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Elevated reproductive toxicity effects of diclofenac after withdrawal: Investigation of the therapeutic role of melatonin

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ABSTRACT

Although there are several reports on the toxic actions of sodium diclofenac (DF), there is dearth information on its effect on the male reproductive system. Therefore, the study investigated the effects of DF and melatonin in male rats. Twenty rats were used in this study, which lasted for 6 weeks. The control group (vehicle treated) received normal saline (0.1 ml/day, *p.o.*). In the experimental groups, DF was administered during the first (group 2) and last (group 3) three weeks of the study. However, in group 4, melatonin was administered for 3 weeks, after 3 weeks of treatment with DF. DF and melatonin were administered at 1 and 10 mg/kg b.w./day (*p.o.*) respectively. The results showed that unlike melatonin, DF had no effect on gonadotrophins; however, it caused significant decreases in GNRH and testosterone, but a significant increase in prolactin. Melatonin attenuated the pro-antioxidant and pro-inflammatory effects of DF, which caused significant decreases in SOD, TAC, CAT, but significant elevations in LDH, MDA, uric acid and CRP. Moreover, the hormone reversed the adverse effect of DF on sperm count, sperm motility and sperm morphology. There were slight evidence of the precipitation of imbalance in lipid metabolism by DF and the antidyslipidaemic action of melatonin. Compared to DF, DF recovery showed more adverse effects on prolactin, testosterone, LDH, MDA, UA, CRP, semen parameters (except sperm motility), TC, LDL-c, HDL-c and phospholipid. The histological results agreed with the biochemical assays. In conclusion, the reproductive toxicity effects of DF seem to escalate after withdrawal; however, these effects could be attenuated by treatment with melatonin.

1. Introduction

The clinical relevance of diclofenac (2-[2,6-dichloranilino] phenylacetic acid) cannot be underestimated. The drug is a well-known non-steroidal anti-inflammatory, analgesic, and antipyretic agent [1], which has been widely used in the management of several chronic disease conditions, such as degenerative joint disease, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis [2,3], actinic keratosis [4], among others. Like other NSAIDs, diclofenac acts by inhibiting the synthesis of prostaglandin via attenuating cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) production with comparable equipotency [5]. It was documented that diclofenac is about 3–1000 times more effective on a molar basis, relative to other NSAIDs, in its ability to inhibit the activity of COX [6]. The administration of the drug has been associated with neurotoxicity [7], hepatotoxicity [8–10], nephrotoxicity [11], and recently, reproductive damage [12], to mention a few.

Sperm are particularly susceptible to reactive oxygen species (ROS) - induced damages because they don't have DNA repair mechanisms.

Asides, they possess low levels of cytoplasmic antioxidant enzymes and contain high levels of polyunsaturated fatty acids [13,14]. Nevertheless, small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities [15]. Although diclofenac is an anti-inflammatory agent, its administration has been associated with an imbalance in the antioxidant enzyme system and hence the instigation of oxidative stress [10,16]. ROS stimulates the activation of nuclear factor kappa-B (NF- κ B) [17], which up-regulates the production of inflammatory cytokines, such as tumor necrosis factor- α , interleukin-1 β (IL-1 β) [18], *etc.* Studies have shown that inflammatory cytokines elevate plasma free fatty acids levels, resulting to dyslipidaemia - a condition that has been linked to male infertility [19]. Therefore, any pharmacological agent with antioxidant, anti-inflammatory, and antidyslipidaemic effects could have therapeutic value in promoting fertility in subjects with compromised physiological status.

There are strong evidence that melatonin (N-acetyl-5-methoxytryptamine) has antioxidant [20], anti-inflammatory [21], and antidyslipidaemic [22] effects. Specifically, the hormone has been reported

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to show protective effects in testis damaged by chemotherapy [23], testicular torsion [24] etc. By binding to its receptors in the testes, melatonin directly regulates testicular functions [25] and testosterone secretion [24]. Apart from the testes, melatonin receptors have been found in hypothalamic neurons, which regulate the secretion of pituitary gonadotrophs [26].

As there is dearth information on the effects of diclofenac on the male reproductive system, the present study investigated for the first time, the effects of diclofenac and melatonin on reproductive hormones, pro-oxidant/antioxidant markers, inflammatory indices and lipid profiles in experimental animals.

2. Materials and methods

2.1. Drugs and chemicals

Melatonin and diclofenac sodium were purchased from Sigma chemical company (St. Louis, MO, USA) and Wuhan Grand Pharmaceutical Company (Wuhan, Hubei, China) respectively, while sodium pentobarbital was procured from Nicholas Piramal Ltd., Thane, Maharashtra, India.

Diagnostic kits for the determination of gonadotrophin releasing hormone, follicle stimulating hormone, luteinising hormone, prolactin, testosterone, uric acid and c - reactive protein were obtained from Fortress Diagnostics Limited, United Kingdom. While analytic kits for the determination of superoxide dismutase, catalase, total antioxidant capacity, lactate dehydrogenase and malondialdehyde were purchased from Elabscience Biotechnology Company Ltd., Wuhan, Hubei, China.

2.2. Experimental animals and care

Twenty (20) adult male Wistar rats weighing between 170 and 220 g were used for this study. They were acquired from the Animal Holding Unit of the Biochemistry Department, University of Ilorin, Ilorin, Nigeria. The rats were kept in wooden cages at a room temperature and photo-periodicity of 27–30 °C and 12 h light/12 h dark respectively. They were given standard pelletised diet (Ace Feed PLC, Ibadan, Nigeria) and water *ad libitum* daily, and were weighed weekly. After two weeks of acclimatisation, the rats were randomly allotted to separate groups. The rats were well-catered for in accordance with the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' documented by the National Academy of Sciences [27] and approved by the Ethical Board of the resident university of the authors.

2.3. Experimental design

Twenty (20) adults male Wistar rats were used for this study, which lasted for 6 weeks. They were divided into four (4) groups, of five (5) rats each, which included: group 1 - Control; group 2 - Sodium diclofenac (DF) treated; groups 3 - Diclofenac recovery (DF rec); and group 4 - Diclofenac + Melatonin (DF + Mel). DF tablets, which were dissolved in normal saline was administered at 1 mg/kg body weight (b.w.)/day (*p.o.*) [28], while melatonin was administered at 10 mg/kg b.w./day (*p.o.*) [29]. The control group was administered normal saline (0.1 ml/day) throughout the duration of the experiment. Groups 2 was administered saline during the first three weeks and DF during the last three (3) weeks of the experiment; however, in groups 3 and 4, DF was administered during the first three (3) weeks, afterwards, they were administered normal saline (0.1 ml/day) and melatonin respectively during the subsequent three weeks.

2.4. Biochemical analyses

Twelve (12) hours after administration on the last day of the experiment, the rats were anaesthetised with sodium pentobarbital (40 mg/kg, *i.m.*) [30,31]. Thereafter, they were dissected and blood was

collected by cardiac puncture into heparinised sample bottles, which were centrifuged at 4000 rpm for 15 min, at - 4 °C using a cold centrifuge (Centurion Scientific Ltd., Chichester, West Sussex, UK). The supernatant plasma samples were collected into separate plain bottles prior to the analyses. The biochemical analyses were performed according to the manufacturers' instruction.

2.5. Determination of low density lipoprotein cholesterol (LDL-C) level

Low density lipoprotein cholesterol was evaluated using the formula below [32]:

$$\text{LDL-C (mg/dl)} = \text{TC} - (\text{HDL-C} + \text{TG}/5)$$

Keys: TC - total cholesterol; HDL-C - High density lipoprotein cholesterol; TG - Triglyceride.

2.6. Determination of epididymal sperm parameters

At the termination of the experiment, the two (2) testes of the rats were carefully excised. Afterwards, the epididymal sperm parameters, viz; sperm count, sperm motility, sperm morphology, and sperm viability were determined [33].

2.7. Histological assessment of the testicular tissue

The right testes of the rats were removed during sacrifice and they were fixed in 10% (v/v) neutral phosphate-buffered formalin (pH 7.4), dehydrated, and embedded in paraffin wax. Afterwards, they were sectioned (5 mm in thickness) and stained with haematoxylin and eosin [34] and then examined under light microscope (Manufacturer: Olympus Optical Co., Ltd. Shinjuku, Tokyo, Japan).

2.8. Data analyses

Data were analysed using statistical package for social sciences (SPSS) version 20.0. Statistical evaluations of the differences between the group mean values were tested by one way analysis of variance (ANOVA) and Tukey *post - hoc* test for multiple comparisons. The results were expressed as mean \pm standard error of mean (SEM), and statistical significance was considered at $p \leq 0.05$.

3. Results

3.1. Effects of diclofenac sodium (DF) and melatonin on gonadotrophin releasing hormone (GNRH), follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin, and testosterone

Compared to the control group, there were significant decreases in GNRH in DF and DF rec groups ($p = 0.028$ and 0.004 respectively) (Fig. 1a). Moreover, in the group 4 (DF + Mel), relative to control, DF and DF rec groups, significant increases in FSH ($p = 0.010$, 0.021 , 0.050 respectively) (Fig. 1b) and LH ($p = 0.000$, 0.000 , 0.000 respectively) (Fig. 1c) were recorded. Although there was a significant increase in prolactin in the DF rec relative to the DF group ($p = 0.050$) (Fig. 1d), a significant decrease in testosterone was observed in former relative to the latter ($p = 0.016$) (Fig. 1e). Moreover, the plasma level of testosterone was found to be significantly reduced in the DF rec compared to the control and DF + Mel groups ($p = 0.003$, 0.050 respectively).

3.2. Effects of diclofenac sodium (DF) and melatonin on lactate dehydrogenase (LDH), malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase (CAT)

In the DF rec, relative to the control group, there were significant elevations in LDH activity ($p = 0.030$) (Fig. 2a) and MDA level ($p =$

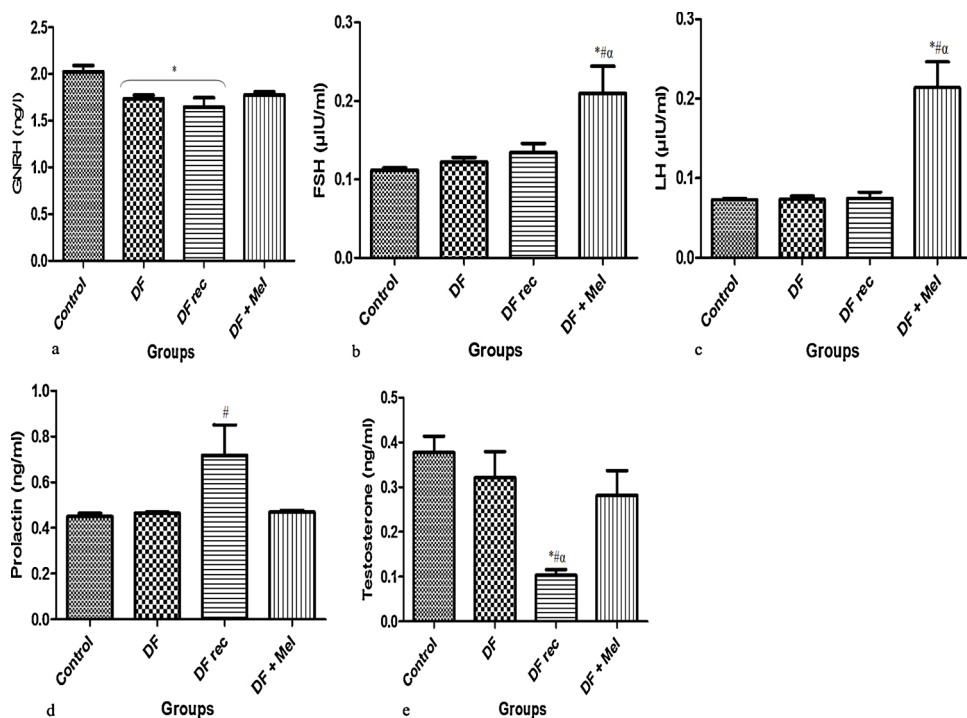


Fig. 1. a–e: Effects of diclofenac sodium (DF) and melatonin on gonadotrophic hormone (GNRH); follicle stimulating hormone (FSH); Luteinising hormone (LH); prolactin; and testosterone in Wistar rats. Values are expressed as mean ± SEM. * $p \leq 0.05$ is significant compared to control group; # $p \leq 0.05$ is significant compared to DF group; ^α $p \leq 0.05$ is significant – DF rec vs DF + Mel. Data were analysed using analysis of variance and Tukey *post hoc* test for multiple comparisons.

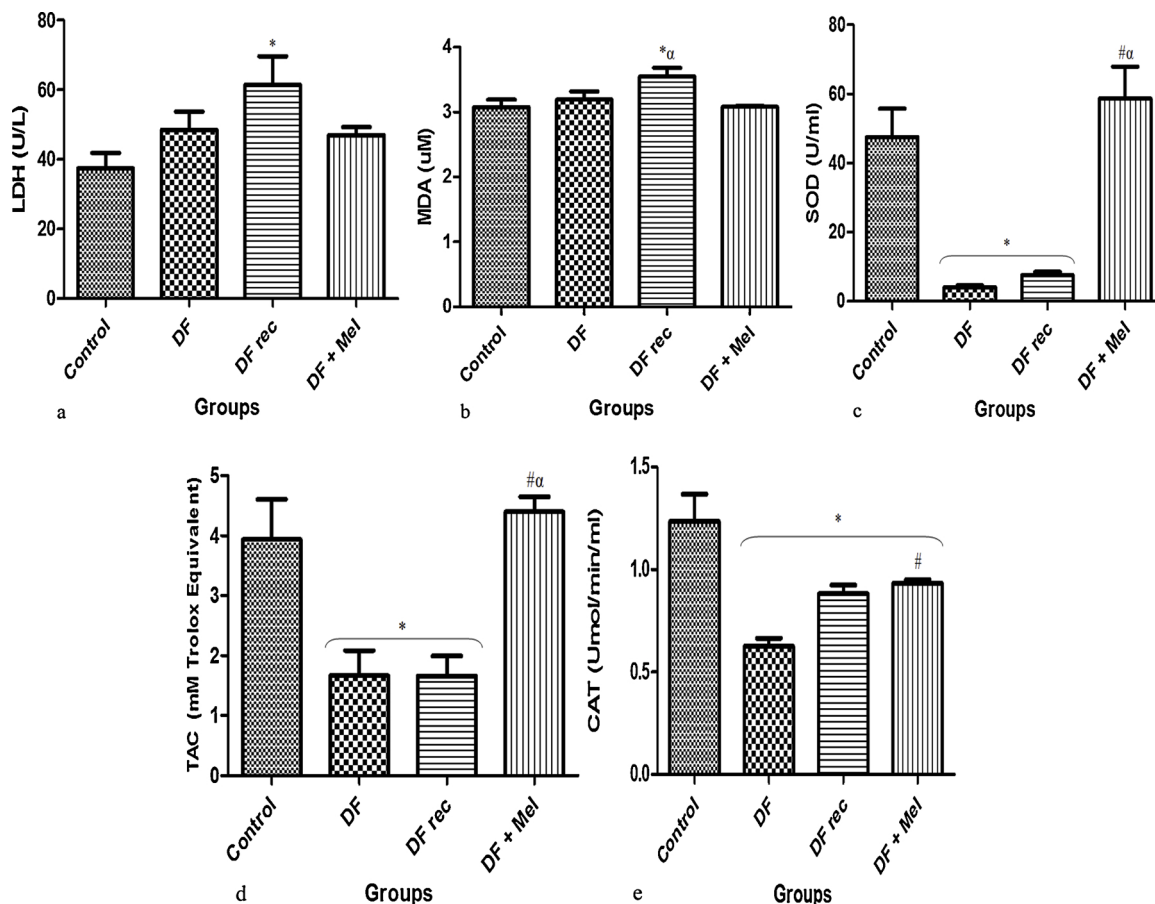


Fig. 2. a–e: Effects of diclofenac sodium (DF) and melatonin on lactate dehydrogenase (LDH); malondialdehyde (MDA); superoxide dismutase (SOD); total antioxidant capacity (TAC); and catalase (CAT) in Wistar rats. Values are expressed as mean ± SEM. * $p \leq 0.05$ is significant compared to control group; # $p \leq 0.05$ is significant compared to DF group; ^α $p \leq 0.05$ is significant – DF rec vs DF + Mel. Data were analysed using analysis of variance and Tukey *post hoc* test for multiple comparisons.

Table 1
Effects of diclofenac sodium (DF) and melatonin on semen parameters in Wistar rats.

Groups/Parameters	Sperm count ($\times 10^6$ /mL)	Sperm motility (%)	Sperm viability (%)	Sperm morphology (%)
Control	305.66 \pm 2.32	71.44 \pm 2.00	78.17 \pm 3.21	90.74 \pm 0.83
DF	282.22 \pm 5.08*	74.70 \pm 0.89	66.64 \pm 2.80*	86.31 \pm 1.99
DF rec	251.20 \pm 5.68*#	71.59 \pm 1.47	50.11 \pm 1.39*#	81.52 \pm 1.34*
DF + Mel	446.78 \pm 6.72*# α	81.83 \pm 1.70*# α	87.61 \pm 0.56*# α	86.64 \pm 0.63

Values are expressed as mean \pm SEM. * $p \leq 0.05$ is significant compared to control group; # $p \leq 0.05$ is significant compared to DF group; $\alpha p \leq 0.05$ is significant – DF rec vs DF + Mel. Data were analysed using analysis of variance and Tukey *post hoc* test for multiple comparisons.

0.028) (Fig. 2b). Moreover, there was a significant increase in MDA level ($p = 0.029$) in the former, compared to DF + Mel group. Also, in the DF and DF rec groups, compared to control group, significant reductions in SOD activity ($p = 0.001, 0.002$ respectively) (Fig. 2c) and TAC ($p = 0.011, 0.011$ respectively) (Fig. 2d) were recorded. Relative to DF and DF rec, there were significant increases in SOD activity ($p = 0.000, 0.000$ respectively) and TAC ($p = 0.003, 0.003$ respectively) in DF + Mel. In addition, significant decreases in catalase activity were noted in the DF ($p = 0.000$), DF rec ($p = 0.017$) and DF + Mel ($p = 0.045$), compared to the control group (Fig. 2e). However, relative to DF group, a significant increase in CAT activity ($p = 0.041$) in DF + Mel was observed.

3.3. Effects of diclofenac sodium (DF) and melatonin on semen parameters

In the DF + Mel group, relative to control, DF and DF rec, there were significant increases in sperm count ($p = 0.000, 0.000, 0.000$ respectively), sperm motility ($p = 0.001, 0.025, 0.001$ respectively) and sperm viability ($p = 0.034, 0.019, 0.002$ respectively) (Table 1). Compared to the control group, there were significant reductions in sperm count ($p = 0.027$) and sperm viability ($p = 0.011$) in the DF group. Moreover, significant decreases in sperm count and viability was recorded in DF compared to the control ($p = 0.000, 0.000$ respectively), and DF rec ($p = 0.003, 0.000$ respectively) groups. In addition, a significant decrease in sperm morphology was recorded in DF rec, compared to the control group ($p = 0.001$) (Table 1).

3.4. Effects of diclofenac sodium (DF) and melatonin on uric acid (UA) and c - reactive protein (CRP)

Relative to the control, DF and DF + Mel groups, there were significant increases in UA ($p = 0.000, 0.000, 0.001$ respectively) (Fig. 3a) and CRP ($p = 0.013, 0.011, 0.038$ respectively) (Fig. 3b) in DF rec group. Moreover, a significant increase in the plasma level of UA was recorded in the DF + Mel group compared to DF group ($p = 0.039$).

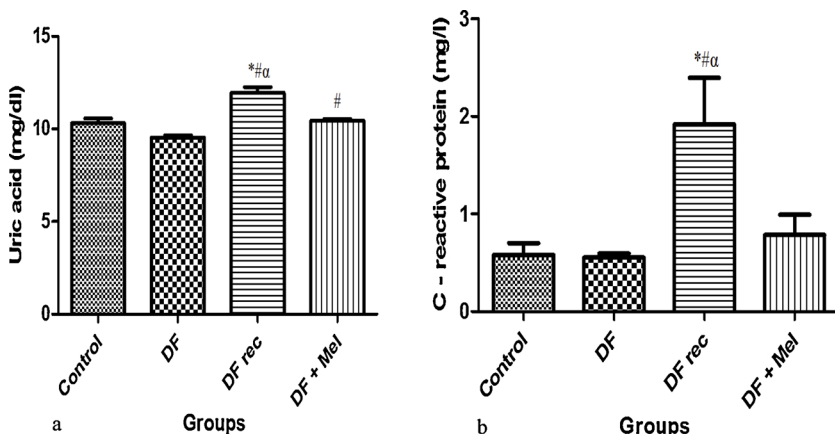


Fig. 3. a–b: Effects of diclofenac sodium (DF) and melatonin on uric acid and c- reactive protein (CRP) in Wistar rats.

Values are expressed as mean \pm SEM. * $p \leq 0.05$ is significant compared to control group; # $p \leq 0.05$ is significant compared to DFgroup; $\alpha p \leq 0.05$ is significant – DF rec vs DF + Mel. Data were analysed using analysis of variance and Tukey *post hoc* test for multiple comparisons.

3.5. Effects of diclofenac sodium (DF) and melatonin on lipid indices

In the DF rec, compared to control, DF and DF + Mel groups, there were significant elevations in TC ($p = 0.020, 0.003, 0.047$ respectively) and LDL-c ($p = 0.002, 0.001, 0.003$ respectively) (Table 2). However, relative to DF and DF rec groups, significant decreases in TG ($p = 0.050, 0.046$ respectively) were recorded in DF + Mel group. Also, significant diminutions in phospholipid level were recorded in DF ($p = 0.000$), DF rec ($p = 0.000$) and DF + Mel ($p = 0.000$), relative to the control group. In addition, compared to DF group, a significant reduction in phospholipid was observed in DF rec ($p = 0.012$). Relative to the control group, there was a significant decrease in HDL-c in DF rec group ($p = 0.047$) (Table 2).

3.6. Effects of diclofenac sodium (DF) and melatonin on the histoarchitecture of the testicular tissue

In the control group (Fig. 4A), the testicular tissue shows the presence of spermatogonium (blue arrow), sertolli cell (purple arrow), leydig cell (yellow arrow), well-arranged seminiferous tubules and well-organised distribution of cells in the seminiferous epithelium, while in the DF group (Fig. 4B), degeneration of the seminiferous tubule (green arrow) and the smooth muscle (red arrow) and some degree of cell loss (brown arrow) were observed. The DF rec (Fig. 4C) shows total cell degeneration while DF + Mel (Fig. 4D) shows few distinct scattered cells in the seminiferous tubules, and also degeneration (green arrow) and disordered arrangement of the seminiferous tubules.

4. Discussion

Decades back, researchers observed that in either fasting or fed state, measurable concentration of DF does not persist beyond 12 h post-administration. However, the scientists were surprised that the pharmacokinetic behaviour of the drug is inconsistent with its extended biological effects [35–37]. In the present study, the results revealed that three weeks after the stoppage of the administration of DF, the adverse effects of the drug lingered, evident by significantly deviations in

Table 2
Effects of diclofenac sodium (DF) and melatonin on lipid indices in Wistar rats.

Groups/ Parameters	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High density lipoprotein cholesterol (mg/dl)	Low density lipoprotein cholesterol (mg/dl)	Free fatty acids (mg/dl)	Phospholipids (mg/dl)
Control	59.46 ± 1.98	48.85 ± 2.58	7.40 ± 1.13	61.83 ± 2.26	822.26 ± 67.44	50.55 ± 1.68
DF	56.54 ± 1.74	49.66 ± 0.58	6.27 ± 0.58	60.20 ± 2.00	759.92 ± 13.23	18.15 ± 1.64 [*]
DF rec	69.21 ± 1.63 ^{#α}	49.89 ± 2.76	3.75 ± 0.53 [*]	75.44 ± 1.60 ^{#α}	706.72 ± 16.73	10.57 ± 1.52 [#]
DF + Mel	60.74 ± 2.74	41.99 ± 0.45 ^{#α}	6.82 ± 1.14	62.31 ± 2.58	693.01 ± 2.69	13.22 ± 1.07 [*]

Values are expressed as mean ± SEM. ^{*}*p* ≤ 0.05 is significant compared to control group; [#]*p* ≤ 0.05 is significant compared to DF group; ^α*p* ≤ 0.05 is significant – DF rec vs DF + Mel. Data were analysed using analysis of variance and Tukey *post hoc* test for multiple comparisons.

testicular histoarchitecture, and in most of the biochemical markers that were assayed for, compared to the pre-withdrawal action.

Diclofenac was observed to have no effects on the plasma levels of gonadotrophins, however, its administration was accompanied with significant suppression in GNRH and testosterone, and a significant increase in prolactin secretion. There are reports in literature on the central [7] and peripheral [8,9,11] effects of DF. Chronic administration of DF instigates oxidative stress, and hence the production of free radicals, which could no doubt cross the blood-brain barrier and cause dysfunction in the hypothalamic secretion of GNRH. However, it was surprising that there was no corresponding depression in the plasma level of FSH and LH. On the other hand, the administration of melatonin was characterised by significant increases in FSH and LH. This could be attributed to the presence of the receptors for the hormones in the pituitary [38]. Nevertheless, exogenous administration of melatonin has been previously reported to cause an increase [39], a decrease [40] or to have no effect [41] on gonadotropin secretion. Unlike GNRH and the gonadotrophins, elevated endogenous levels of prolactin have been associated with infertility [42,43]. Al-Daghistani and M Abdel-Dayem (2006) observed a significant increase in prolactin in azoospermic and oligospermic subjects with hypergonadotrophins levels [44]. Almost half of hyperprolactinaemic patients with erectile dysfunction were reported to have normal plasma level of testosterone [45]. The significant increase in prolactin level and the significant decrease in testosterone which was observed in DF rec group, but was not recorded in DF + Mel, attested that melatonin could promote fertility in DF treated individuals. It was expected that the significant decrease in testosterone in DF rec group should have possibly stimulated the anterior pituitary (*via* a negative feedback mechanism) to release more LH, which could act on the interstitial cells of Leydig and so promotes testosterone secretion, however, this was not the case. Hence, it could be suggested that DF might have also caused dysfunction in the pituitary, even though it was not evident in the assessment of FSH and LH in this study.

The hormonal profile corresponded fairly with the semen analysis results. DF showed no notable effect on the sperm motility and viability,

as the values obtained were comparable to that of the control rats. This could be related to the insignificant levels of gonadotrophins recorded in DF and DF rec groups. However, there were significant decreases in sperm count and sperm morphology in DF rec, but not DF group. Vyas and colleagues reported that sperm density (in epididymis and testis), sperm count, sperm motility and testicular cell population dynamics were reduced in a dose-dependent manner after DF treatment. But, they did not assay for any reproductive hormone. Contrary to the effects of DF, melatonin was noted to significantly increase sperm count, sperm motility and sperm viability, compared to what was recorded in the other experimental animal groups. Melatonin has receptors in the testes, and as such, the hormone directly regulates testicular functions [26] and testosterone secretion [25]. Even though there was no significant increase in testosterone in the melatonin treated rats, the interference of the hormone with testicular functions could be linked with its effects on the semen indices.

Small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities [15]. Nevertheless, excessive production of free radical could be damaging on sperm integrity. Sperm are particularly susceptible to ROS-induced damages because they don't have DNA repair mechanisms. Besides, they possess low levels of cytoplasmic antioxidant enzymes and contain high levels of poly unsaturated fatty acids [13,14]. In our previous study, DF administration was associated with oxidative stress [9]. Moreover, in the present research, in the DF and DF rec groups, there were comparable depressions of endogenous TAC, CAT and SOD. However, there were significant increases in LDH and MDA in latter compared to the former. The reason for the sustained adverse effects of DF after the stoppage of administration could not be accounted for in this study. Increased plasma LDH has been considered as an indicator of acute or chronic tissue damage [46], while, elevated MDA (a product of lipid peroxidation) is an indicator of oxidative stress [47]. The combined significant increases in LDH and MDA in DF rec, compared to the control group, which was not observed in DF + Mel, and the significant decrease in MDA in the latter, compared to the former, presents an evidence of the anti-lipid peroxidative and hence tissue protective effects of melatonin against DF insult. This finding is

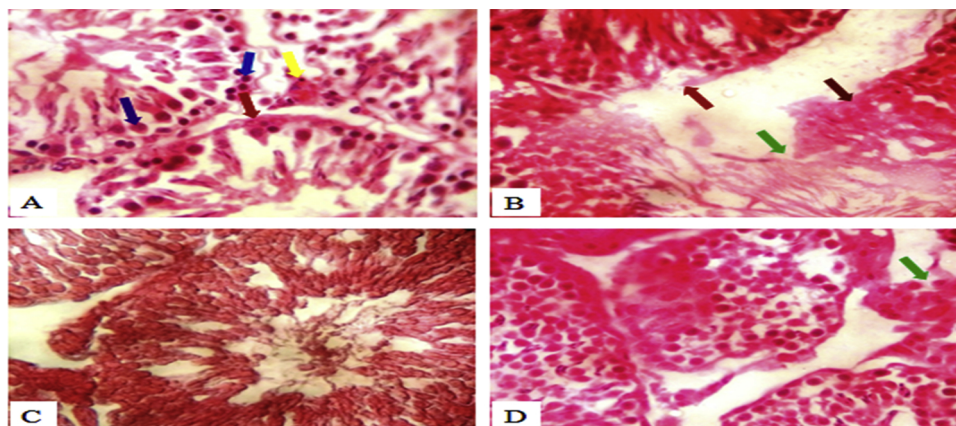


Fig. 4. (A–D): Photomicrographs (haematoxylin and eosin stain; X40) of the testes of male rats, showing the effects of diclofenac sodium and melatonin.

further supported by the significant elevations in SOD and TAC in DF + Mel group, relative to DF and DF rec. Melatonin and its metabolites have been documented to be powerful scavengers of oxygen and nitrogen free radicals [48]. It should however be noted that there are also reports on the pro-oxidant action of the hormone in specific conditions [49]. Besides from melatonin, omega-3 fatty acids, sodium selenite, vitamin C and E, triiodothyronine, among other have been noted for their protective actions against diclofenac and heavy metal insults [50,51].

Inflammation is considered as one of the results of imbalance in the antioxidant enzyme system. The pathways that activate the production of inflammatory mediators are all instigated by oxidative stress [52]. As such, a positive association often exists between oxidative stress and inflammatory status [53,54]. DF has a renowned anti-inflammatory property. The drug tends to be administered for a long duration in the management of chronic disease conditions. Prolonged administration of diclofenac has been associated with oxidative stress, which has a pathogenic link with inflammatory events [9]. Even though UA is constitutively present in normal cell, it has been tagged as one of the primary endogenous markers that are released from damaged cell [55]. UA activates NF- κ B (a pro-inflammatory master switch) that mediates the production of inflammatory markers [56]. In consonance with our result on prolactin, testosterone, LDH, MDA, and sperm morphology, there were significant elevations in UA and CRP in DF rec, relative to DF group. Moreover, significant increases in UA and CRP were recorded in the DF rec, compared to the control and DF + Mel groups. Although there was no significant difference in the plasma levels of the inflammatory markers (UA and CRP) in the DF + Mel group, relative to the control, the significant decreases in these markers in the former compared to DF rec group, suggests that melatonin has anti-inflammatory property. The administration of the hormone has been documented to be accompanied with a reduction in pro-inflammatory cytokines [21].

Besides from the contributing effects of hormonal imbalance, oxidative stress and pro-inflammatory events to male reproductive dysfunction, dyslipidaemia has been considered to be implicated in male infertility. Cholesterol and lipid homeostasis are crucial for male fecundity [57,58]. It was reported that 65% of infertile men had triglyceridaemia and/or hypercholesterolaemia [19]. In the present study, the significant elevations in the plasma levels of TC and LDL-c in DF rec group, relative to the other experimental animal groups, and the significant decrease in HDL-c in DF rec, compared to the control group, further affirmed the elevated reproductive toxicity effect of diclofenac after its withdrawal, as earlier stated. However, the significant deviations in these markers in DF rec, compared to DF + Mel group, attested to the hypocholesterolaemic effect of melatonin, which could be as a result of the augmentation of cholesterol clearance mechanisms [59]. Esquifino et al. suggested that the antidyslipidaemic effect of melatonin, which was further evident in this study by the increase in the plasma phospholipids, may be related to its ability to enhance lecithin-cholesterol acyltransferase (LCAT)-mediated cholesterol esterification [22]. Unlike other lipid indices, the TG result showed no after-withdrawal effect of DF. It is suggested that further studies could evaluate biochemical profile in the testicular tissue.

The histological results agree with the biochemical assays. Compared to DF, DF rec showed more adverse effects on prolactin, testosterone, LDH, MDA, UA, CRP, semen parameters (except sperm motility), TC, LDL-c, HDL-c and phospholipid. The significant fluctuations in the endogenous status of these markers would no doubt be connected to the total degeneration of cells in the seminiferous tubules in the testes of DF rec group. Although some degree of degeneration was also noticed in DF group, it was not to the extent of that which was indicated in DF rec group. As earlier stated, excess production of free radicals as a result of imbalance in the antioxidant enzyme system has been noted to be detrimental to sperm cells and testicular health [15]. Post-administration with melatonin after DF treatment in DF + Mel

group was characterised with cell regeneration, even though there was still an evidence of the disruption of the histoarchitecture of the testicular tissue by DF.

5. Conclusion

The reproductive toxicity effects of DF seem to escalate after withdrawal however, these effects could be attenuated by treatment with melatonin.

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