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Research Article

AMIODARONE INDUCED OXIDATIVE STRESS IN STRESS - VULNERABLE ORGANS OF ADULT MALE RATS

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ABSTRACT

Objective: Amiodarone used as an antiarrhythmic agent bears a structural resemblance to thyroid hormones containing about one-third iodine by weight. The pro-oxidant potentialities of amiodarone induced changes were studied.

Materials and Methods: Male adult Wister rats were divided into two groups of eight animals each, and amiodarone was supplemented orally for 30 days against control. The urinary iodine content of both the groups was measured. Animals were sacrificed after completion of treatment; investigated parameters were adrenal morphology and histology, adrenal Δ^5 3β hydroxy steroid dehydrogenase (HSD) and serum cortisol level. Superoxide dismutase (SOD), catalase and lipid peroxidation (LPO) level were assayed in the liver, kidney and testis along with their histology. Serum glutamic-oxaloacetic transaminase (SGOT) and glutamic-pyruvate transaminase (SGPT) were measured. Obtained results were interpreted against the control group of rats.

Results: Urinary iodine level was high after the amiodarone exposure. Hypertrophied cortex with enhanced Δ^5 3β HSD activity in adrenal caused elevated serum cortisol level. Amiodarone exposure had increased LPO level with a concomitant rise in catalase and SOD activities in liver, kidney and testis in comparison to control ($p<0.001$). Simultaneously kidney showed shrinkage of the glomerulus, in liver the area surrounding the central canal found disrupted and in the testis seminiferous tubules, and germ cells were disorganized in comparison to control. SGOT and SGPT level were found elevated in the treated group.

Conclusion: Amiodarone exposure develops stress for the metabolism and deiodinization of amiodarone releasing excessive iodine in circulation that in turn generates reactive oxygen species and free radicals resulting cellular damage of stress vulnerable organs.

Keywords: Amiodarone, Excess iodine, Hypertrophied adrenal, Cellular damage, Stress-vulnerable organs, Reactive oxygen species.

INTRODUCTION

Amiodarone is a benzofuran derivative containing a phenol moiety with two atoms of covalently bound iodine having structural resemblance to thyroid hormones [1]. It is amphiphilic with both hydrophobic portions presented by tertiary amine, for this reason it is highly lipophilic drug. The drug is highly soluble in chloroform and methanol but poorly soluble in water [2]. It is used worldwide for treatment of cardiac dysfunction such as arrhythmia. In spite of its therapeutic effect, it has multi organ side effects [3]. The commercial availability of amiodarone is in tablet as well as an intravenous formulation. Amiodarone has been reported as an enhancing agent in free radicals generation and also in mitochondrial hydrogen peroxide production [4]. It has relatively high iodine content; about 37% of its molecular mass is occupied by iodine [5]. Chronic treatment is associated with 40-fold increases in plasma and urinary iodide levels [1]. Each molecule of amiodarone contains two iodine atoms. It is estimated that amiodarone metabolism in liver releases approximately 3 mg of inorganic iodine to the systemic circulation per 100 mg of amiodarone [6]. The average iodine content in human male diet is about 0.15 mg/day [7]. Thus, a daily dose of 100 mg amiodarone intake can certainly increase iodine load in the physiological system.

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. ROS form as a natural by-product of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. Examples include oxygen ions and peroxides. Cumulative effects of ROS may result in significant damage to cell structures and have been implicated as an underlying agent in various pathological conditions [8]. ROS are also generated by exogenous sources such as ionizing radiation [9]. There are also reports on excessive iodine induced ROS mediated oxidative stress [1,10].

During oxidative stress, the oxidation exceeds the antioxidant symptoms in the body secondary to a loss of the balance between them [11]. It causes hazardous events such as lipid peroxidation (LPO), oxidative DNA damage etc. To ameliorate this cellular oxidative stress human/animal body has an intricate antioxidant defense mechanism facilitated by superoxide dismutase (SOD), catalase and glutathione – enzymes that are important in the elimination of free radicals and are considered to play a significant role in quenching ROS and thereby display a modulatory role in different diseased condition [8].

Information on amiodarone that bears a structural resemblance to thyroid hormones containing 39% iodine by weight induced generation of ROS and associated disorders in stress vulnerable organs are scanty. The current study thus examines the effects of the amiodarone exposure on the development of oxidative stress if any in stress vulnerable organs as adrenal, kidney, liver, and testis.

MATERIALS AND METHODS

Reagents

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), HCL, chloroform was purchased from Sisco Research Laboratories (SRL), Mumbai, India; bovine serum albumin (BSA), ethylenediaminetetraacetic acid (EDTA), $MnCl_2$, isooctane, cortisol were purchased from Sigma Chemical Company, Steinheim, Germany; Ethanol, ß-estradiol and triton X-100 used in the study are of analytical grade and were was purchased from LOBA Chemie Pvt. Ltd, Mumbai, Maharashtra, India; nicotinamide adenine dinucleotide phosphate (NADPH), EDTA-$MnCl_2$, Marcaptoethanol from Alfa Aesar.

Maintenance of animals

3-month-old adult male albino rats of the Wistar strain weighing 110 ± 10 g were used. The animals were maintained according to national

guidelines and protocols, and the study was approved by the Institutional Animal Ethics Committee. The animals were housed in hygienic polypropylene cages as well as maintained in a controlled environment at temperature 22°C ± 2°C and relative humidity (40-60%) in an animal house with a constant 12 hrs light/12 hrs dark schedule. The animals were fed a standardized diet, which consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined sesame oil, 0.25% shark liver oil, and water ad libitum [12]. The rats were divided into two groups of eight animals each: The experimental group were orally administered amiodarone for 30 days and was paired with a control group. Control rats were fed on a normal laboratory standardized diet whereas experimental rats received amiodarone. Feed consumption, corrected for wasted feed was noted regularly whereas body weights were measured after every seven days. During the last week of the treatment, animals in both groups were kept in metabolic cages for 24 hrs to collect urine over xylene for the analysis of iodine.

All the animals were sacrificed 24 hrs after the last feeding (i.e., during 9-10 am on the day of the experiment to avoid any discrepancy that may arise for diurnal variation) following standard protocols and ethical procedures. Blood samples were collected, and serum was kept separated for hormone assay.

Dose of amiodarone

Amiodarone at the dose of 0.17 mg/kg body weight/day [13]was given orally for 30 days, dissolving in 5% dextrose solution because of its solubility in organic solvents (Amiodarone was collected in the 100 mg and 200 mg ampoule, from registered medical stores). The control group was provided with the same quantity of water.

Organ weight

Just after sacrifice kidney, adrenal and testis were dissected out and weighed. The relative weight of organs (g/mg) was expressed per 100 g body weight.

Histological studies

The adrenal gland, testis, kidney and liver after dissection were fixed in bouins fluid for 24 hrs. These were processed and embedded in paraffin wax. 5 µ thick sections were obtained on cutting in microtome. These sections were then stained by hematoxylene and eosin and examined under light microscope.

Measurement of LPO (in the liver, kidney and testis)

Tissue was taken in 1 mL of phosphate buffer and was homogenized at 4°C. Following homogenization, 1 mL homogenate was mixed thoroughly with TBA-TCA-HCL mixture. The solution was heated for 10 minutes. After cooling the precipitate was removed by centrifugation at 5000 rpm for 30 minutes. The absorbance of the sample was determined at 435 nm against a blank that contained all the reagents minus the tissue homogenate following the method of Ohkawa *et al.* [14].

Measurement of catalase activity (in the liver, kidney and testis)

Tissue was quickly removed and washed in ice cold normal saline, dried on filter paper. Homogenization was carried out at 4°C in ice cold 0.2 M sucrose solution. The crude homogenate was centrifuged at 3000 rpm for 20 minutes at 4°C. The pallet was discarded, and the supernatant was further centrifuged at 8000 rpm for 30 minutes. The post mitochondrial supernatant thus obtained was utilized for the catalase measurement after the method of Aebi, [15].

Measurement of SOD activity (in the liver, kidney and testis)

Tissue was taken in phosphate buffer and was homogenized. The crude homogenate was centrifuged at 2500 rpm for 30 minutes. Then in the cuvette the following solutions were subsequently added - 1 ml TDA, 60 µl NADPH, 40 µl EDTA-$MnCl_2$ and 0.5 mL sample. In the control, the sample was replaced by equal volume of the medium used for enzyme solution. The absorbance was taken at 640 nm over a 5 minutes period. Marcaptoethanol was added later, and the decrease in absorbance was noted for about 10 minutes following the method of Marklund and Marklund [16].

Measurement of adrenal Δ^5 3β- hydroxyl steroid dehydrogenase (HSD) activity

The tissue was homogenized with homogenizing fluid containing 20% spectroscopic grade glycerol, 5 mM potassium phosphate and 1 mM EDTA at a tissue concentration of 100 mg/mL homogenizing mixture and centrifuged at 10,000 rpm at a constant temperature of 4°C. The supernatant was used for the assay purpose. The activity was determined by optical measurement of the rate of reduction of NAD. The reaction system contain in a final volume of 3.0 mL. 100 µM of sodium pyrophosphate, 0.5 Mm of NAD, 30 µg of substrate of 3β each in 0.02 mL of purified doxin and a suitable quantity of enzyme (200-500 µl) to initiate the reaction at final pH of 9.1. The reactions were carried out in the silica cuvettes of 1.0 cm light path, in a spectrophotometer at 340 mµ absorbance. The activities were measured at 15 seconds interval against a blank containing all components except the steroid. One unit of enzyme activity is the amount causing change in absorbance of 0.001/min when enzyme serves as substrate [17].

Fluorometric determination of serum cortisol [18]

Standard cortisol solution was prepared by dissolving 1 mg cortisol in 2.5 ml ethanol and volume was made up to 25 mL by distilled water. In 10 mL glass stopper extraction tube 0.5 mL serum was taken. For blank 0.5 mL distilled water and for standard 0.4 µg and 0.08 µg cortisol/0.5 mL distilled water were taken respectively in separate extraction tube. In each tube 1.5 mL isooctane was added, mixed thoroughly and was centrifuged for separating the layers. The top isooctane layer was discarded by aspiration. Then to each tube 3 mL chloroform was added and mixed thoroughly on a vortex for extraction and centrifugation. The top aqueous layer was discarded by aspiration. Then 0.25 mL of 0.1 N sodium hydroxide was added to each tube, mixed well and centrifuged. The top layer was discarded by aspiration. 2.5 mL of chloroform was taken in a separate set of 5 mL glass stopper tube containing 3 mL of acid alcohol mixture was mixed and centrifuged. Then 2.5 mL of bottom layer from each tube was taken in the cuvette and was kept for 45 minutes. The fluorescence was measured with the spectrofluorometer at 462 µm (excitation) and 518 µm (emission) wave length by setting the fluorometer at an arbitrary point of 80 with high standard (0.08 µg). The concentration of cortisol (µg/dl) was calculated by multiplying the conc. of standard with sample.

Estimation of urinary iodine level

Urine sample was digested followed by subsequent ashing, and iodine was measured by its catalytic action on the reduction of ceric ion (Ce^{++}) to cerous ion (Ce^{3+})[19], maintaining internal quality control.

Protein estimation

Proteins were estimated by the method of Lowry *et al.* [20] using BSA as the standard protein.

Statistical analysis

Results were expressed as mean ± standard deviation. Differences between-group was established using t-test. A value of $p<0.001$ was interpreted as statistically significant. Statistical analyses were performed using Origin 89 (Northampton, MA, USA) and MS-Office Excel 2007 software packages.

RESULTS

Body weight

The body weight of the control animals increased progressively throughout the period of investigation, with a net body weight gain of +44.178% (Table 1). However, the net body weight gain of the animals fed with amiodarone was only +16.78% at the end of the total experimental period.

Food intake and water consumption

Total food consumption by weight was similar in all the groups of experimental and control animals. Average food consumption of the

control rats was 14.08 ± 0.83 g/day and that of the rats administered with amiodarone was 13.55 ± 0.76 g/day; similarly when the water intake pattern was recorded, the average intake in control group was 10.15 ± 0.63 mL/day/rat whereas amiodarone treated group was 9.67 ± 0.57 ml/day/rat respectively (Table 2).

Adrenal, testis and kidney weight

The weight of adrenal glands was significantly increased (p<0.001) after amiodarone administration as compared to their respective control group (Table 3) while the relative testicular and kidney weight were significantly reduced in the experimental groups (p<0.001).

Serum glutamic-pyruvate transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) activities

SGPT and SGOT activities were increased significantly (p<0.001) when compared to amiodarone treated group after the exposure (Table 4).

LPO level in liver, kidney and testis

LPO level was significantly increased (p<0.001) in the liver, kidney and testis of amiodarone-treated rats as compared to their respective control (Table 5).

Table 1: Amiodarone induced alteration in percentage of gain in body weight

Groups	Initial body weight (g)	Final body weight (g)	Percentage of gain in body weight (g%)
Control	112.86±4.1	162.72±5.4	44.178
Amiodarone treated	116.25±5.5	135.67±6.2	16.785*

Values are mean±SD of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001) denoted by asterisk. SD: Standard deviation

Table 2: Amiodarone induced alteration in food intake and water consumption

Groups	Food intake (g/day/rat)	Water consumption (ml/day/rat)
Control	14.08±0.83	10.15±0.63
Amiodarone treated	13.55±0.76	9.67±0.57

Values are mean±SD of 8 rats. When the comparison between the two was done following t-test, no significant difference between the two groups was found. SD: Standard deviation

Table 3: Amiodarone induced alteration in relative weight of the adrenal gland, testis, kidney

Group	Adrenal gland weight (mg)	Testis weight (g)	Kidney weight (g)
Control	11.728±0.73	1.504±0.094	0.638±0.03
Amiodarone treated	14.788±0.92*	1.38±0.086*	0.594±0.02*

Values are mean±SD of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001) denoted by asterisk. SD: Standard deviation

Table 4: Amiodarone induced alteration in SGPT and SGOT activities

Group	SGPT activity IU/l	SGOT activity IU/l
Control	1.31±0.13	1.51±0.75
Amiodarone treated	1.94±0.51*	1.87±0.98*

Values are mean±SD of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001) denoted by asterisk. SGPT: Serum glutamic-pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SD: Standard deviation

Antioxidant enzyme activities

Figs. 1 and 2 demonstrates antioxidant enzyme activities *viz*. SOD and catalase in liver, kidney and testis of control and amiodarone treated experimental animals. Amiodarone administration caused a statistically significant (p<0.001) stimulation in the activity of those enzymes over the respective control values.

Adrenal 3β HSD activity

Adrenal 3β HSD was significantly (p<0.001) increased in the amiodarone exposed groups compared to controls (Fig. 3).

Urinary iodine excretion pattern

The urinary iodine content of amiodarone treated group was 41.25 ± 2.21 µg/dl and in the control group was 32.03 ± 1.78 µg/dl and was significantly (p<0.001) increased compared to control (Fig. 3).

Histopathological studies of liver, kidney, testis and adrenal

Histological assessments performed on studied organs from the different groups are presented in Plate 1. Adrenal cell mass showed a hypertrophied cortex upon comparison to control. The seminiferous tubules and germ cells in the testis were disorganized whereas in kidney shrinkage of glomerulus and in the liver the area surrounding the central canal was disrupted in the treated groups as compared to control groups.

DISCUSSION

Amiodarone, a structural analogue of thyroid hormone, is a well-known anti-arrhythmic agent used in the treatment of various kinds of life threatening cardiac arrhythmias and ultimately in the prevention of death caused due to cardiac arrest [21]. Long term use of amiodarone can induce adverse effects on various organ systems such as kidney, liver, testis, skin, adrenal, thyroid, alveolar tissue and even in myocardial tissue [22]. However, effects of amiodarone as cellular/molecular stressor are less highlighted in the available literature. In this investigation, attempt was made to study the effect of the amiodarone exposure on some stress vulnerable organs such as adrenal, liver, testis and kidney by evaluating certain invasive and non-invasive parameters after its exposure.

The body weight gain percentage was significantly decreased in amiodarone treated rats as compared to the control group . Similar observations were also reported in other studies [23]. The accumulation of amiodarone occurs mostly in adipose tissue [24] because of its lipid solubility. The underlying pathophysiology of this observation is due to excess iodine as incorporated in the amiodarone molecule (37.5% of organic iodine by molecular weight), which may cause thyroid dysfunction manifesting by hypothyroidism or hyperthyroidism and are characterized clinically by decreased growth and weight loss respectively. In hypothyroid condition, both syntheses of proteins and growth rate is retarded; as in the later, a large amount of the energy produced is dissipated as heat, with less in the form of the required energy-rich phosphate compound hence resulting in weight loss despite a normal appetite [25]. However, the food intake and water consumption showed no significant difference in amiodarone exposed rats when compared to their respective control groups.

Table 5: Amiodarone induced alteration in LPO in liver, kidney and testis

Group	(nmole TBARS/mg protein)		
	LPO in liver	LPO in kidney	LPO in testis
Control	9.05±1.74	8.78±1.86	4.1±0.61
Amiodarone treated	14.95±1.88*	13.72±2.25*	13.3±1.12*

Values are mean±SD of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001) denoted by asterisk. LPO: Lipid peroxidation, SD: Standard deviation, TBRAS: Thiobarbituric acid reactive substances

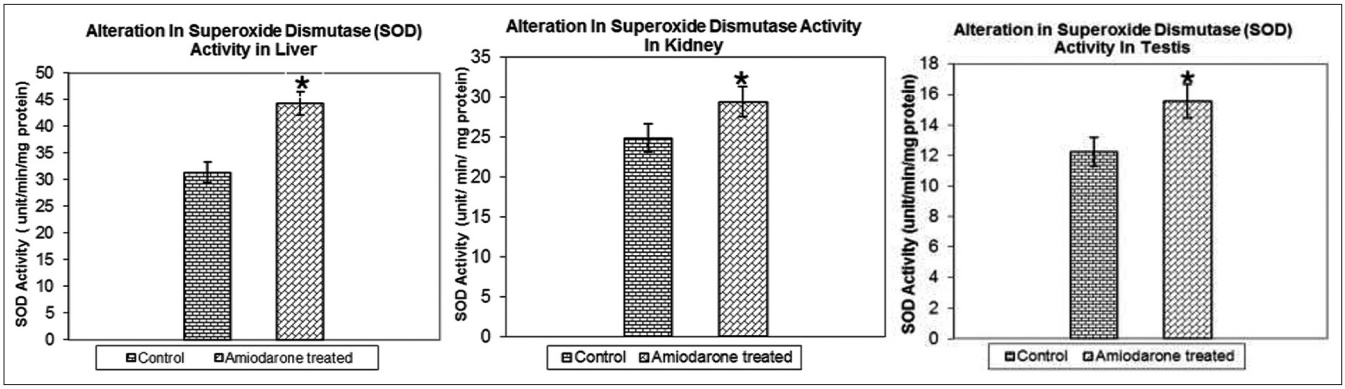

Fig. 1: Changes in amiodarone-induced alterations in superoxide dismutase in different organs. Values are mean ± standard deviation of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001)

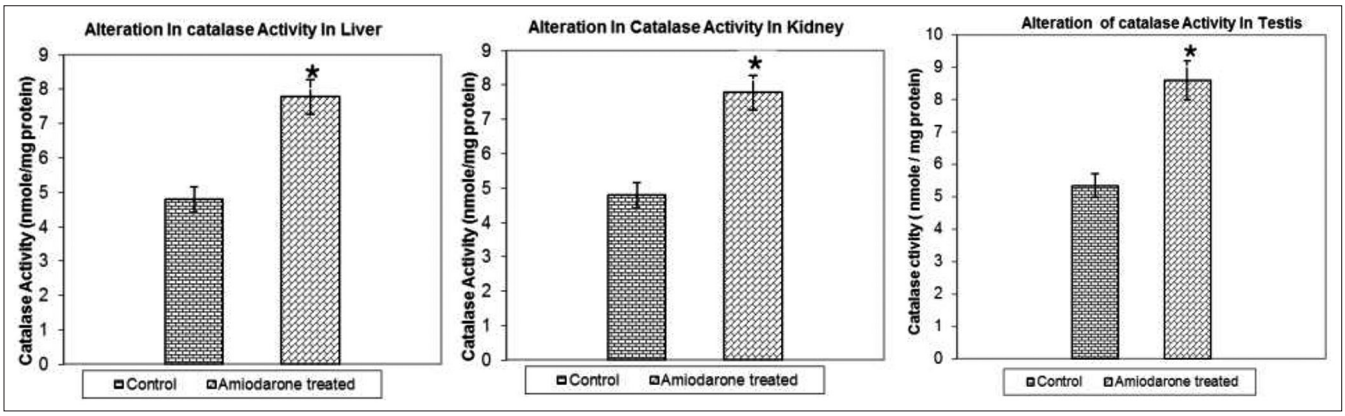

Fig. 2: Changes in amiodarone induced alterations in catalase in different organs. Values are mean ± standard deviation of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001)

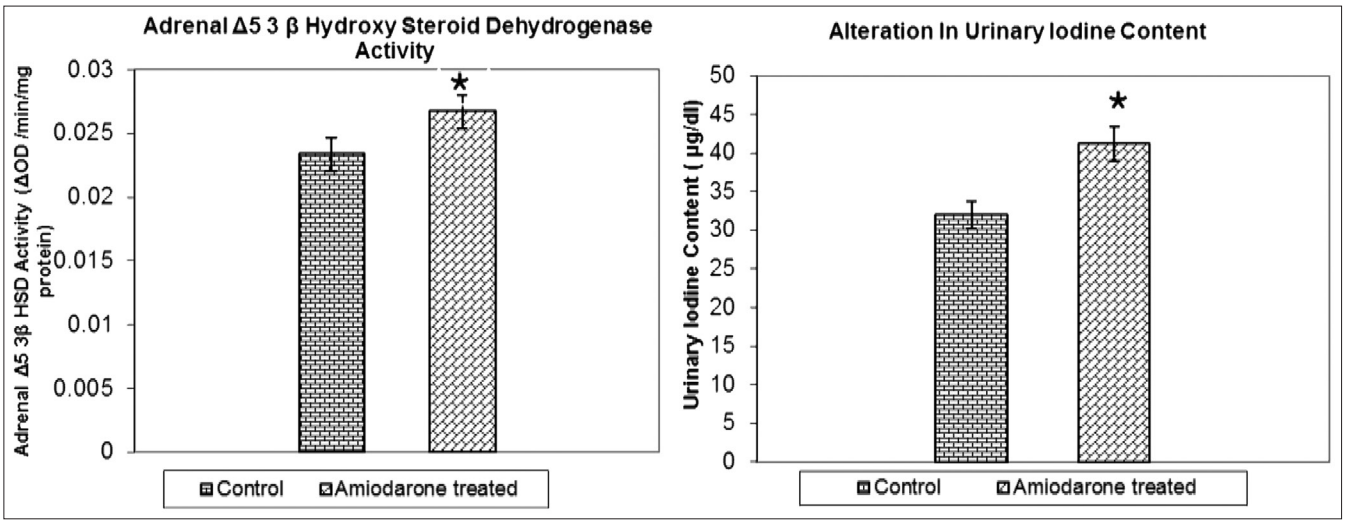

Fig. 3: Changes in amiodarone-induced alteration in adrenal 3 β hydroxy steroid dehydrogenase activity and urinary iodine content in experimental groups compared to control groups. Values are mean ± standard deviation of 8 rats. When the comparison between the two was done following t-test, a significant difference between the two groups was found (p<0.001)

The weight of the adrenal gland was found to be increased in amiodarone treated group of rats in comparison to the control group. The increase in adrenal weight may be for the development of stress induced by excess iodine in amiodarone resulting in the release of more glucocorticoids through hypothalamo-pituitary-adrenal axis [26]. Similar observations were also found in studies on calves feeding excess iodine [27]. Amiodarone has been reported to induce pheochromocytoma, an adrenal gland tumor [28]. However the weight of kidney was decreased in rats treated with amiodarone, which may be for the increase in urinary N-acetyl-glucosamine and alkaline phosphatase excretion that corresponds with tubular alterations resulting in weight reduction and was confirmed by electron microscopy [29]; testis weight was also concomitantly decreased that corresponds to the studies of Dobs *et al.* [30] who reported atrophic

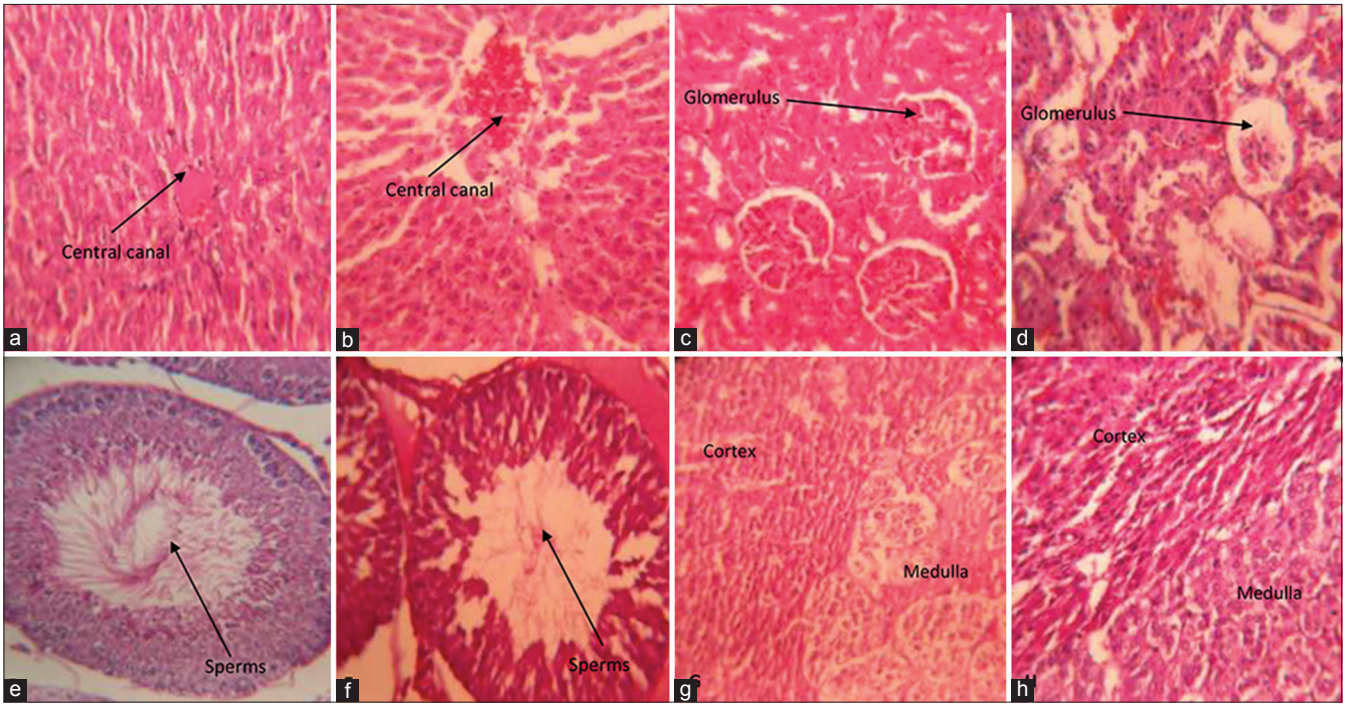

Plate 1: Photomicrographs of paraffin-embedded H and E - stained sections (a) control liver, (b) treated liver; (c) control kidney, (d) treated kidney, (e) control testis, (f) treated testis, (g) control adrenal, (h) treated adrenal

testes commonly observed in amiodarone-treated men possibly for the reduction of spermatogenesis, which ultimately results in a decrease in testis weight since it is very vulnerable to oxidative stress [31].

Adrenal Δ^5 3β HSD is a regulatory enzyme that converts 17 hydroxy pregnenolone to 17 hydroxy progesterone, which leads to the production of cortisol in the steroidogenic pathway [32]. Δ^5 3β HSD was measured in adrenal gland and found that its activity was increased in treated group as compared to control group, suggesting that the this Δ^5 pathway is stimulated under the influence of amiodarone, which is in line with the earlier findings that during stress the weight of this endocrine gland is increased and adrenal Δ^5 3β HSD activity is elevated [33]. In this study, the cortisol level was found to be increased, and it is the reflection of higher activity of rate limiting enzyme though the stimulation of hypothalamic pituitary adrenal axis. It might be for the free radicals that are produced during the metabolism of amiodarone liberating excess iodine. It has been previously reported that amiodarone, containing excess iodine generates free radicals *in vitro* and caused a significant increase of NADPH and Fe^{3+} induced LPO in the liver microsomal fraction [34].

LPO in liver, kidney and testis were increased significantly in amiodarone treated animals measured by tissue thiobarbituric acid reactive substances production as compared to the respective control group. As widely known, LPO refers to oxidative degradation of lipids. It is a process in which free radicals steal electrons from the lipid in the cell membrane. LPO is one of the adverse effects of amiodarone therapy that occurs due to free radical mediated chain oxidation [35]. Malonaldehyde (MDA) levels thus formed indicate the intoxication and generation of oxidative stress in those organs. Excess iodine has been proved to generate stress in adrenal [26] and also in some target tissues of thyroid hormone such as hepatocytes [36]. The amiodarone when deiodinized after metabolism releases excess iodine in circulation, that might generate free radicals and those in the liver, kidney and testis can enhance the rate of LPO evidenced by high MDA levels. In our present study, MDA levels in liver, kidney and testis increased significantly in comparison to control.

The ROS that are generated as a result of metabolism of amiodarone are counteracted by antioxidant defense systems e.g. glutathione peroxidase, SOD, catalase etc. present in the physiological system. Amiodarone treated animals showed a higher level of SOD activity; SOD catalyses the dismutation of superoxide [O_2^-] ions into oxygen and hydrogen peroxide. They are important antioxidant marker in nearly all cells exposed to oxygen and destroy the highly reactive oxygen radical by converting it into less reactive hydrogen peroxide [37]. As proposed earlier [10], the free radicals production after administration of high dose of iodide could overshadow the normal cellular defense mechanism against those free radicals. Excess iodine has been reported to cause oxidative stress and changes in SOD activities. Hence, the amiodarone induced oxidative damage followed by free radical formation, which may concomitantly increase the SOD activity as observed in this study in order to neutralize those ROS in these organs.

On the other hand, catalase is the enzyme, which is present mainly in the peroxisomes of mammalian cells and increased catalase activity parallels to an increase in hydrogen peroxide. In our study, catalase activity of kidney, liver and testis was found to be significantly increased in amiodarone treated group as compared to control the group. Amiodarone has already been known to be highly lipophilic and is concentrated in many tissues and cells, including hepatocytes in the liver and thus iodide are subsequently released over a long period [38]. The cause of amiodarone-induced hepatotoxicity appears to be for the direct damage to lipid bilayers and disturbance of lysosomal and/or mitochondrial function suggesting mitochondrial injury and dysfunction [39] that has been reflected by high SGOT and SGPT activities compared to their controls. The kidneys are vulnerable to damage as a result of perfusion and the increased concentrations of excreted compounds that occur in renal peri-tubular cells as a result of amiodarone administration [40]. The elevated level of catalase in those organs is to protect the cell against oxidative stress induced by amiodarone. An increase in catalase activity has been suggested to reflect a high production of radicals such as H_2O_2 [40]. These results demonstrate that amiodarone accumulates in the kidneys of rats that in turn and release excess iodine causing marked alterations in the activity of this antioxidant enzymatic activity. Excess iodine released from amiodarone causes toxic effects in different organs [41] by generating rather producing free radicals due to its pro-oxidant nature that in-turn increases catalase activity as observed in this study.

Histoarchitectural alterations of adrenal gland, liver, kidney and testis were observed in treated groups as compared to control. In adrenal, cell mass and cell size increased associated with hypertrophied cortex; seminiferous tubules and germ cell layer were disorganized in testis; glomerulus were shrinked in the kidney cortex and in liver the area surrounding the central canal found disrupted in the amiodarone-induced groups possibly for the development of cellular oxidative stress after amiodarone exposure. Amiodarone has been suggested to cause ultra structural effects on the thyroid gland and cytokine production [41-43]. These findings are consistent with the observations of our study.

Urinary iodine concentration in amiodarone treated groups found significantly high as 37% of its total molecular weight contains iodine [5]. Amiodarone is metabolized releasing free iodine in circulation, which results in iodine overload, the causative factor for the cellular and biochemical alterations of the studied organs as found in this study. As 90% of the body's iodine is excreted through urine [44] and thus, the iodine excretion pattern in amiodarone exposed group was found high, this may also be due to its extremely long terminal half-life [1].

Therefore amiodarone not only develops stress in adrenal but also in other important organs as the liver, kidney and testis as evidenced by their cellular changes as well as their enhanced SOD, catalase activities and LPO levels with increased SGOT and SGPT activities. It may thus be concluded that chronic amiodarone exposure develops cellular oxidative stress in stress vulnerable organs for the metabolism and deiodinization of amiodarone releasing excessive iodine in circulation that in turn might form ROS and free radicals resulting cellular damage.

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