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Biochemical and Histomorphometric Response of Gallic Acid on Gene Expression Levels in Annular Puncture-induced Intervertebral Disc Degeneration using Rabbit Model

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ABSTRACT

Intervertebral disc (IVD) degeneration is a complex multifactorial condition arising from changes in disc morphology, extracellular matrix and neurovascular composition. This study investigated the effect of Gallic acid on the histoarchitecture of the intervertebral disc of rabbit subjected to annular puncture. Twenty (20) New Zealand white rabbits were divided into 4 groups (*n*=5). Group A (non-punctured) received normal saline orally as *placebo*; Group B underwent annular puncture (punctured control group) and received normal saline orally for 8 weeks; Group C received 500 mg/kg bw of Gallic acid orally for 8 weeks immediately after puncture; Group D received 500 mg/kg bw of Gallic acid orally after 4 weeks post-puncture for 4 weeks. After the experimental period, the disc height index, histomorphometric studies, biochemical analysis, gene expression for collagen type I, collagen type II, Aggrecan and Matrix Metaloproteinase-13, and expression level of Bax were measured and analysed. There was a significant difference in disc height index among the punctured-treated groups compared to the punctured non-treated group. The morphology of the intervertebral disc was preserved and significant restoration in the structural architecture of both the nucleus pulposus and annulus fibrosus was observed in group C compared to group B. However, the oxidative stress markers (catalase, superoxide dismutase and glutathione) collagen type II and aggrecan levels were significantly increased with a corresponding decrease in malondialdehyde, collagen type I, Matrix Metaloproteinase-13, and Bcl-2-associated X protein (Bax) levels. Gallic Acid administration prevents and restores annular punctured disc degeneration attributed to its antioxidant and anti-inflammatory potential.

Keywords: gallic acid, intervertebral disc degeneration, gene expression, antioxidant, histology

INTRODUCTION

Low back pain causes more global disability than any other condition in the world involving huge financial burden due to the cost of working, time losses and medical treatment [1]. At some stage in life, about 80% of people usually experience low back pain, with prevalence ranging from 15% to 45% [2]. Degenerative lumbar disc disease often induces discogenic pain which ultimately leads to chronic low back pain [3] differs from radicular pain because of disc prolapse whose pain originates from nerve root compression [1]. Lumbar disc degeneration is a complex process arising from changes in the intervertebral disc's cellular, matrix, endplate, and neurovascular components [3]. In an attempt to address the pathogenesis and treatment modalities, several animal models have been established that significantly contribute to the etiology of disc degeneration and the disease burden of low back pain. The similarities in the morphological and developmental composition of the intervertebral disc with the underlying pathophysiology are a prerequisite for comparing and contrasting the merits of different models [1].

The intervertebral disc is the largest avascular complex multicomponent tissue composed of an external fibrous ring, the Annulus Fibrosus (AF), and an internal hydrated gel-like material, the Nucleus pulposus (nucleus pulposus) and cartilaginous Vertebral End Plate (VEP) [4]. The annulus fibrosus is a fibrocartilaginous tissue rich in the collagen of type I and II and assembled in different locations and species as lamellae fibers oriented at varying degrees to adjacent lamella [5]. The main cell types of the AF are fibroblasts that synthesize not only the lamellar collagen, but also proteoglycan (PGs), elastin, and other non-collagenous proteins [6]. The annulus fibrosus's tough fibrous composite structure encapsulates the gelatinous nucleus pulposus and offers the mechanical strength and resilience needed to enable the disc to rebound from axial, rotational and bending loading deformations. In healthy discs, the nucleus pulposus consists of a large amount of proteoglycan and hydrated gel mainly of the type II collagen [7]. Aggrecan is the most abundant form of proteoglycan in the nucleus pulposus but due to its high anionic charge, it consumes and retains high water molecules within the nucleus pulposus thereby maintaining a high hydrostatic swelling pressure that offers resistance to disc deformation and disc height maintenance [4, 7].

During development, the notochord differentiates into the cells of the nucleus pulposus which are present in humans throughout childhood, but disappear with maturity and are replaced by cells similar to chondrocytes [6]. Nucleus pulposus notochordal cell loss is an important early phase of degenerative disc disease [1].

Degeneration of the intervertebral disc is a major cause of lower back pain affecting major socio-economic impact in Western societies [8]. Human intervertebral disc degeneration is a complex and incompletely known multifactorial process involving mechanical stresses, gene contributions, cellular senescence, and nutritional changes due to limited vascular supply [9]. Since only the outermost layers of the annulus fibrosus contain blood vessels, the nucleus pulposus cells rely on the diffusion of capillary bud nutrients in the cartilaginous endplate to fulfil their metabolic needs [10]. Cells in the nucleus pulposus are therefore metabolically impaired by inadequate vascular and nutrient supply thereby resulting in intervertebral disc degeneration characterized by endplate calcification, distortion in morphological structure and microvascular disease [10].

Oxidative stress, induced by overproduction and aggregation of free radicals, is the leading cause of several degenerative diseases such as cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases [11]. Polyphenols are an essential group of naturally occurring antioxidants, with numerous biological functions, such as antifungal, anticancer, antibacterial, anticholesterol, antibacterial, antiulcer and antibacterial [11]. Gallic acid (3, 4, 5-trihydroxy-benzoic acid) ($C_6H_2(OH)_3COOH$) is a type of phenolic acid found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants [12]. It is a naturally emerging low molecular weight triphenolic substance with potent antioxidant properties and acts as an active triggering agent for apoptosis [11]. Gallic acid derivatives have been used in various phytomedicines with a range of biological and pharmacological roles, including cancer cell apoptosis, free radical scavenging, and pathways for cell signaling [11]. The connections between its

prooxidant and antioxidant potentials are attributed to the wide range of gallic acid uses [11]. Gallic acid is a class of polyphenols present in tea and grapes and is widely available in nature [13].

Gallic acid can be used as a catalyst in the synthesis of experimental alkaloid mescaline and the pharmaceutical industries as a method in the Folin-Ciocalteau test for evaluating the phenol content of specific analyses [14, 15]. Its compounds are used as antioxidants in several food and pharmaceutical industries, because they exhibit certain properties, such as anti-inflammation, antitumor, antioxidation, and bacteria inhibition [16]. Gallic acid has been observed to have an anti-inflammatory effect on nucleus pulposus cells [16]. This study therefore aimed at investigating the antioxidant and anti-inflammatory effects of Gallic Acid on the histomorphometric and gene expression levels of annular puncture-induced intervertebral disc degeneration in rabbit models.

MATERIALS AND METHODS

Drugs/chemicals

Gallic acid was obtained from Sigma Company (St. Louis, MO, USA) with 99% purity, dissolved in distilled water and given orally in a dose of 500 mg/kg. All other chemicals used in this study were of analytical grade.

Care and maintenance of animals

Twenty (20) 5-month-old adult New Zealand white Rabbits (*n*=5) (weighing between 1.5-2.0 kg) were used in this study after being authenticated in the Department of Animal Production and Health of the Federal University of Technology, Akure. The rabbits were then cautiously evaluated to be free of any diseased condition before the commencement of the experiment by physical observation after two weeks of acclimatization. The rabbits were fed with growers' marsh (pellets), purchased from a feed store- Agro feeds and flour mills, and water during the period of the experiment. The rabbits went through an acclimatization period of 14 days. The processes of protocols for using experimental animals followed the Guide for the Care and Use of Laboratory Animals and ethical approval of the Center for Research and Development of the Federal University of Technology, Akure (FUTA/ETH/24/147).

Surgical techniques and experimental protocol

The surgical technique was performed using the Young-Joon [17] procedure. Briefly before the procedure, each rabbit was anaesthetized with intramuscular injection of ketamine (35 mg/kg body weight), followed by diazepam (5 mg/kg body weight). The furs were shaved from the mid back and right flank. The rabbits were then placed in the lateral oblique prone position, and the injection field was sterilized with an alcohol sponge. Initially, the L5-L6 disc was identified through manual palpation of the interspinous space from the mid back and pelvic rim. A 5 cm lateral approach between the iliac crest and the last rib was performed. Blunt dissection of muscles and retroperitoneal exposure of the entire lumbar spine, with further dissection of the three most caudal intervertebral discs (L3/L4 to L5/6), was performed. The first two lumbar discs (L1/L2 and L2/L3) were left without dissection for control purposes. The three most caudal intervertebral discs were punctured manually three times with a 21-gauge needle with 360^0 clockwise rotation and were kept in each position for 30 seconds. After the annular puncture, the retroperitoneal space was irrigated and washed with saline solution, and the muscle layers and skin were then sutured back with chromic and silk sutures respectively. The rabbits were administered antibiotics to prevent infections in the surgical areas and then placed in their cages after close observation for recovery and ambulation.

Experimental design

This experiment was divided into four groups each containing five animals.

- Group A received distilled water for 8 weeks (Positive control group) (non-punctured group)
- Group B received distilled water for 8 weeks immediately after puncture (Negative control group) (punctured group).

- Group C received oral administration (via oral cannula) of 500 mg/kg bw of Gallic acid for 8 weeks (non-punctured).
- Group D received oral administration (via oral cannula) of 500 mg/kg bw of Gallic acid for 4 weeks after 4 weeks post-puncture of the intervertebral disc.

The rabbits were sacrificed after 8 weeks of the experiment. Upon sacrifice, the discs were extracted and histological, collagen fibers, and biochemical analysis were performed. All the animals were sacrificed by cervical dislocation.

Determination of Percentage Disc Height Index

Lateral plain radiographs of the lumbar spine were scanned and digitally stored with a radiograph machine by a Veterinary Radiologist (collimator-to-film distance, 50 cm; exposure, 5 mAs; penetration power, 44 kVp). During the radiographs, special care was taken to minimize axial rotation of the disc space by holding the rabbit in the lateral decubitus position while ensuring the X-ray beam was maintained straight. In addition, each rabbit was treated with a consistent amount of ketamine (35 mg/kg) and an intramuscular injection of xylazine (5 mg/kg) to provide a similar degree of muscle relaxation to minimize differences in disc height. Vertebral body height and disc height were measured using Scion Imaging Software 4.0 (Scion Corporation, Chicago, USA) and analyzed using an image analysis program (Image J) (National Institutes of Health, USA).

The intervertebral disc height was expressed as the disc height index using the method by Masuda *et al.*, The average intervertebral disc height was calculated using measurements obtained from the anterior, middle, and posterior portions of the intervertebral disc and was divided by the average of adjacent vertebral body heights. Changes in the disc height index (DHI) were expressed as percentage disc height index and normalized to the measured pre-operative intervertebral disc height (percentage DHI=post-operation DHI/pre-operation DHI×100).

Biochemical assay

The blood from the animal was put in a homogenizer with 1 ml of 5% sucrose solution and homogenized properly. Iintervertebral disc tissue was homogenized and centrifuged accordingly for biochemical analysis. The homogenates were collected in a 5 ml plain serum bottle for enzyme assay; superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione (GSH) levels.

An assay of superoxide dismutase activity in the intervertebral disc was determined according to the method described by Nishikimi *et al.* [19]. Assay of catalase activity in the intervertebral disc was obtained by the method described by Sinha, [20]. An assay of intervertebral disc reduced glutathione concentration in blood was done by the method of Beutler *et al.* [21]. Lipid peroxidation was evaluated based on Malondialdehyde level, MDA in RBCs was determined using the method described by Stocks and Donnandy [22].

Determination of expression levels of protein (Immunohistochemical analysis)

The expression levels of Bax protein were determined using Le Maitre *et al.*, [23] protocol. Representative intervertebral disc degeneration areas were chosen based on the staining of the tissue sections with Hematoxylin and Eosin. Formalin-fixed, paraffin-embedded 3 μm thick sections were de-paraffinized and rehydrated. Endogenous peroxidase activity was blocked by incubating the sections with 3 % H_2O_2 for 10 min followed by digestion with 0.01 % protease K for 10 min. Non-specific binding sites were blocked by incubation with confining liquid for 10 min after which the sections were incubated with rat polyclonal antibody to Bax (Cell Signaling Inc., Danvers, MA) at 4 °C for 12 h. After thorough washing, the sections were incubated with biotinylated goat anti-rabbit IgG at 4 °C for 60 min and then in Streptavidin-HRP for 10 min. The final colour reaction was developed by incubation with the chromogenic substrate 3, 3'-diaminobenzidine (0.5 mg/mL in Tris). The sections were counterstained with Hematoxylin and mounted for examination

with an O-max microscope coupled to Image J software.

Statistical analysis

Data were analysed using Statistical Package for the Social Sciences version 24.0 computer soft package (SPSS Inc.; Chicago U.S.A.). Longitudinal X-ray data were analysed using two-way analysis of variance (ANOVA) followed by multiple comparisons using the Bonferroni method. Quantitative real-time data were analyzed using a student t-test. Data were presented as mean ± standard error of the mean. The level of significance was considered at $p< 0.05$.

RESULTS

Radiological analysis

The percentage disc height index of the punctured group showed a significant decrease compared to the non-punctured group (group A) ($p< 0.05$) (Fig.1). The reduction of the disc height observed in this result observed degenerative changes of the intervertebral disc after 4 weeks of puncture. However, there was a significant increase in the % disc height index of the group administered with gallic acid for 4 weeks after puncture for 4 weeks (group D) compared with the punctured group that received distilled water (Group B) ($p< 0.05$) (Fig.1). Although, the administration of Gallic acid immediately after puncture for 8 weeks (group C) showed no significant difference in the % disc height index compared with non-punctured group (group A) (Fig.1).

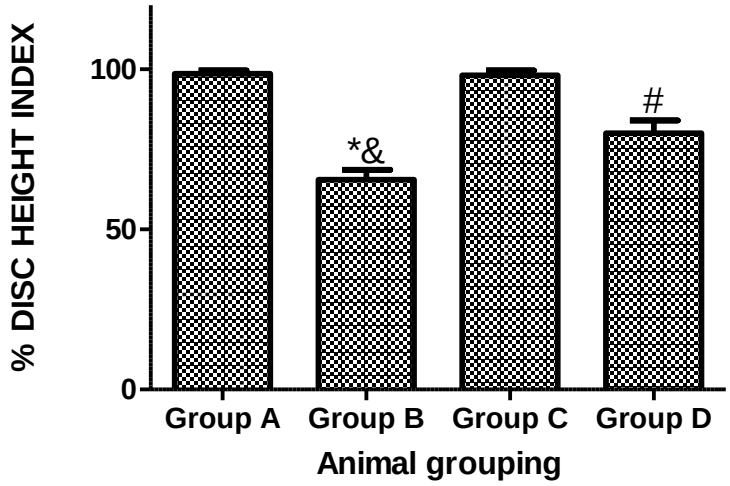

Figure 1: Effect of GA on percentage disc height index (%DHI) in annular punctured induced disc degeneration in rabbit (n=5). **: p < 0.05 as compared to group A; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and C.*

Histomorphological outcome

The photomicrograph of the punctured group that received distilled water (Group B) showed visible fissures within the disc morphology with the absence of chondrocytes-like cells in the nucleus pulposus and disorganization of the architectural arrangement of collagen fibres within the inner and outer layers annulus fibrosus and less distinct demarcation in the border between the annulus fibrosus and nucleus pulposus when compared with non-punctured disc (Group A). These observations are distinguishing characteristics of a degenerating intervertebral disc. However, the photomicrograph

of the punctured group that received GA after 4 weeks of puncture (Group D) showed notable restoration in the morphology of the disc produced as a result of degeneration by re-organizing the collagen fibers arrangement and the presence of few chondrocytes like cells within the nucleus pulposus compared with the punctured group that received distilled water (Group B).

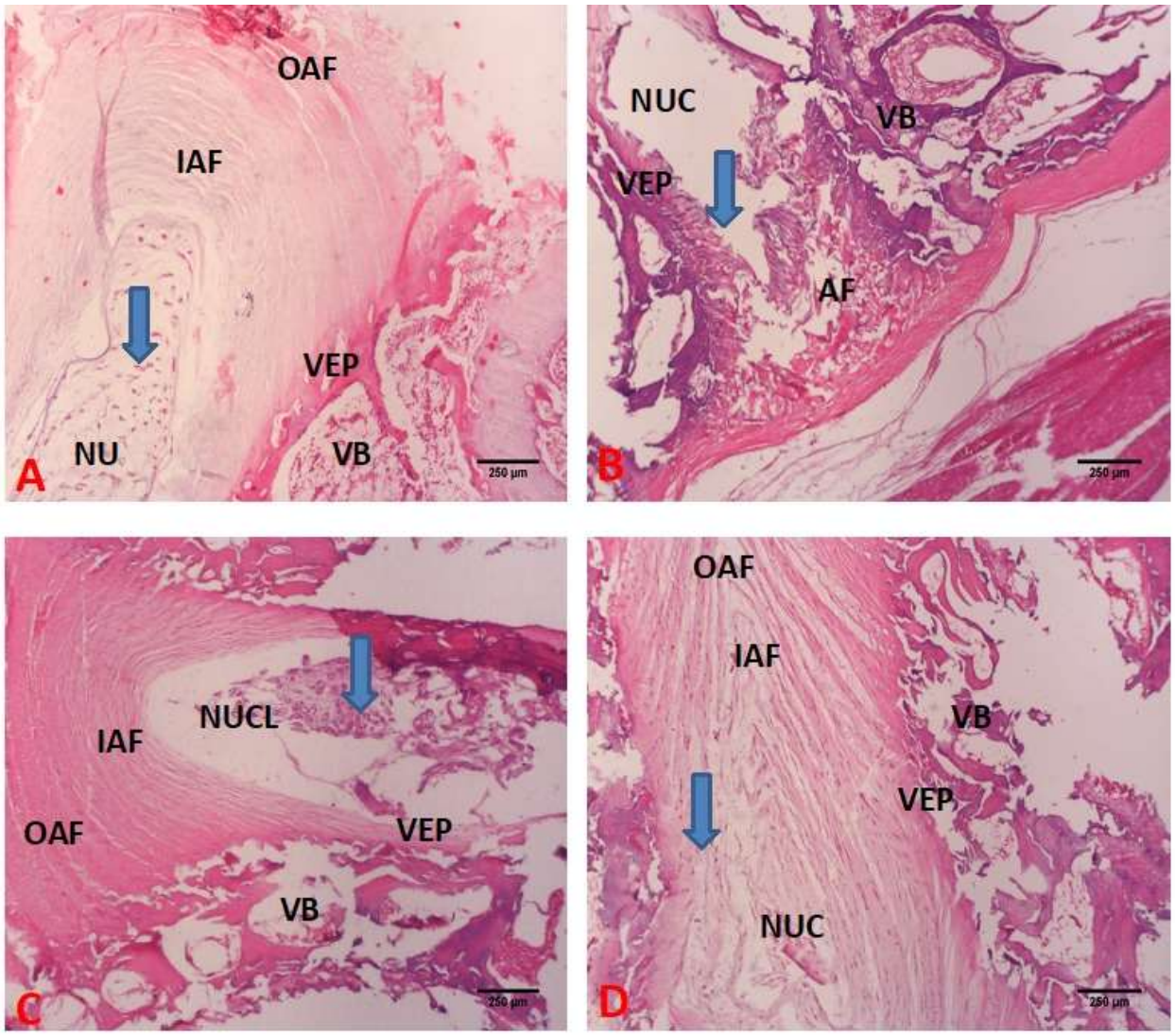

Figure 2: Photomicrograph of intervertebral disc histomorphology in rabbits administered with Gallic acid in the punctured and non-punctured treated groups. Group A showed no pathological intervertebral disc morphology with numerous chondrocyte-like cells in the NP (arrow), intact collagen fibers organization within the inner annulus fibrosus (IAF) and outer annulus fibrosus (OAF); Group B showed the presence of fissure (arrow), absence of chondrocyte-like cells in the nucleus pulposus and disorganization of fibers within the inner annulus fibrosus and outer annulus fibrosus; Group C showed normal morphology of the intervertebral disc similar to Group A; Group D showed restored chondrocyte-like cells (arrow) in the nucleus pulposus, restored collagen fibers organization within the inner annulus fibrosus and outer annulus fibrosus.

CLCs: chondrocytes-like cells; VB: Vertebral Bone; VEP-Vertebral End Plate; NP- Nucleus pulposus; IAF: Inner Annulus Fibrosus; OAF: Outer Annulus Fibrosus; VB: Vertebral Bone. H and E: x100.

Histological grading scores

The histological grading score as previously described by Boos *et al.,* [24] was used to ascertain the degree of degeneration of the punctured intervertebral disc within the photomicrograph. The level of degeneration varied from 4 (normal) to 14 (severe degeneration). In this present study, the punctured group that received distilled water showed an increase in the grading score (10.5) compared with the control (5) (Fig. iii). In addition, the punctured group that received GA immediately after puncture for 8 weeks (group C) showed no difference in the grading scores compared with the control (5). However, the histological grading score within the group that received GA 4 weeks after puncture for 4 weeks was lower compared with group B (Fig. iii). However, the grading score of the group that received gallic acid for 4 weeks after 4 weeks post-puncture was higher compared with groups A and C respectively (Fig. iii).

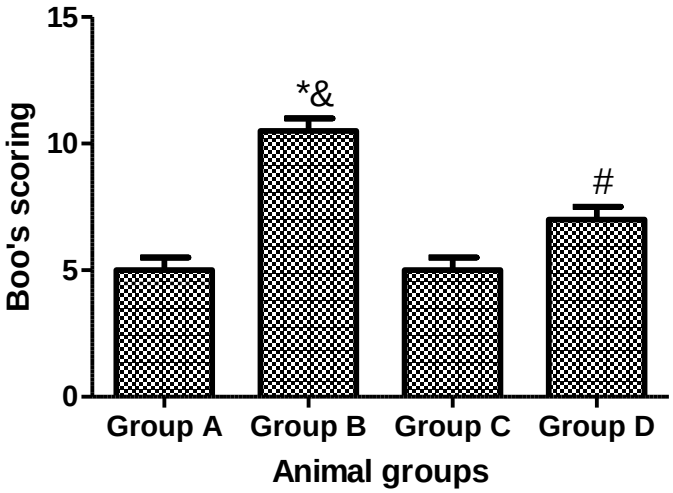

Figure 3: Effect of Gallic Acid on histological grading score in annular punctured induced disc degeneration in rabbit (n=5). *: $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to groups A and C.

Morphometric analysis of chondrocyte-like cells on intervertebral disc

One of the major predictive features to assess the level of intervertebral disc degeneration is the number of chondrocyte-like cells within the annulus fibrosus (inner and outer) and nucleus pulposus. In this present study, the punctured group that received distilled water (group B) showed a significant decrease in the chondrocyte-like cells within the nucleus pulposus and inner annulus fibrosus compared to the control ($p< 0.05$) (Fig.3). The number of chondrocyte-like cells within the nucleus pulposus and inner annulus fibrosus in the punctured group that received gallic acid immediately after puncture for 8 weeks (group C) showed no significant difference compared to the control (group A). Although, the increase in number of chondrocyte-like cells in the nucleus pulposus and inner annulus fibrosus of group D was significant compared to group C and A ($p< 0.05$) (Fig.3). However, there was no significant difference in the number of chondrocyte-like cells in the outer annulus fibrosus across all the groups.

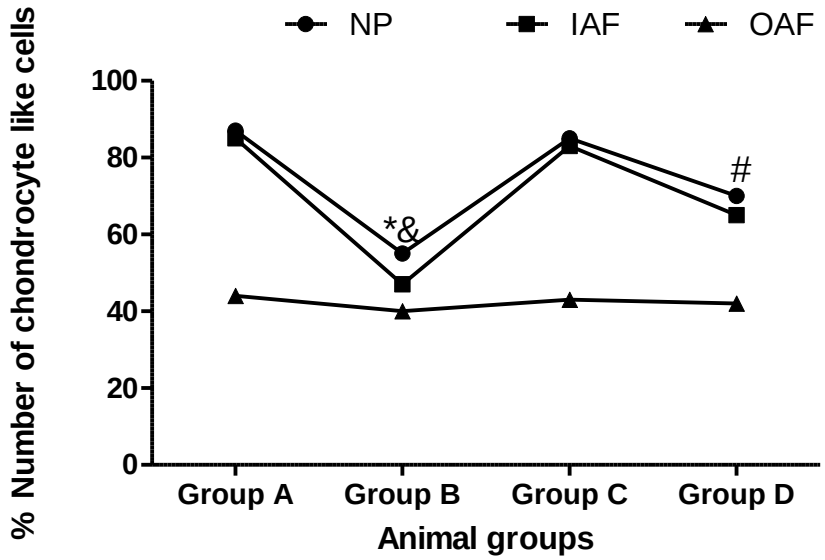

Figure 4: Effect of Gallic Acid on morphometric analysis of chondrocyte-like cells in annular punctured induced disc degeneration in rabbit (n=5). **: p < 0.05 as compared to group A; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and C.*

Biochemical Evaluations of Antioxidant markers

The result revealed that the levels of CAT, SOD and GSH were significantly decreased with a corresponding increase in the level of MDA among the punctured group that received distilled water (group B) compared to the control (group A) ($p< 0.05$) (Fig.6). In addition, there was a significant

increase in the levels of CAT, SOD and GSH with a corresponding decrease in MDA level among the group that received gallic acid for 4 weeks after puncture for 4 weeks (group D) compared to the punctured group that received distilled water (group B) ($p< 0.05$) (Fig.6). However, the treated group that received gallic acid immediately after puncture for 8 weeks (group C) showed similar levels of CAT, SOD, GSH and MDA compared to the non-punctured control (Group A) (Fig. 6).

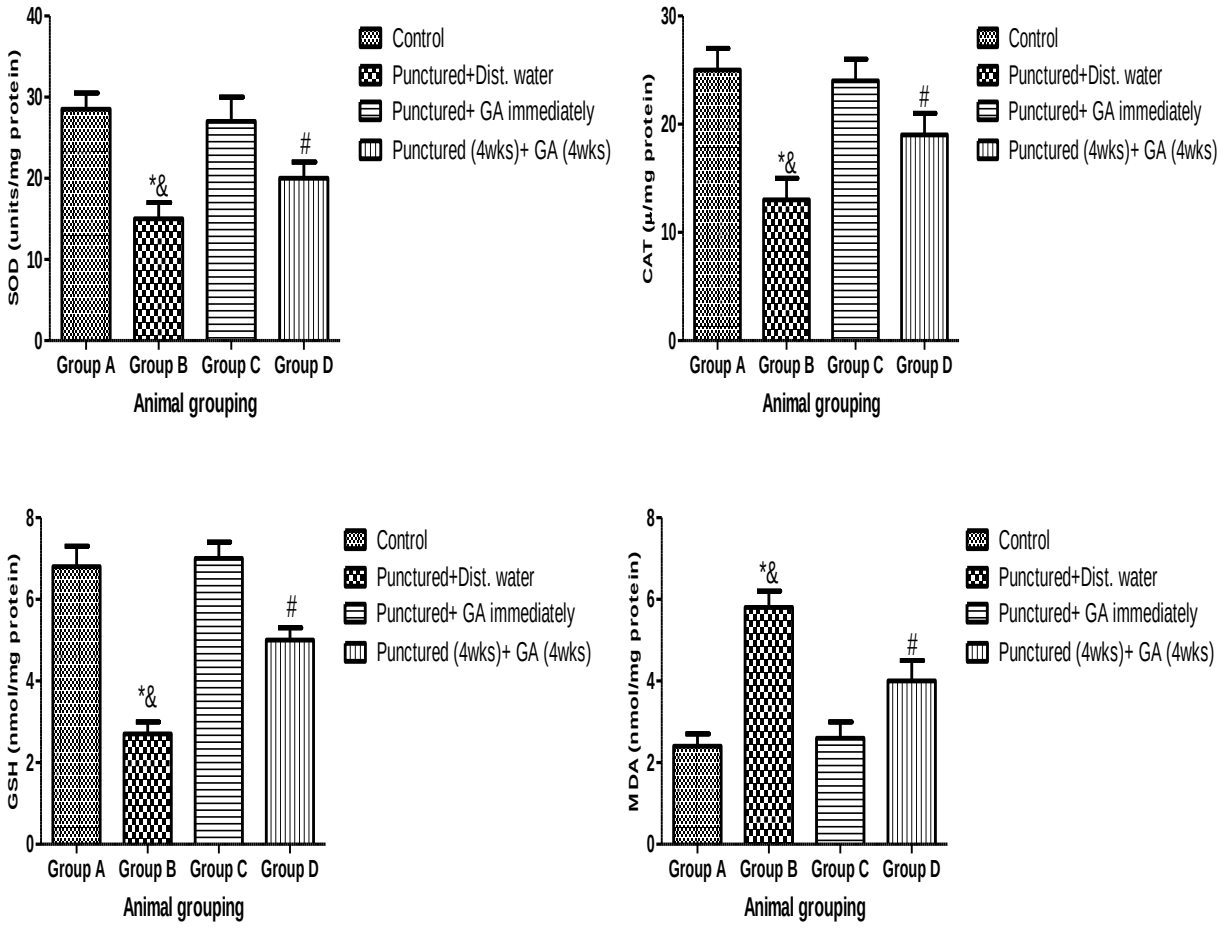

Figure 5: Effect of Gallic Acid on Antioxidant Enzymes; catalase level in annular punctured induced disc degeneration in rabbit (n=5); * $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to groups A and C

Gene expression levels outcome

The present study showed that the gene expression levels of collagen type I and matrix metalloproteinase -13 were significantly elevated with corresponding decline in expression levels of aggrecan and collagen type-II within the punctured group that received distilled water (group B) compared to the non-punctured control group (Group A) ($p< 0.05$) (Fig.4). In addition, the group that received gallic acid for 4 weeks after puncture for 4 weeks (Group D) showed that the gene expression levels of collagen type I and matrix metalloproteinase -13 were significantly decrease with corresponding increase in expression levels of aggrecan and collagen type-II compared to punctured group that received distilled water (group B) ($p< 0.05$) (Fig.4). Although, the gene expression levels of collagen type I and matrix metalloproteinase -13 were significantly decrease with corresponding increase in the expression levels of aggrecan and collagen type-II in the group that received gallic acid for 4 weeks after puncture for 4 weeks compared to the non-punctured control group (group A) and the group that received gallic acid immediately after puncture for 8 weeks (group C) ($p< 0.05$) (Fig.4). However, there was no significant difference in the relative gene expression levels

among the group that received gallic acid immediately after puncture for 8 weeks (group C) compared to the non-punctured control group (group A).

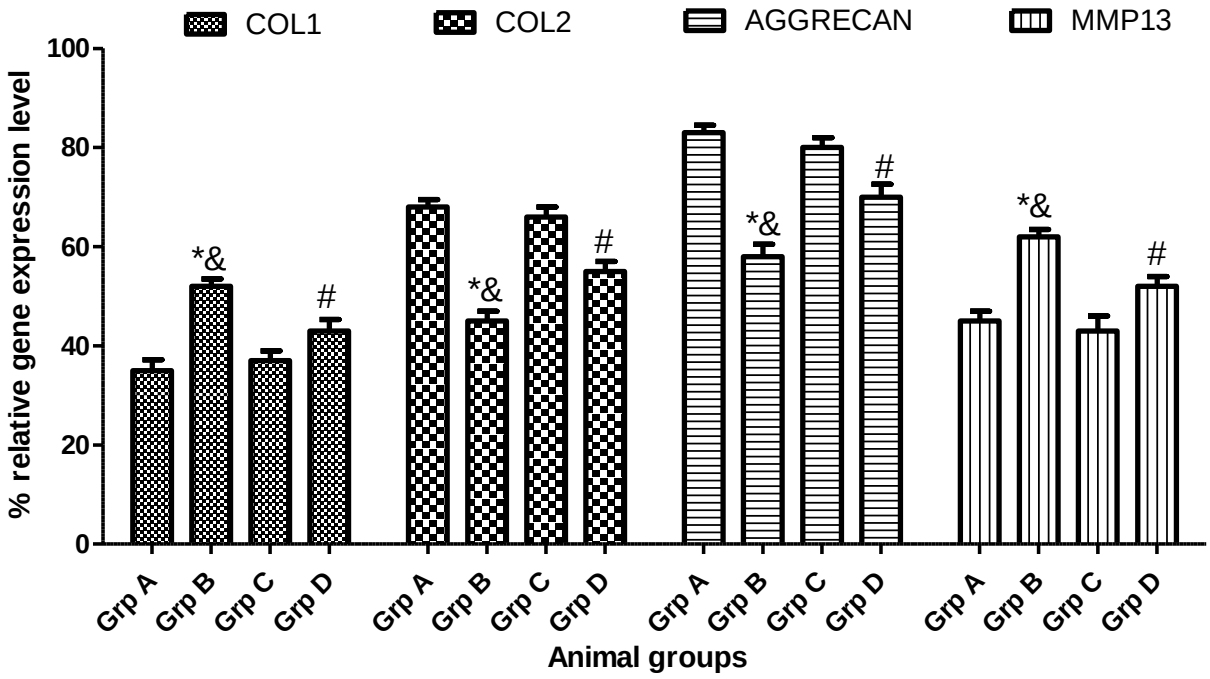

Figure 6: Effect of Gallic Acid on levels of gene expression in annular punctured induced disc degeneration in rabbit (n=5). *: $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to groups A and C.

Immunohistochemical analysis

The immunohistochemical analysis in this present study showed that Bax expression level was significantly higher within the punctured group that received distilled water (group B) compared to the control (group A) ($p< 0.05$) (Fig.5). In addition, the expression level of Bac in the group that received gallic acid for a duration of 4weeks after 4 weeks post-puncture (group D) was significantly decreased compared to the punctured group that received distilled water (group B) ($p< 0.05$) (Fig.5). However, the treated group that received gallic acid immediately after puncture for 8 weeks (group C) showed similar expression level of Bax compared to the non-punctured control group (group A) (Fig. 5).

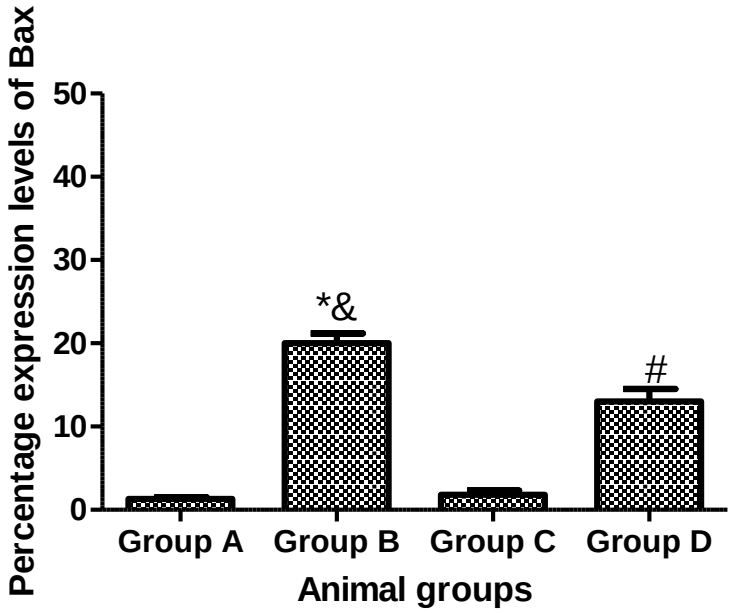

Figure 7: Effect of Gallic Acid on expression levels of Bax in annular punctured induced disc degeneration in rabbit (n=5). *: $p < 0.05$ as compared to group A; &: $p < 0.05$ as compared to group C; #: $p < 0.05$ as compared to groups A and C.

DISCUSSION

The annular puncture model of disc degeneration has been reported to induce early and evident degenerative signs similar to characteristics observed in human degenerated discs [9]. This model is relatively simple, cost economical and reproducible allowing researchers to investigate the pathogenesis of intervertebral disc degeneration [18]. Radiological and histomorphological outcomes have been described as key tools in validating the annular puncture model by strongly predicting the severity of disc degeneration [25]. In this present, Radiological assessment using X-rays revealed that the percentage disc height index of the punctured group showed a significant decrease compared to the non-punctured group. The reduction of the disc height observed in this study deduces degenerative changes of the intervertebral disc after 4 weeks of puncture. The annular puncture model has been reported to cause a slow progressive irreversible disc degeneration starting from 2 weeks till at least 8 weeks post-surgery similar to human intervertebral disc degeneration [7, 25]. However, the administration of gallic acid immediately after puncture showed no significant difference in the percentage disc height index compared with the non-punctured group suggesting the preventive ability of gallic acid in the narrowing of the disc height after puncture. The present study also observed that the percentage disc height index of the group administered with gallic acid 4 weeks after puncture for another 4 weeks showed a significant improvement in the percentage disc height index compared to the punctured group that received distilled water only. It could be deduced that gallic acid can preserve and restore the narrowing of the disc and its components thereby maintaining the shock-absorbing function of the disc since previous observation has revealed that gallic acid serves as an antioxidant agent by protecting the cell against oxidative damage.

The morphological integrity of the punctured group in this present study showed several distortions similar to degenerated discs in humans ranging from disruption and expulsion of nucleus pulposus contents, decrease in disc height, disorganization in

the architecture and border between the annulus fibrosus and nucleus pulposus, decrease /absence of chondrocyte-like-cells in the nucleus pulposus and complete obliteration of cavity compared with the control and gallic acid only group. However, administration of gallic acid four weeks after puncture revealed that the morphology and structural conformation of the disc tissue was restored significantly compared to the punctured group that received distilled water only. The histological observation was supported by the morphometric analysis results and level of degeneration as evaluated using the grading score in this present study. Quantification of the number of chondrocyte-like cells in the annulus fibrosus and nucleus pulposus of the punctured rabbits that received distilled water was significantly decreased compared with the non-punctured control group supporting the distortion and pathological outcome of the histomorphology results. However, the treated groups that received gallic acid after four weeks of puncture showed a significant increase in the number of chondrocyte-like cells compared with the punctured control group that received distilled water. Furthermore, the severity of degeneration using the grading score in this present study showed a significant increase in the extent of degeneration of the punctured group that received distilled water only compared with the control thereby ascertaining the histological and morphometric quantification results of degenerated disc characteristics observed in this study. In addition, there was a significant decrease in the severity of degeneration after treatment with gallic acid compared with the punctured control groups that received distilled water suggesting the restorative attribute of gallic acid in reversing the ongoing degeneration process in the disc tissue probably due to its anti-inflammatory and antioxidant properties.

The alteration of the structural organization of the disc tissue especially within the nucleus pulposus was initiated by hydration. Alteration in the nucleus pulposus cell phenotype was carried out on the expression levels of molecules that were previously shown to be modulated during the onset of intervertebral disc degeneration or osteoarthritis (collagen type I, matrix metalloproteinase -13, aggrecan and collagen type-II. The corresponding transcript levels by real-time polymerase chain reaction analysis were done to evaluate the expression of the molecules. The present study showed a significant increase in collagen type-II and aggrecan expression with a corresponding decrease in collagen type I and matrix metalloproteinase -13 in the gallic acid-treated group compared to the punctured group that received distilled water suggesting that nucleus pulposus cells undergo a process of dedifferentiation previously described in cultured articular chondrocytes and osteoarthritic joints [30]. Previous studies showed that matrix metalloproteinase -13 is known to degrade collagens and glycosaminoglycans [31]. Therefore, an increase in matrix metalloproteinase -13 could be a major contributor to intervertebral disc degeneration previously reported in cartilage degradation during osteoarthritis [28].

The pro-oxidant and antioxidant characteristics of gallic acid have been linked to its anticancer and apoptosis-inducing properties [32]. It was reported that gallic acid the cellular integrity of Reactive Oxygen Species (ROS) was modulated in a dose-dependent and time-dependent manner since an increased level of ROS could result in mitochondrial potential loss, the release of cytochrome c and caspases 3, 8, and 9-activation [33]. In this present study, the Bax expression level was significantly increased within the punctured group that received distilled water compared to the control. However, the expression level of Bax in the group that received gallic acid for 4 weeks after 4 weeks post-puncture was significantly decreased compared to the punctured group that received distilled water thereby attenuating apoptosis, inflammation, and matrix degradation in chondrocyte cells within the nucleus pulposus. Therefore, the treatment of gallic acid could effectively kill chondrocyte-like cells through the process of apoptosis. The quality control mechanism of apoptosis helps in the maintenance of the internal environment of the tissue by removing defective cells. These defective cells usually undergo apoptosis either through the death receptor pathway or the mitochondrial pathway [34]. The pro-apoptotic Bcl-2-associated X protein (Bax) induces permeabilization of the mitochondrial membrane, the release of cytochrome c and ultimately initiates activation of caspase-9 which eventually activates effector caspase-3 leading to apoptosis.

Previous studies have revealed the antioxidant and neuroprotective role of gallic acid in the involvement of antagonist receptor activation, thereby preventing neurotoxicity and/or excitotoxicity as a result of brain injury [35, 36]. The present study showed a significant decrease in the levels of CAT, SOD and GSH with a corresponding increase in MDA level in the punctured group that received distilled water compared to the control group. It can be deduced that annular puncture significantly decreased tissue antioxidant levels due to increased reactive oxygen species. However, treatment with gallic acid restored the antioxidant enzyme levels compared to the punctured group that received distilled water only. Previous observations showed that polyphenolic acids (such as gallic acid) significantly elevate the levels of expression of antioxidant enzymes and their activities in the brain[37]. Furthermore, it has been proven that gallic acid exerts its neuroprotective and antitumor properties by altering antioxidant/pro-oxidant balance [38] capable of controlling ROS-induced toxicity through an increase in the levels of antioxidant enzymes [38]. Since ROS initiate pro-inflammatory pathways thereby deteriorating the deleterious oxidized environment [39], the use of gallic acid is important by scavenging free radical molecules inhibiting lipid peroxidation, and stimulating the activity of endogenous antioxidant agents.

CONCLUSION

The use of Gallic Acid showed a restorative and preventive potential on puncture-induced intervertebral disc degeneration by enhancing the synthesis of matrix proteoglycan, ameliorating antioxidant enzyme levels and restoring the disorganized structural architecture of the discs. These results validate the use of Gallic Acid as a potential supporting regimen in the treatment of degenerative disc disease attributed to low back pain.

Ethics approval

The experimental procedures were conducted following the NIH guidelines for the care and use of laboratory animals in line with guidelines Health Research and Ethical Committee of the College of Medicine, University of Lagos and ethical approval of the Center for Research and Development of the Federal University of Technology, Akure (FUTA/ETH/24/147).

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