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MORIN ATTENUATES DUTASTERIDE/TAMSULOSIN-INDUCED HEPATIC OXIDATIVE STRESS IN RAT

Olayinka, E. T.[1], Adewole, K. E.[2]*

[1]Biochemistry Unit, Department of Chemical Sciences, Faculty of Natural Sciences, Ajayi Crowther University, PMB 1066, Oyo, Oyo State, Nigeria. Email address: ebenezertundeolayinka2gmail.com. [2]*Department of Biochemistry, Faculty of Basic Medical Sciences, University of Medical Sciences Ondo City, Ondo State Nigeria.

*Author for correspondence; e-mail address: kayowolemi@gmail.com

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ABSTRACT

Dutasteride-Tamsulosin (DUT-TAM) is a combination drug for the treatment of symptoms of prostate enlargement (benign prostatic hyperplasia, BPH). Despite the efficacy, it is associated with some side effects, including hepatotoxicity. Therefore, this study investigated the attenuative effects of morin on DUT-TAM-induced organ toxicity. Twenty four male rats were divided into 4 groups (A-D) consisting of 6 animals each. Group A animals (control) were given olive oil, Group B animals were administered with DUT-TAM (5.4 mg/kg body weight of dutasteride + 3.4 mg/kg body weight of tamsulosin), Group C were given morin (100 mg/kg body weight) while group D animals were administered DUT-TAM and morin (5.4 mg/kg body weight dutasteride + 3.4 mg/kg body weight of Tamsulosin and 100 mg/kg body weight of morin). All the administrations were carried out orally for 14 days. DUT-TAM caused a significant increase in plasma bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by 62%, 45% and 18% in the DUT-TAM treated group respectively, compared with the control ($P<0.05$). However, treatment with morin significantly decreased the DUT-TAM-induced increase in plasma bilirubin concentration as well as AST and ALT activities. Furthermore, DUT-TAM administration decreased the activities of hepatic superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), as well as hepatic concentration of ascorbic acid and reduced glutathione (GSH) by 58%, 54%, 59%, 46% and 63% respectively, but increased malondialdehyde (MDA) level by 49% relative to the control ($P<0.05$). However, treatment with morin significantly ameliorated the observed changes in these antioxidant parameters ($P<0.05$). These data suggest that morin protects against hepatic toxicity, as well as oxidative stress induced by dutasteride-tamsulosin in rats.

Keywords: Dutasteride, Tamsulosin, Prostate enlargement, Oxidative stress, Liver

INTRODUCTION

Dutasteride-Tamsulosin (DUT-TAM) is a front-line drug for managing prostate enlargement (benign prostatic hyperplasia, BPH) common in men older than 40 years (Miller and Tarter, 2009; Kurczewski *et al*., 2017). It is a combination of 2 drugs, dutasteride and tamsulosin; while dutasteride (5α, 17β)-N {2, 5, bis (trifluoromethyl) phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide), (Figure 1), is a 5α-reductase inhibitor (5ARI), tamsulosin, (R)-5[2-[[2-(2-Ethoxyphenoxy) ethyl] amino] propyl]-2-methoxybenzenesulfonamide monohydrochloride, (Figure 2), is an α1-adrenergic blocking agent (α-blocker), Roehrborn *et al*., (2015). Benign prostatic enlargement, which results from benign prostatic hyperplasia, leads to lower urinary tract symptoms (LUTS), especially in men above 40 years (Kurczewski *et al*., 2017). Treatment with DUT-TAM improves the symptoms of BPH and LUTS, which helps in cutting down BPH-associated surgical procedures. Although various studies have confirmed the efficacy of dutasteride, negative effects have however been reported, these include reduced libido, ejaculatory dysfunction, impotence and gynecomastia (Roehrborn *et al*., 2002). For, tamsulosin, the unwanted effects include asthenia, dizziness, headache and postural hypotension (Roehrborn *et al*., 2008). Long-term treatment with dutasteride therapy has been found to cause increased blood glucose, glycated haemoglobin, total cholesterol and low density lipoprotein levels, with increased activity of plasma alanine amino transferase (ALT) and aspartate aminotransferase (AST) (Traish *et al*., 2017). Dutasteride has also been reported to cause liver inflammation and negative alteration of the histological architecture of mice liver, an indication that it is possibly hepatotoxic (Mohamad *et al*., 2017).

Several indices, such as inflammation, diet, genetic modification, and oxidative factors have been implicated in the aetiology of BPH (Minciullo *et al*., 2015). Oxidative stress and damage to tissue of the prostate may cause abnormal cell growth which can lead to hyperplastic growth. Inflammation of the prostate can result in production of reactive oxygen species (ROS) and reactive nitrogen species, (RNS) (Hamid *et al*., 2011). Convincing scientific evidences suggest that micronutrient supplementation, including plant-derived molecules, may help to restore systemic antioxidant which can enhance the treatment results of BPH subjects (Udensi and Tchounwou, 2016). Morin (Figure 3) is a bioflavonoid chemically referred to as 3,5,7,2´,4´-pentahydroxy flavone, it was first isolated from the plant family *Moraceae*, which are commonly used as traditional medicinal herbs (Sreedharan and Venkatachalam, 2009). Morin has also been found in *Prunus dulcis*, *Chlorophora tinctoria*, (Ricardo *et al*., 2001), *Psidium guajava* (Rattanachaikunsopon and Phumkhachorn, 2010), tea, cereal grains, fruits and in vegetables (Kang *et al*., 2004). Studies have revealed that morin exhibits antioxidant properties and offers protection towards hydrogen peroxide-induced oxidative stress (Zhang *et al*., 2009). It has also been reported that morin exhibits antioxidative, antiinflammatory and anti-proliferative potentials in different experimental models (Middleton *et al*., 2000). The feasibility of co-administration of anticancer drugs for treating BPH and antioxidants as a strategy to protect tissues from agents-inducing oxidative stress has been demonstrated (Joshi *et al*., 2007). Based on the pharmacological properties of morin, this study hypothesizes that morin can protect against possible hepatotoxic effects of DUT-TAM. The objective of this study therefore, is to evaluate the ameliorative potentials of morin on dutasteride-tamsulosin-induced hepatotoxicity and oxidative stress in rats.

MATERIALS AND METHODS

Chemicals and Reagents

Dutasteride and tamsulosin hydrochloride (Duodart®) is a product of Catalent, Schorndorf GmbH, Germany. 1-chloro-2, 4-dinitrobenzene (CDNB), adrenaline (epinephrine), Glutathione (GSH), 5', 5'-dintrobenzoic acid (DTNB) and thiobarbituric acid (TBA) are products of Sigma Chemical Company (London, UK). Assay kits for AST, ALT and total bilirubin are products of Agape Diagnostics, Switzerland GmbH. Other reagents and chemicals were of analytical grade and were purchased from British Drug House, London.

Experimental Rats

Male Wistar rats (120-140g) used for this study were purchased from Ladoke Akintola University, College of Health Sciences, Osogbo, Nigeria. The animals were kept in woody wire meshed cages, given free access to commercial animal feed (Vital feed®) and clean water *ad libitum*. They were acclimatized for two weeks before the onset of treatment. Animal treatment conformed to the guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use in line with the rules of the Ethical Committee of the Ajayi Crowther University, Oyo, Oyo State Nigeria.

Experimental Design (Grouping of Rats and Administration of Drug)

Twenty four animals were randomly grouped into 4 (A-D), n= 6 per group. Group A which served as the control were given olive oil, Group B animals were administered with DUT-TAM (5.4 mg/kg body weight of dutasteride and 3.4 mg/kg body weight of tamsulosin), animals in Group C were given morin (100 mg/kg body weight), and Group D animals were administered DUT-TAM and morin (DUT-TAM, 5.4 mg/kg body weight of dutasteride + 3.4 mg/kg body weight of tamsulosin + Morin, 100 mg/kg body weight). The dose of the drug (DUT-TAM) is the therapeutic dose, while that of morin is the recommended dose from literature (Kuzu *et al*., 2019; Olayinka *et al*., 2019). The vehicle for dissolving both morin and DUT-TAM was olive oil and all administration was by oral gavage, using oral intubator, once per day for 14 days. Rats were then sacrificed 24 hr following the last administration.

Preparation of Plasma and Post Mitochondrial Fraction

Animals were sacrificed by cervical dislocation,

and Ganjoo, 2015).

Determination of Hepatic Reduced Glutathione Content

The GSH level in the samples was estimated by the method described by Jollow *et al*. (1974). Briefly, distilled water (900 µl), 100 µl of sample and 1.5 ml of sulphosalicylic acid (4%) were mixed, left on the bench for 5 min and filtered; the filtrate (500 µl) was added to 2 ml of phosphate buffer (0.1 M) and, 250 µl 0.04% DTNB (in 0.1M phosphate buffer, pH 7.4). The absorbance was read at 412 nm, with a blank reference, while the GSH level was interpolated using the standard curve generated from using different concentrations of GSH.

Estimation of Hepatic Ascorbic Acid Level

The ascorbic acid concentration in hepatic tissue was estimated using the method described by Jagota and Dani (1982). Briefly, 500 µl of sample and 800 µl of 10% TCA were mixed vigorously, by shaking, and then placed on ice for about 5 min. The mixture was centrifuged at 2000× g for 10 min, after which 1000 µl of the resulting supernatants and 200 µl of diluted Folin's reagent (Folin's reagent: distilled H_2O, 1:10) were added together and stirred vigorously. The absorbance of the resulting blue-colored solution was recorded after 10 min at 760 nm. The hepatic ascorbic acid concentration (µg/ml) was obtained by interpolation from the ascorbic acid standard curve generated using varying concentrations of standard ascorbic acid

Assessment of Hepatic Lipid Peroxidation

Hepatic lipid peroxidation was estimated by the quantification of malondialdehyde (MDA) levels with the aid of 2-thiobarbituric acid in organ homogenates (Varshney and Kale, 1990). Briefly, 3.2 ml Tris-HCl buffer, 1.0 ml of 30% TCA, 0.8 ml of samples, 1.0 ml of 0.75% TBA were mixed. The temperature of the mixture was raised to 95 °C and maintained at same for 1 hr using a water bath. The mixture was then centrifuged at 3000 rpm, after cooling on ice. The supernatant was retrieved and the absorbance read against distilled water blank at 532 nm. The level of lipid peroxidation in nmole/mg protein was estimated using the following equation:

MDA =

$$\frac{Abs \times Volume\ of\ reaction\ mixture}{E532 \times Volume\ of\ sample \times mg\ protein}$$

Where: E_{532} = molar extinction coefficient for MDA, which is 1.56×10^5/M/cm

Histopathology of the Liver

Histopathological study was done as described by Krause (2001). Briefly, liver samples were fixed in 10% formalin for 24 hr and dehydrated through ascending grades of ethanol, and fixed in paraffin wax. Sections of about 5 µm, were cut on a microtome and stained in aqueous dyes. Balsam mounting medium was put on the liver section and thin glass cover slips were placed on the mounting medium and the underlying tissue sections allowed to dry and later observed using x 720 magnification of the microscope and the photomicrographs were taken in a bright field.

Statistical Analysis

The data were expressed as Mean ± SD. The data were analyzed using one way analysis of variance, with Duncan multiple range test used to compare control and treatment groups using SigmaPlot® (Systat Software Inc, CA, USA). For statistical significance, $p < 0.05$ were considered.

RESULTS

Protective Effects of Morin on DUT-TAM-Induced Hepatotoxicity in Rats

Table 1 shows data on the ameliorative potential of morin on DUT-TAM-induced alterations in the concentrations of bilirubin, as well as the activities of ALT and AST in the plasma of rats. DUT-TAM significantly increased the level of bilirubin in the plasma by 62% and the activities of AST and ALT by 45% and 18% respectively, relative to the control. Morin caused a significant increase in these parameters when administered alone. However, administration of morin with DUT-TAM (co-treated group) significantly ($P<0.05$) ameliorated the increased plasma bilirubin, AST and ALT levels relative to DUT-TAM treated group.

and blood was obtained via ocular puncture into heparinized sample bottles. From the blood samples, plasma was obtained by centrifugation for 10 minutes at 4000 xg (Celcom bench centrifuge), and kept at -20 °C for subsequent biochemical analyses. After the rats have been carefully dissected, the livers were removed, cleared of fat and washed in KCl (0 °C and 1.15%). The organs were homogenized in potassium phosphate buffer, (0 °C, 0.01 M, pH 7.4), centrifuged at 12,000 × g for 10 min at 0 °C and the post-mitochondrial fractions (PMF) obtained for further analyses.

Determination of Protein Concentration

Concentration of protein was estimated as described by Gornall *et al*., (1949). The reaction mixture was made up of 2 ml of biuret reagent plus 0.5 ml of suitably diluted sample, in the blank, 0.5 ml of distilled water was used in place of sample. Estimation of the protein concentration was done by interpolation from the standard curve prepared using varied concentration of bovine serum albumin.

Assays for Biomarkers of Hepatotoxicity

The plasma levels of biomarkers of liver damage ALT, AST and bilirubin were determined using Agape Diagnostics® following the manufacturer's protocol. Activities of plasma AST and ALT were estimated using the method of Reitman and Frankel (1957) while that of bilirubin was done as described by Tietz *et al*., (1994).

Determination of Hepatic Catalase Activity

Hepatic catalase activity was estimated according to the method described by Sinha (1972). The assay mixture which was made up of 2.5 ml of 0.01 M phosphate buffer (pH 7.0), 2 ml H_2O_2 solution (800 µmoles), 0.5 ml of suitably diluted liver PMF (1:50) was mixed, and 1 ml of it was added into 2 ml acetic acid/dichromate solution, at intervals of 1 min, to estimate the quantity of the hydrogen peroxide left. The chromic acetate generated was quantified at 570 nm with the amount of hydrogen peroxide left obtained by interpolation from the hydrogen peroxide standard curve. The activity of catalase was expressed as micromole of hydrogen peroxide used up every 60 sec, by 1 mg of protein.

Determination of Hepatic Superoxide Dismutase Activity

The activity of superoxide dismutase (SOD) was estimated using the method of Misra and Fridovich (1972). Five hundred (500 µl) of the sample was diluted with 4.5 ml of distilled H_2O. A portion of the diluted sample (200 µl) was mixed with 2.5 ml of carbonate buffer (0.05 M, pH 10.2) and allowed to stay in the spectrophotometer to equilibrate, 0.3 ml of freshly prepared epinephrine (0.3 mM) was added to the mixture, following which the mixture was briskly mixed by inversion, and the rise in the value of absorbance at 480 nm recorded every 0.5 min for 2.5 min. the blank contained distilled water in place of sample. Superoxide dismutase activity was estimated as follows:

Percentage inhibition =

$$\left(\frac{\text{increase in absorbance of sample}}{\text{increase in absorbance of blank}}\right) \text{x } 100$$

A unit of SOD activity is the quantity of SOD required to effect 50% inhibition of the oxidation of epinephrine (adrenaline) to adrenochrome in 1 min (Ahmad et al., 2019).

$$\text{SOD activity (Unit/mg)} = \frac{SOD\ (Unit)}{Protein\ (mg)}\ x\ df$$

df = dilution factor

Determination of Hepatic Glutathione-S-Transferase Activity

The activity of GST was determined as described by Habig *et al.* (1974). Briefly, the constituents of the assay mixture include: 60 µl of 0.1 M GSH, 5.58 ml of 0.1 M phosphate buffer (pH 6.5), 300 µl CDNB (3.37 mg/ ml) and 30 µl of liver PMF. The absorbance of the reaction mixture was recorded at 340 nm against the blank after 60 seconds. The activity of the enzyme in the sample was estimated as follows:

Activity of GST, in µmole/min/mg protein =

$$\left(\frac{Abs/min}{9.6}\right) x \frac{1}{0.03\ x\ protein\ (mg)}$$

Where: 9.6 = molar extinction coefficient of S-2,4-DNP-glutathione conjugate ($mmol^{-1}cm^{-1}$) and 0.03 is the volume of sample used (Chaudhary

Table 1: Protective Effect of Morin on DUT-TAM-induced Changes in the Plasma Level of Bilirubin and the Activities of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)

Treatment Groups	Bilirubin (mg/dL)	AST (U/L)	ALT (U/L)
Control	2.06 ± 0.15	6.50 ± 0.57	97.50 ± 0.58
DUT-TAM	5.40 ± 0.10 (61.85%)*	11.77 ± 0.23 (44.77%)*	119.50 ± 1.05 (18.41%)*
morin	2.13 ± 0.12*	7.18 ± 0.22*	102.00 ± 2.53*
DUT-TAM + morin	3.41 ± 0.10*,[a]	8.22 ± 0.19*,[a]	110.00 ± 1.41*,[a]

DUT-TAM= Dutasteride-Tamsulosin (5.4mg/kg body weight Dutasteride + 3.4mg/kg body weight of Tamsulosin); Morin = 100mg/kg body weight morin
The results are expressed as Means ± SD for six rats in each group
*= Significantly different from the control (P<0.05)
[a]= Significantly different from Dutasteride-Tamsulosin group (P<0.05)
Values in parenthesis represent percentage (%) increase

Ameliorative Effect of Morin on DUT-TAM-Induced Alterations in Hepatic Catalase and Superoxide Dismutase in Rats

Table 2 shows data on the protective effect of morin on DUT-TAM induced alterations in hepatic catalase and SOD activities in rats. Hepatic SOD and catalase activities were significantly reduced in DUT-TAM group by 58% and 54% respectively, compared to control (P<0.05). Administration of morin alone has no effect on the activity of SOD but significantly lowered the activity of catalase relative to control. Co-treatment with morin and DUT-TAM, however, significantly (P<0.05) ameliorated the reduction in hepatic SOD and catalase relative to DUT-TAM treated rats.

Table 2: Protective Effect of Morin on DUT-TAM-induced Changes in the Activities of Hepatic Superoxide Dismutase (SOD) and Catalase in Rats

Treatment Groups	Superoxide dismutase (Units)	Catalase (μmole H_2O_2 consumed/min)
Control	14.60 ± 0.55	0.26 ± 0.01
DUT-TAM	6.60 ± 0.89 (54.76%)*	0.12 ± 0.01 (53.84%)*
Morin	13.00 ± 0.71	0.22 ± 0.01*
DUT-TAM + morin	10.80 ± 0.84*,[a]	0.20 ± 0.01*,[a]

DUT-TAM = Dutasteride-Tamsulosin (5.4mg/kg body weight Dutasteride + 3.4mg/kg body weight of Tamsulosin); morin = 100mg/kg body weight morin
The results are expressed as Means ± SD for six rats in each group
*= Significantly different from the control (P<0.05)
[a]= Significantly different from Dutasteride-Tamsulosin group (P<0.05)
Values in parenthesis represent percentage (%) decrease

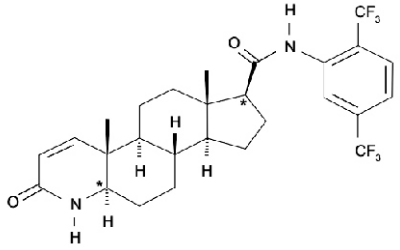

Figure 1: Structure of Dutasteride

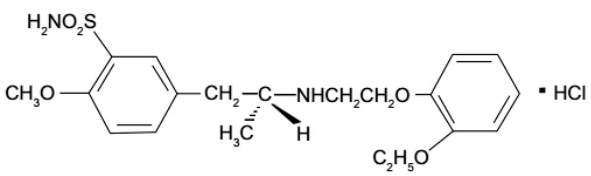

Figure 2: Structure of Tamsulosin

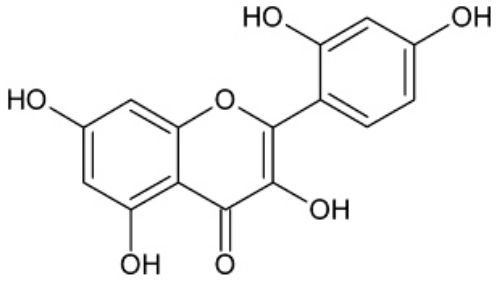

Figure 3: Structure of Morin

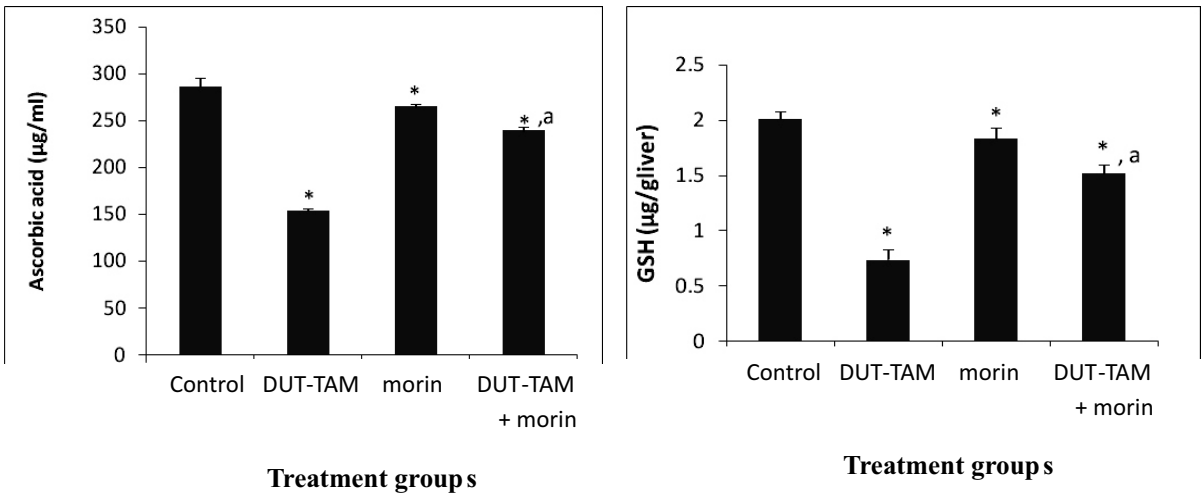

Figure 4: Protective Effect of Morin on Dutasteride/Tamsulosin-induced Changes on the Level of Hepatic Ascorbic Acid and Reduced Glutathione (GSH) in Rats

DUT-TAM= Dutasteride-Tamsulosin (5.4mg/kg body weight Dutasteride + 3.4mg/kg body weight of Tamsulosin); Morin = 100mg/kg body weight morin
The results are expressed as Means ± SD for six rats in each group
*= Significantly different from the control ($P<0.05$)
[a]= Significantly different from Dutasteride-Tamsulosin group ($P<0.05$)

Protective Effect of Morin on DUT-TAM-Induced Alterations in the Concentrations of Hepatic Ascorbic Acid and Reduced Glutathione in Rats

The protective capacity of morin on DUT-TAM-induced alterations in the concentrations of ascorbic acid and reduced glutathione are presented in figure 4. Following treatment with DUT-TAM, the hepatic ascorbic acid and reduced glutathione levels were significantly decreased when compared with the control group. The combined treatment of morin and DUT-TAM significantly ($P<0.05$) attenuated the reduction in hepatic ascorbic acid and GSH levels relative to DUT-TAM group.

Protective Capacity of Morin on DUT-TAM-Induced Alterations in Hepatic Glutathione-S-Transferase and Lipid Peroxidation

The protective ability of morin on changes caused by dutasteride/tamsulosin in rats' hepatic GST and lipid peroxidation are presented in figure 5. Results showed that GST activity was significantly reduced ($P<0.05$) in the DUT-TAM treated group, compared with the control animals. Administration of morin with DUT-TAM significantly ameliorated induced decrease in hepatic GST activity relative to the DUT-TAM treated group. However, the hepatic MDA was significantly increased ($P<0.05$) by DUT-TAM treatment, compared with the control. Co-

treatment of morin and DUT-TAM significantly reduced the elevated hepatic MDA relative to the DUT-TAM group.

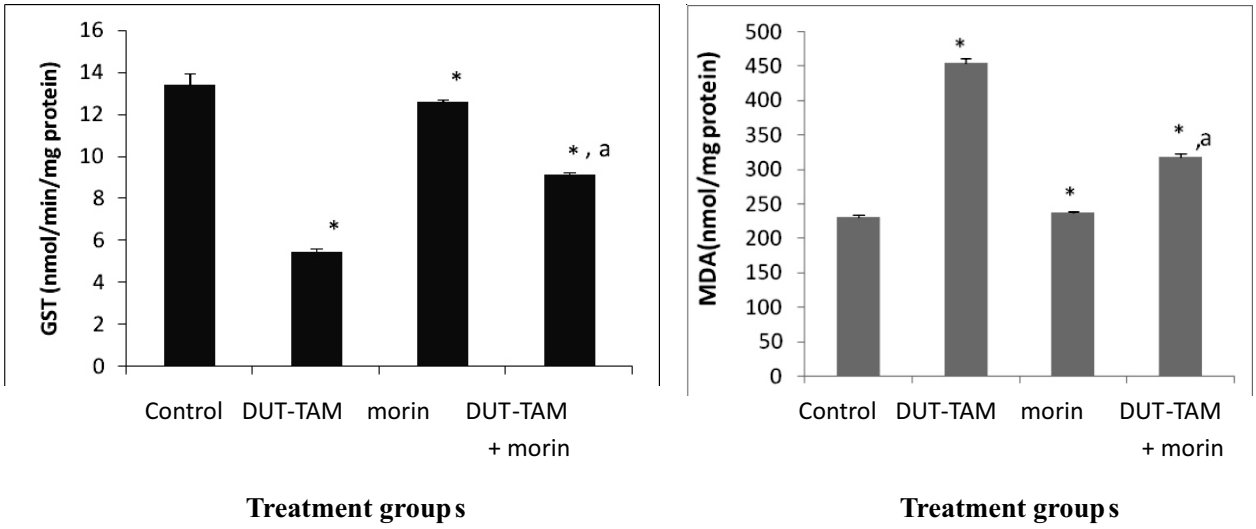

Figure 5: Protective Effect of Morin on DUT-TAM-induced Changes in the Activity of Hepatic Glutathione-S-Transferase and Level of Hepatic Lipid Peroxidation in Rats

DUT-TAM = Dutasteride/Tamsulosin (5.4mg/kg body weight Dutasteride + 3.4mg/kg body weight of Tamsulosin); morin= 100mg/kg body weight morin
The results are expressed as Means ± SD (range) for six rats in each group
*= Significantly different ($P<0.05$) from the control
[a]= Significantly different ($P<0.05$) from DUT-TAM group

Effect of DUT-TAM and Morin on Liver Histology of Rats

The photomicrographs of hepatic tissue after treatments, based on experimental grouping, are shown in figure 6. The sections show normal hepatic parenchyma, normal portal tract and vessels with no inflammatory cells seen in all the treated groups and the control animals.

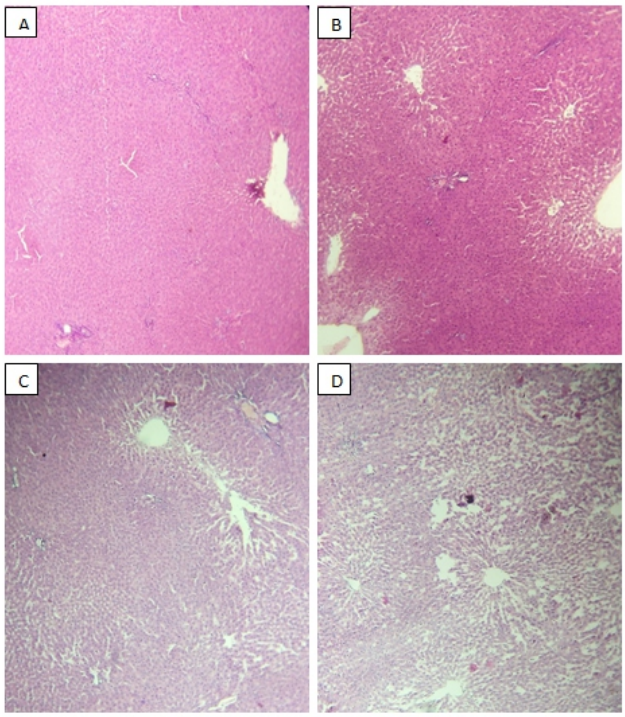

Figure 6: Photomicrographs of Hepatic Tissue Showing the Morphology of Liver (hematoxylin and eosin stain with magnification of ×720, and scale of 0.44mm)

A = Control group, B = Dutasteride-Tamsulosin group, C = morin group and D = morin + Dutasteride/Tamsulosin group

DISCUSSION

In this study, it was observed that DUT-TAM induced liver changes and altered the antioxidant status. However, co-treatment of DUT-TAM with morin offers protection against the observed hepatic damage. The significantly elevated plasma bilirubin level in the DUT-TAM treated animals suggests impairment in hepatic function. Morin administration with DUT-TAM was able to attenuate liver impairment occasioned by elevated plasma bilirubin level. Increased plasma bilirubin occurs when the liver cells are damaged, and there is obstruction of the intra and extra-cellular biliary tracts (Tredger and Sherwood, 1997; Renner, 2003).

Plasma aminotransferases (ALT and AST) are routinely used for assessing the integrity of the liver. The activities of these marker enzymes are usually raised above normal level when there is damage or necrosis to the hepatic cells (Zimmerman and Seeff, 1970). However, ALT is more specific for the liver tissue because the other enzymes and metabolites could be released as a result of damage to other organs. The elevated plasma ALT and AST by DUT-TAM is an indicator of hepatotoxicity. Co-treatment with morin plays highly significant protective role by attenuating the liver impairment brought about by the elevated plasma AST and ALT occasioned by DUT-TAM. Treatment with morin has been reported to prevent liver damage and to inhibit over-expression of radicals induced by various inflammatory stimuli (Milenkovic *et al*., 2012).

DUT-TAM treatment also led to a significant reduction in the activity of hepatic SOD and catalase. Morin was however able to ameliorate this decrease. The increase level of SOD activity after co-treatment with morin implies that morin is able to enhance the level of production of this antioxidant enzyme in the liver of rats. Catalase and SOD co-operate to prevent the deleterious effects of free radicals. Superoxide dismutase catalyzes the conversion of superoxide radical to hydrogen peroxide, while hydrogen peroxide is converted to innocuous oxygen and water. Accumulating hydrogen peroxide concentration inactivates SOD activity and this may leave the liver exposed to more hydrogen peroxide and hydroxyl-radical induced oxidative damage (Adaramoye *et al*., 2005). Morin, a free radical scavenger, decreases the levels of oxidative stress markers (Milenkovic *et al*., 2012). Ascorbic acid is a known primary antioxidant (Frei, 1991), and this vitamin is thus an important systemic antioxidant which effectively mops up toxic free radicals in the body (Olayinka and Ore, 2014), but at the same time it is possibly very vulnerable to oxidation (Buettner and Moseley, 1993). From different studies, the systemic level of ascorbic acid has been shown to be a function of the systemic level of GSH (Stocker *et al*., 1986). DUT-TAM treatment significantly decreased the level of ascorbic acid, which in turn implies that an overwhelming effect of DUT-TAM-induced oxidative stress is expressed on systemic antioxidant indices. However, morin offers significant protection against the observed DUT-TAM-induced hepatic oxidative stress.

DUT-TAM induced reduction in hepatic GST activity and GSH. Glutathione-S-transferase uses GSH in the metabolism and detoxification of various compounds, such as carcinogens and other xenobiotics, as well as various free radicals (Farombi *et al*., 2002). Glutathione conjugation is thus a protective mechanism by the body through which noxious radicals are scavenged as GSH conjugates. The reduced activity observed for GST is in tandem with hepatic GSH diminution; validating that DUT-TAM-induced toxicity is associated with systemic reduction of GSH (Aniya and Naito, 1993). Generally, GSH has been used as an important factor in assessing tissue susceptibility to oxidative onslaught, and the reduction in the level of GSH confirms the toxicity of chemicals (Adewole and Adebayo, 2017).

DUT-TAM induces significant increase in malondialdehyde (MDA) levels. Elevated hepatic MDA levels suggests an increased peroxidation of lipids (Olayinka *et al*., 2015). Generally, it has been shown that lipid peroxidation causes disruption of membrane structure, and thus, disruption of membrane fluidity and functions which may consequently lead to cell death (Devi *et al*., 2004). On treating with morin, the increase in the level of MDA was ameliorated. Thus, from investigations, morin has the capacity to inhibit oxidative stress

caused by peroxidation of lipid by inhibiting the lipid peroxidation processes.

CONCLUSION

From this study, it may be suggested that dutasteride/tamsulosin impedes hepatic antioxidant system and causes degenerative changes. However, morin, a very potent antioxidant positively modulates the effect of the drug on the antioxidant status by ameliorating the oxidative damage and effectively protects against dutasteride/tamsulosin-induced hepatic oxidative stress, possibly as a result of its intrinsic antioxidant properties. The result obtained from this study serves as a basis for further studies to validate the protective effect of morin on DUT-TAM-induced hepatic oxidative stress in other experimental models, especially in human subjects.

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