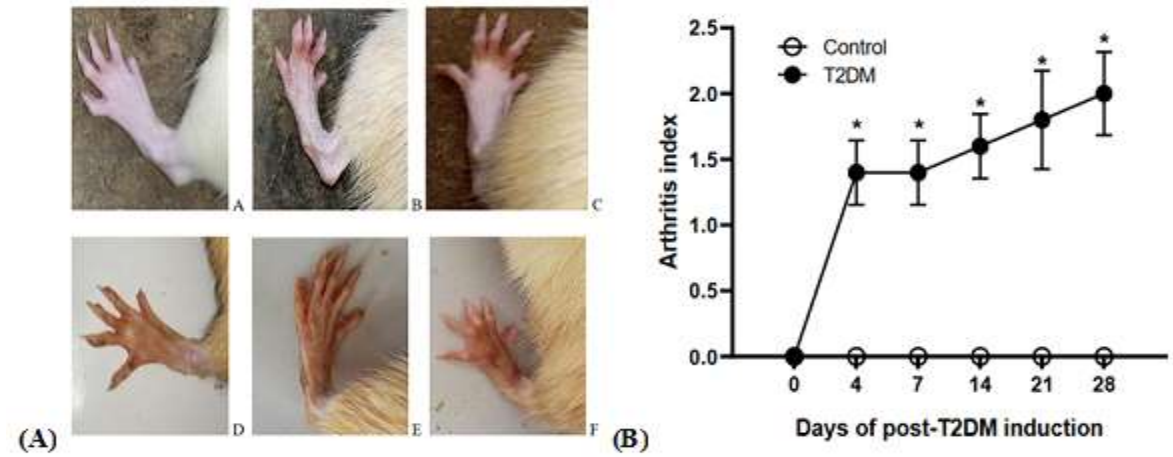

Fig. 6: (A) Photograph of Arthritis index; A = Control (No evidence of erythema and swelling); B – F = T2DM (Erythema and swelling on hind paw; evidence of arthritis). (B) Arthritis index of control and diabetes group; Values are mean±SEM, n=5, P<0.05, *indicates significant differences between control and T2DM group.

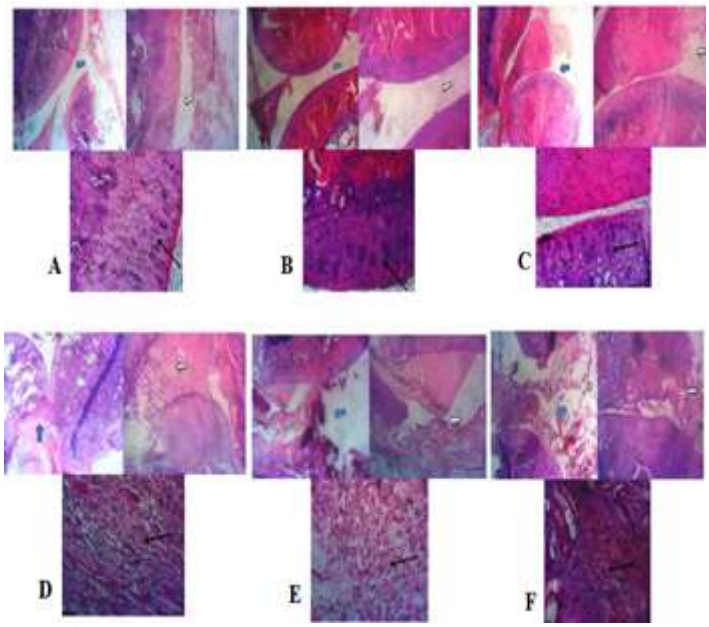

Plate 1: (A-F) Photomicrograph of H & E-stained knee joint showing normal synovial joint surface (blue arrow), synovium without infiltration by inflammatory cells (white arrow), and a well-maintained structure of the articular cartilage (black arrow) in control (Plate A) and at day 4 (Plate B), slight structural change in the synovium (white arrow) (Plate C) at day 7, acute inflammation of the synovial joint (blue arrow), moderate structural derangement and inflammatory infiltration in the synovium (white arrow) and abnormal changes in the articular cartilage structure (black arrow) at day 14 (Plate D). At day 21, severe inflammation, significant structural derangement in the synovial joint (blue arrow), and moderate synovium inflammatory infiltration (white arrow) were noted as well as abnormal articular cartilage structure and loss of cells (black arrow) (Plate E). By day 28, the knee joint displayed severe inflammation, severe structural derangement in the synovial joint (blue arrow), total disorganization of the synovium structure due to high inflammatory infiltration (white arrow), and abnormal articular cartilage architecture with a loss of cells (black arrow) (Plate F).

A=Control; B=Day 4 post-T2DM (Confirmation of T2DM); C=Day 7 post-T2DM induction; D=Day 14 post-T2DM; E=Day 21 post-T2DM; F=Day 28 post-T2DM. Synovial joint surface (blue arrow), synovium (white arrow), articular cartilage (black arrow)

Histological findings and MANKIN osteoarthritis score of control and diabetes group: Histological evaluations on days 4, 7, 14, 21 and 28 revealed a progressive deterioration of the knee joint structure from day 7 following T2DM induction, with increasing synovium derangement, and cartilage abnormalities over time compared to control (Plate 1). MANKIN scores for osteoarthritis showed significant ($P < 0.05$) induction of osteoarthritis in T2DM group on days 14, 21 and 28 compared with control, respectively (Fig. 7). Further suggesting that 14 days post-type II diabetes induction in Wistar rats could predispose to osteoarthritis and give rise to type II diabetes-induced osteoarthritis phenotype.

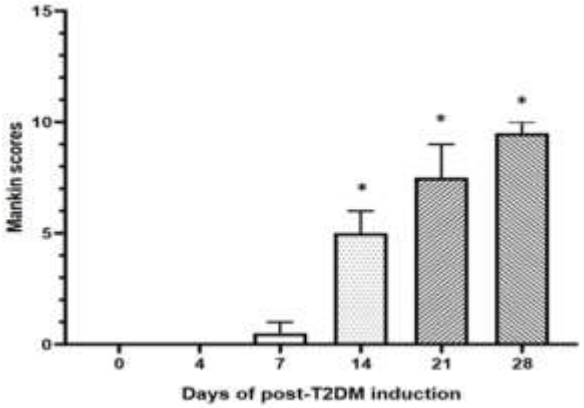

Fig. 7: Mankin scores for osteoarthritis in diabetes group on days 0, 4, 7, 14, 21 and 28. Values are mean±SEM, n=5, P<0.05, *indicates significant differences compared with control.

Conclusion: In conclusion, this study shows an experimental model in Wistar rats that exhibits type II diabetes-induced osteoarthritis phenotype. The osteoarthritis indices observed, increased in severity from days 21 to 28 exhibiting signs of increased morbidity and likely mortality. This study therefore suggests that experimental type II diabetes-induced osteoarthritis occurs in Wistar rats by day 14 post-induction of diabetes using nicotinamide and streptozotocin, with signs and symptoms consistent with diabetes-induced osteoarthritis in human.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from corresponding author.

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