Melatonin supplementation in rat ameliorates ovariectomy-induced oxidative stress

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Key words: MELATONIN, OXIDATIVE STRESS, CORTICOSTERONE, ESTROGEN REPLACEMENT THERAPY, OVARIECTOMY

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ABSTRACT

Objective The present study aims to determine the potential of melatonin supplementation in ameliorating tissue oxidative stress, elevated serum corticosterone and hepatic and renal dysfunction.

Materials and methods Adult Wistar rats, either ovariectomized or sham-operated, served as experimental or control groups, respectively. Rats received either melatonin, estrogen, progesterone or a combination of melatonin and estrogen for a period of 15 days. Tissue oxidative stress, serum markers of hepatic and renal dysfunction and serum corticosterone level formed the parameters of assay in all groups at the end of the treatment schedule.

Results Ovariectomized rats showed significant increases in levels of tissue lipid peroxidation, serum levels of glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase, acid phosphatase and corticosterone and significant decrement in enzymatic and non-enzymatic antioxidant status. All parameters showed maximal reversal to control levels on supplementation with high-dose melatonin or estrogen + melatonin treatment.

Conclusion Melatonin supplementation proved better than estrogen replacement therapy, with the higher dose being more effective in preventing ovariectomy-induced increases in oxidative stress and serum levels of marker parameters of hepatic and renal dysfunction and corticosterone titer. Overall, melatonin supplementation therapy qualifies as a more potent and safe alternative to estrogen replacement therapy in alleviating postmenopausal increases in oxidative stress and hepatic and renal dysfunction.

INTRODUCTION

Oxidative stress plays a pivotal role in the pathogenesis of various diseases including atherosclerosis, diabetes, neurodegerative disorders, cancer and aging[1,2]. The generated free radicals lead to alterations in various enzymes, cellular signaling proteins and cell membrane composition[2]. Estrogen deficiency, following natural menopause or surgical menopause (ovariectomy), has been shown to be associated with an increase in the production of lipid peroxides and a deficient antioxidant defence, resulting in the pathogenesis of several alterations that commonly affect menopausal women[3,4].

Gonadal hormones and oxidative stress share an interesting relationship, well established by some recent studies[5,6]. With reference to oxidative stress-induced cardiovascular impairment in postmenopausal women, there have been reports suggesting a significant decrease in oxidative protein damage under hormone replacement therapy (HRT)[7,8]. Still other studies have reported inhibition of formation of lipid peroxides in liver tissue *in vitro* by estrogen supplementation in bilaterally ovariectomized rats[9]. However, recent *in vitro* studies on antioxidant potentials of estrogen are not adequately supportive of *in vivo* models that tend to provide conflicting results[10].

Melatonin, a known and powerful free radical scavenger, can act at two levels: either by scavenging the free radicals generated directly, similar to the action of antioxidants, or by inducing expression and activity of major antioxidant

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enzymes[2]. Due to its highly acclaimed antioxidant potentials, melatonin has found wide application in various age-related diseases such as Alzheimer's, Parkinson's, diabetes mellitus, and rheumatoid arthritis[2]. Melatonin, recognized as an anti-aging hormone, shows an actual decline in association with menopause[11]. There are unjustified reports of melatonin as a replacement agent in postmenopausal women implicating the need for focused studies[12,13].

There are no reports on the competence of melatonin to alleviate surgical menopause-induced oxidative stress. Further, there is also a dearth of studies involving a combination of melatonin and estrogen as a replacement/supplementation therapy. Therefore, the present study in this context evaluates the dose-dependent potential of melatonin supplementation, individually as well as in combination with estrogen, in combating ovariectomy-induced oxidative stress in major organs and increases in serum corticosterone titer and markers of hepatic and renal dysfunction.

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MATERIALS AND METHODS

Experimental animals

Female albino Wistar rats of 200–250-g body weight were used for the study. Animals were maintained under a 12 : 12 h light : dark cycle and 21–23°C temperature regimen throughout in accordance with CPCSEA guidelines. The animal experiments were approved by the animal ethical committee of the Department of Zoology, The M.S. University of Baroda, Vadodara (approval no 827/ac/04/CPCSEA). Throughout the experimental period, the animals were provided with standard rat chow (M/S Pranav Agro Limited, Baroda) and water *ad libitum*.

Ovariectomy

Experimental animals underwent bilateral ovariectomy through a single mid-ventral incision under appropriate anesthesia[14]. For sham operation, ovaries were located by inducing a ventral incision but were not removed from the body. Following surgery, animals received postoperative care and rest for 20 days for recovery and abatement of residual sex steroid levels in the circulation.

Experimental design

Animals for experimentation consisted of eight groups of six rats each:

(1) Group I (SO): sham-operated control animals treated with saline (vehicle) for 15 days;
(2) Group II (OVX): ovariectomized (OVX) animals administered saline (vehicle) for 15 days;
(3) Group III (OVX + E2): OVX animals given 17β-estradiol (E2) replacement (i.p.) at a dose of 30 μg/kg body weight daily for a period of 15 days;
(4) Group IV (OVX + P4): OVX animals given progesterone (P4) replacement (i.p.) at a dose of 20 mg/kg body weight daily for a period of 15 days;
(5) Group V (OVX + ML): OVX animals given low-dose melatonin (ML) as an alternative supplementation (i.p.) at a dose of 1 mg/kg body weight daily for a period of 15 days;
(6) Group VI (OVX + MH): OVX animals given high-dose melatonin (MH) supplementation (i.p.) at a dose of 10 mg/kg body weight daily for a period of 15 days;
(7) Group VII (OVX + E2 + ML): OVX animals given melatonin (1 mg/kg) and E2 (30 μg/kg) daily for a period of 15 days;
(8) Group VIII (OVX + E2 + MH): OVX animals given melatonin (10 mg/kg) and E2 (30 μg/kg) daily for a period of 15 days.

Biochemical estimations

At the end of the experimental period, animals were food-deprived overnight and sacrificed by decapitation. Blood was drawn from the jugular vein and serum separated for various assays. Liver, muscle, kidney, uterus and adipose tissue were dissected, washed in saline and blotted dry before weighing.

Evaluation of oxidative stress involved assessing the levels of lipid peroxidation and enzymatic and non-enzymatic antioxidants. Lipid peroxidation was determined as per the method of Beuge and Aust[15], reduced glutathione (GSH) by the method of Beutler and colleagues[16], superoxide dismutase (SOD) by the method of Marklund and Marklund[17], catalase by the method of Sinha[18] and glutathione peroxidase (GPx) by the method of Rotruck and colleagues[19].

Other biochemical parameters and hormones were assayed using relevant kits: corticosterone, estrogen, progesterone (Immuno-Technology & Steroid Laboratory Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, Munirka, New Delhi), serum glutamic pyruvic transaminase (SGPT, Agappe Diagnostics Ltd.), serum glutamic oxaloacetic transaminase (SGOT, Crest Biosystem Ltd.), alkaline phosphatase (ALP; Rekon diagnostics Pvt Ltd.), acid phosphatase (ACP; Aspen Laboratories), urea (DiaSys Diagnostic Systems GmbH and Co, Germany) and creatinine (Nicholas Piramal India Limited, India).

Statistical analysis

Statistical analysis of the data involved one-way ANOVA followed by Bonferroni's multiple comparison tests. The values represent mean ± standard error of the mean as derived by the use of Graph Pad Prism version 3.0 for Windows (Graph Pad Software, San Diego, CA, USA).

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RESULTS

Serum markers of hepatic dysfunction

Ovariectomy resulted in significant increases in serum levels of SGPT, SGOT, ALP and ACP (Table 1). Treatments with 17β-estradiol, progesterone or melatonin showed a decrease in the levels of these marker enzymes but significant decrement occurred only with MH and melatonin–estrogen combinations.

Serum markers of renal dysfunction

Serum levels of urea and creatinine, markers of renal dysfunction, registered significant increases in ovariectomized rats compared to sham-operated controls (Table 2). Both 17β-estradiol and progesterone had similar effects on the levels of both these markers and could reduce levels to a lesser degree while the most significant normalizing action was observed in the OVX + E2 + MH group followed by the OVX + MH, OVX + E2 + ML and OVX + ML groups in that order.

Serum corticosterone

There was a significant increment ($p < 0.001$) in serum corticosterone titer in ovariectomized rats compared to sham-operated control rats (Table 2). Melatonin supplementation therapy (MST) and ERT decreased serum corticosterone levels in ovariectomized animals, with MH being maximally effective in restoring corticosterone to the normal level. All other treatments (ML, E2 + ML, E2 + MH) were equally effective but to a lesser degree compared to MH.

Lipid peroxidation

Ovariectomized animals showed significant increases in the levels of lipid peroxidation in the three tissues compared to sham-operated rats (Figure 1). Maximal decrease in lipid peroxidation in liver occurred with the combination of MH + E2. Next in effectiveness was MH alone or a combination of ML and E2. The muscle lipid peroxidation level in ovariectomized animals was reversed fully by both MH supplementation and by the MH + E2 combination. Renal lipid peroxidation was, however, reduced maximally by MH alone (even below the sham-operated level) in comparison to other treatment groups.

Tissue glutathione and ascorbic acid status

Levels of glutathione (GSH) and ascorbic acid (AA) in all three organs registered a significant decrease in ovariectomized rats, with the depletion of the former being more prominent than that of the latter (Figures 2 and 3). Restoration of GSH contents in general was equally effective by either E2 replacement, MH supplementation or MH + E2 supplementation. Significant depletion in AA content due to ovariectomy occurred only in the kidney compared to liver and muscle. The efficacy of various treatments to increase tissue AA content was in the order MH and MH + E2 > ML + E2 > ML and E2.

Activities of glutathione peroxidase, catalase and superoxide dismutase

Ovariectomized animals showed significant decrements in the activities of all three enzymes in all three organs (Figures 4–6).

Table 1 Serum markers of hepatic dysfunction in control and experimental groups. Data are given as mean ± standard error

	SGPT (U/l)	*SGOT* (U/l)	*ALP* (U/l)	*ACP* (U/l)
SO	52.00 ± 1.15	74.33 ± 2.32	199.33 ± 0.88	9.33 ± 0.24
OVX	59.67 ± 0.88**	81.33 ± 0.88	208.33 ± 3.27	11.67 ± 0.88
OVX + E2	55.00 ± 2.07	77.33 ± 1.20	201.67 ± 2.32	10.85 ± 0.16
OVX + P4	51.00 ± 1.15††	78.67 ± 1.45	205.33 ± 0.33	11.36 ± 0.38
OVX + ML	56.67 ± 0.33	76.67 ± 0.66	206.00 ± 2.51	11.33 ± 1.20
OVX + MH	54.33 ± 1.20	75.00 ± 1.72	202.00 ± 0.57	7.93 ± 0.63
OVX + E2 + ML	55.00 ± 0.57	75.67 ± 2.72	198.67 ± 4.04	10.23 ± 0.19
OVX + E2 + MH	53.00 ± 2.30†	69.67 ± 1.20†††	191.67 ± 2.59†††	8.52 ± 0.44†

**, $p < 0.01$ when compared to sham-operated controls; †, $p < 0.05$; †††, $p < 0.001$ when compared to ovariectomized animals

SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; ALP, alkaline phosphatase; ACP, acid phosphatase; SO, sham-operated controls given saline; OVX, ovariectomized (OVX) animals given saline; OVX + E2, OVX animals given 17β-estradiol (E2) 30 μg/kg; OVX + P4, OVX animals given progesterone (P4) 20 mg/kg; OVX + ML, OVX animals given low-dose melatonin (ML) 1 mg/kg; OVX + MH, OVX animals given high-dose melatonin (MH) 10 mg/kg; OVX + E2 + ML, OVX animals given melatonin (1 mg/kg) + E2 (30 μg/kg); OVX + E2 + MH, OVX animals given melatonin (10 mg/kg) and E2 (30 μg/kg)

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Table 2 Serum levels of corticosterone, urea and creatinine in control and experimental rats. Data are given as mean ± standard error

	Corticosterone (mg/dl)	*Urea* (mg/dl)	*Creatinine* (mg/dl)
SO	8.36 ± 0.30	37.33 ± 1.20	0.44 ± 0.02
OVX	12.62 ± 0.26***	46.00 ± 1.16***	0.54 ± 0.02*
OVX + E2	10.11 ± 0.11**,†††	42.78 ± 0.33	0.44 ± 0.03
OVX + P4	9.70 ± 0.42*,†††	42.33 ± 2.03	0.46 ± 0.02
OVX + ML	10.59 ± 0.29***,†††	41.67 ± 0.88	0.46 ± 0.01
OVX + MH	8.11 ± 0.08***,†††	39.33 ± 0.88††	0.41 ± 0.01†††
OVX + E2 + ML	10.13 ± 0.18†††	42.90 ± 1.49	0.41 ± 0.02†††
OVX + E2 + MH	10.63 ± 0.35†††	38.67 ± 0.88††	0.39 ± 0.02†††

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ when compared to sham-operated controls; ††, $p<0.01$; †††, $p<0.001$ when compared to ovariectomized animals
For abbreviations, see footnote to Table 1

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As a point of interest, muscle seemed to register maximal levels of all the enzymes compared to liver and kidney. In general, the recovery to control levels of all the enzymes in all the organs was best with MH supplementation followed by MH + E2 and ML + E2. Both ML and E2 were least but equally effective.

DISCUSSION

Ovariectomized animals serve as appropriate *in vivo* models to mimic postmenopausal pathophysiological alterations in women[20,21]. Menopause, marked by estrogen deficiency, precipitates remarkable metabolic alterations and oxidative stress, which can lead to various organic illnesses including cardiovascular disorders and bone loss[22,23]. A hypoestrogenic status as in ovariectomized animals contributes to body weight gain, adiposity, metabolic alterations affecting carbohydrates and lipids, as well as predisposition to diabetes due to insulin insensitivity[23,24]. Many of the organic disorders in estrogen-deficient postmenopausal women may be credited to increased oxidative stress, as E2 reportedly has antioxidant potentials. The clear need in the postmenopausal phase in this context would be to contain the escalated oxidative stress to prevent the various serious predicaments/predispositions. The most obvious recourse would be hormone replacement therapy (HRT), which has beneficial effects even on neurobehavioral aspects[25,26]. However, the role of E2 *in vivo* as an anti-oxidant is beset with many contradictory findings and remains at best controversial[27,28]. Moreover, a glaring pharmacological disadvantage of E2 as an antioxidant is its inability to pass through the blood–brain barrier[29]. Critical studies on ERT have reservations on long-term use of E2, thereby suggesting short-term ERT in menopausal women[30,31]. In lieu of this, it is pertinent to look for alternatives for the safer treatment of postmenopausal women.

Melatonin fulfils the criteria as an efficient natural antioxidant with obvious advantages such as solubility in both lipids and water and ability to cross the blood–brain barrier, thereby being unique amongst antioxidants[32]. Its competence to

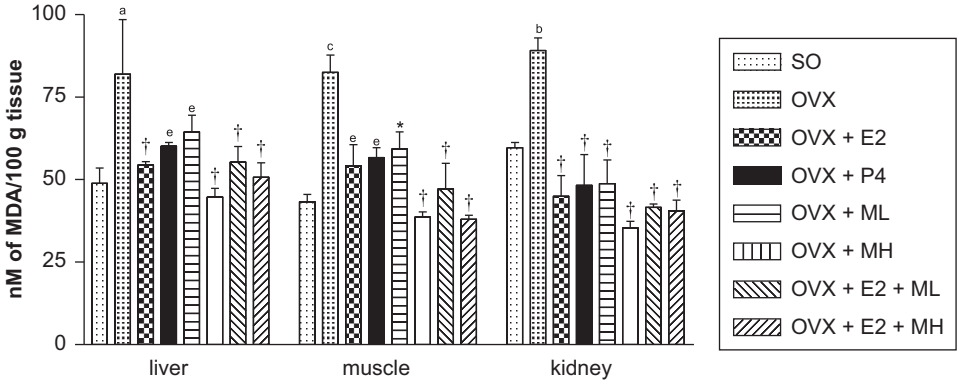

Figure 1 Levels of lipid peroxidation (malondialdehyde assay, MDA) in liver, muscle and kidney of control and experimental rats. Data are given as mean ± standard error. a, $p<0.05$; b, $p<0.01$; c, $p<0.001$ when compared to sham-operated controls. *, $p<0.05$; e, $p<0.01$; †, $p<0.001$ when compared to ovariectomized animals. For abbreviations, see footnote to Table 1

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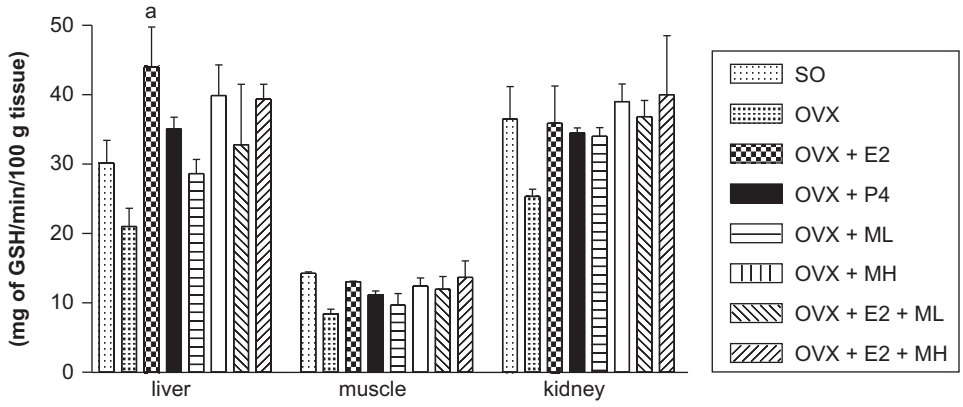

Figure 2 Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats. Data are given as mean ± standard error. a, $p < 0.05$ when compared to sham-operated controls. For abbreviations, see footnote to Table 1

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contain toxicant-induced oxidative stress and associated neurodegenerative disorders, cardiovascular diseases and diabetes merits serious consideration[33]. The present study on MST and its comparison with ERT and a combination of MST and ERT in containing hepatic, muscle and renal oxidative stress post-ovariectomy is in this regard a continuation of our previous evaluation of melatonin in diabetogenic alterations in ovariectomized rats[24]. Our present observations clearly show that MST is very potent and effective in reversing ovariectomy-induced tissue oxidative stress. In keeping with the difficult nature of melatonin in terms of dosage and time of administration gleaned from studies from our laboratory[24,34], two doses (low and high) of melatonin have been employed together with combinations with E2 to compare the effects with ERT.

Our data clearly indicate a nearly doubled increase in lipid peroxidation in liver, muscle and kidney due to ovariectomy. There are reports suggesting increased lipid peroxidation in various tissues following ovariectomy[34,35]. Estrogen is also reported to protect the liver against ischemic/reperfusion injury[36,37]. A decline in ovarian steroid output with aging (< 50 pg/ml) in this context has greater relevance in aging-associated increase in lipid peroxidation. Increased oxidative stress marked by increased lipid peroxidation in ovariectomized rats is substantiated by the observed decrease in endogenous antioxidants. Relatively, the observations of Feng and Zhang[22] of a 50% reduction in brain mitochondrial GSH content and of Oztekin and colleagues[39] of a 25% reduction in hepatic GSH content lend support to our observations of differential changes in GSH content. In fact, the depletion in both GSH and AA seen in our study clearly suggests a prominent role for female sex steroids in resisting the induction of oxidative stress. These changes in non-enzymatic antioxidants parallel the significant decrement in enzymatic antioxidants. Muscle seems to have greater resistance to oxidative stress, as can be deduced from the higher levels of enzymatic antioxidants. Rat liver and bone seem susceptible to greater oxidative stress by way of decreased SOD and GPx after ovariectomy[38]. A decrease in catalase activity in uterus of ovariectomized rats has been mentioned in the literature[39]. A study in late

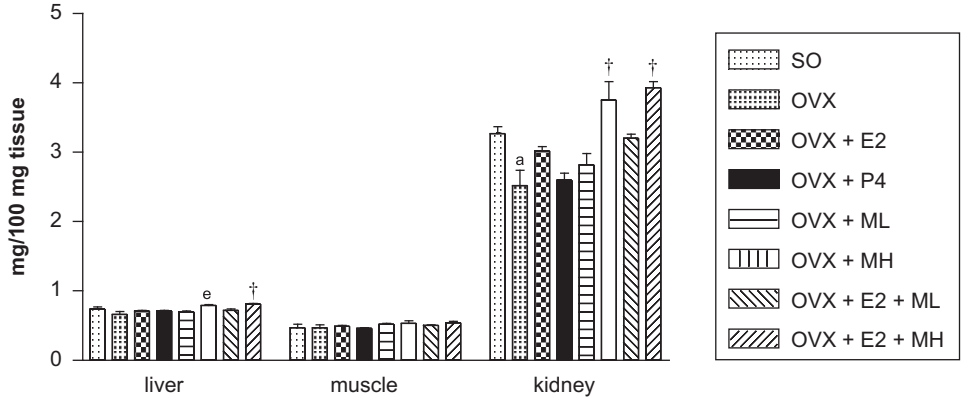

Figure 3 Ascorbic acid content in liver, muscle and kidney of control and experimental rats. Data are given as mean ± standard error. a, $p < 0.05$ when compared to sham-operated controls. e, $p < 0.01$, †, $p < 0.001$ when compared to ovariectomized animals. For abbreviations, see footnote to Table 1

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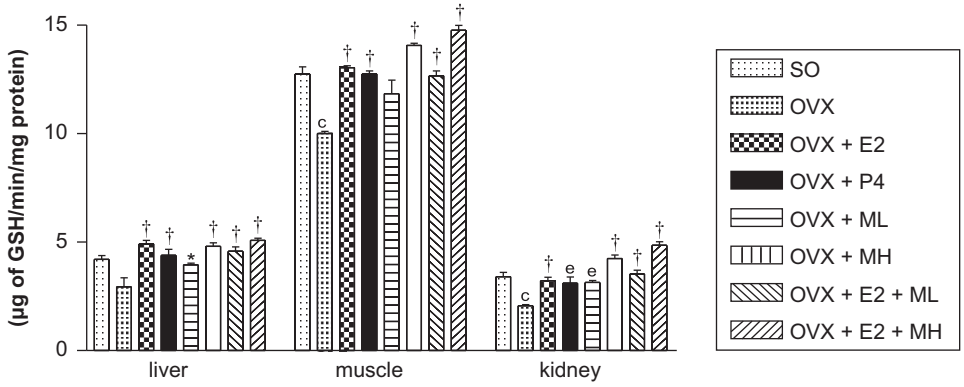

Figure 4 Glutathione peroxidase activity in liver, muscle and kidney of control and experimental rats. Data are given as mean ± standard error. b, $p < 0.01$; c, $p < 0.001$ when compared to sham-operated controls. *, $p < 0.05$; e, $p < 0.01$; †, $p < 0.001$ when compared to ovariectomized animals. For abbreviations, see footnote to Table 1

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menopausal women has observed significantly increased GPx activity in blood compared to premenopausal women[40–43]. Further, Sontakke and Tare[44] recorded a significant decrement in GPx and SOD activity in osteoporotic postmenopausal women, as has also been observed by Maggio and colleagues[45]. Decreased catalase and GPx activities, as witnessed herein, could result in compromised hydrogen peroxide inactivation from the reported inhibition of mitochondrial oxidation by way of decreased activity of complex I and complex IV by ovariectomy[22]. It is likely that superoxide anion concentration would increase, and the observed decrease in SOD activity can further compound the problem. Evidence for inhibition of catalase by superoxide radicals is available[44] and this can result in accumulation of H_2O_2 and consequent SOD inhibition. Inhibited SOD and GPx activities together can escalate the upswing in superoxide radicals. In fact, Muthusami and colleagues[40] have reported an increase in H_2O_2 concentration in bones of ovariectomized animals. The set of changes delineated herein suggests the creation of a vicious circle of increased load of reactive oxygen species (ROS), leading to greater oxidative stress as the long-term consequence.

Compromised antioxidant status with a tendency towards higher pro-oxidant to antioxidant ratio in liver and kidney is likely to erode their functional competence and cause a certain degree of dysfunction. Increased levels of serum markers of hepatic renal dysfunction, observed in the course of the present study, are suggestive of the same. There are hardly any studies that have evaluated the possible effects of estrogen deficiency on hepatic and renal functions in females. We have recently shown a parallel correlation between oxidative stress and hepatic and renal functional impairment in diabetic animals[33]. It is pertinent to note that reports have appeared in the literature demonstrating an age-dependent increase in cytokine production and altered hepatic structure and function[45,46]. Apart from an age-dependent increase, ovariectomy and menopause also contribute to increased cytokine production[3,47]. A mutually protective interrelationship between ROS-mediated oxidative stress and pro-inflammatory cytokines can be inferred from the many reports in this context[48] and, as such, a parallel increase in both has been demonstrated[3]. Even though there are no reports on a ROS–cytokine interrelationship in renal tissue, the same may be

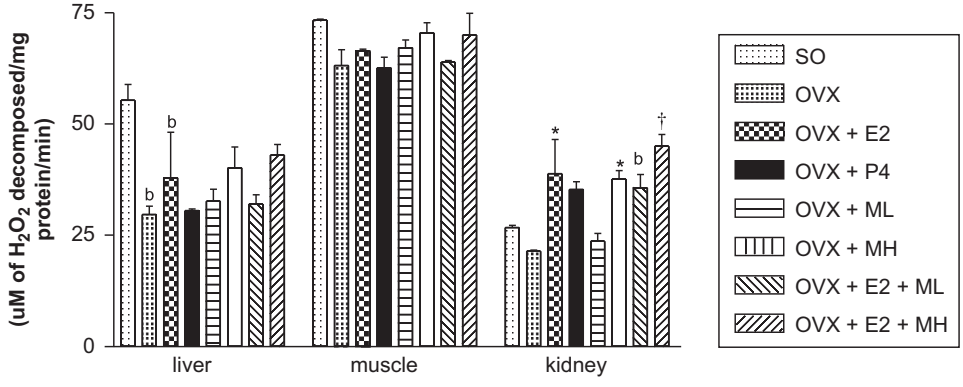

Figure 5 Catalase activity in liver, muscle and kidney of control and experimental animals. Data are given as mean ± standard error. b, $p < 0.01$ when compared to sham-operated controls. *, $p < 0.05$; †, $p < 0.001$ when compared to ovariectomized animals. For abbreviations, see footnote to Table 1

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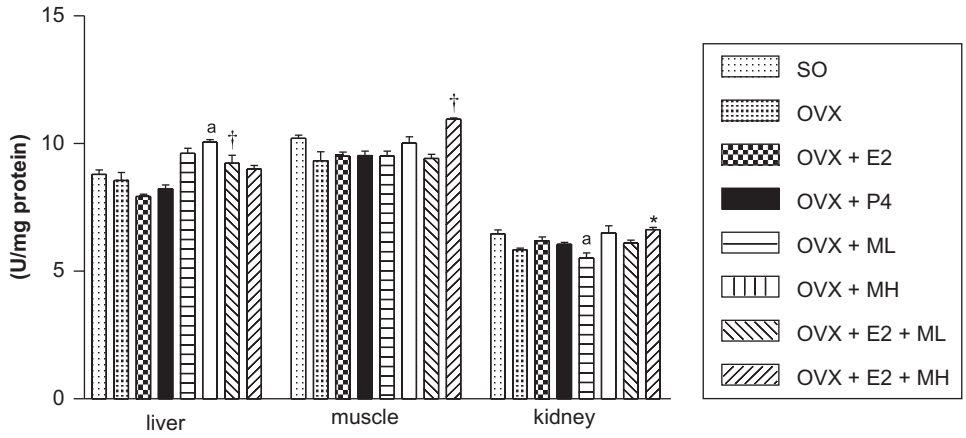

Figure 6 Superoxide dismutase activity in liver, muscle and kidney of control and experimental rats. Data are given as mean ± standard error. a, $p < 0.05$ when compared to sham-operated controls. *, $p < 0.05$; †, $p < 0.001$ when compared to ovariectomized animals. For abbreviations, see footnote to Table 1

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deduced from the herein observed elevation in serum markers of renal function. Apparently, in the light of the reports cited above and the present observations of increased serum markers of hepatic and renal dysfunction, structural and functional alterations of these organs may be inferred in E2-deficient females either due to ovariectomy or menopause.

A major marker of chronic stress in animals and humans is corticosterone/cortisol. The elevated serum cortisol level recorded in ovariectomized rats in the current study confirms the prevalence of heightened stress. Elevated serum cortisol levels are characteristic of aged rats[49] and human subjects[50]. Central disturbances in the hypothalamic–pituitary–adrenal (HPA) feedback system are suggested as being responsible for the age-associated increase in cortisol titer[49]. This defect in the HPA axis, related to decreased sensitivity of hypothalamic glucocorticoid receptors and failure to suppress secretion of adrenocorticotropic hormone (ACTH), thereby contributes to unregulated cortisol secretion from the adrenals[51]. Accordingly, aging-related elevation in cortisol levels, due to altered negative feedback on the HPA axis, has been noted in both animals[52] and humans[53] with such changes being more pronounced in females than in males[54]. Predictably, cortisol, in a cause-or-effect relationship with ROS and cytokines, can contribute to heightened oxidative stress under conditions of estrogen deficiency.

Reversal of sex steroid deficiency in ovariectomized rats by either estrogen or progesterone replacement has revealed a mitigating effect on the cortisol level to the same extent, although not fully brought down to the ovary-intact level. The fact that both estradiol and progesterone can lower the serum cortisol level to the same degree suggests a similarity of action of both sex steroids by improving negative feedback on the HPA axis. Support for this contention comes from the reports of estradiol interference on the activity of neurotransmitters that regulate secretion of corticotropin releasing factor[55] and of estradiol upregulating adrenocorticoid steroid receptors in the hippocampus[56]. Both estrogen and progesterone replacement have also shown reversible effects on endogenous antioxidants (non-enzymatic and enzymatic) and oxidative stress as denoted by lipid peroxidation, although estrogen seems to be more effective than progesterone replacement. Conceivably, estradiol is a more dominant sex steroid in improving anti-oxidant status than progesterone. Although there is significant improvement in endogenous antioxidant status and levels of lipid peroxidation in all three tissues of ovariectomized rats, estrogen replacement is apparently not efficient in recovering the status to the pre-ovariectomy level. Some other workers have also reported the ability of sex steroid replacement to redress the compromised antioxidant system and lipid peroxidation[37,57]. Full reversal seems to need higher replacement doses of estradiol, as deducible from a couple of previous studies[22,37]. This is untenable in the light of the known negative consequence of ERT with a higher dose or a long-term application, as discussed earlier.

Dose-dependent MST, attempted in the present study as an alternative to ERT, provides substantial evidence for its possible use as it has shown significant alleviating effects, with the higher dose being successful in fully nullifying the effects of ovariectomy in all aspects and at times creating a status even better than in ovary-intact animals. A comparison of all the schedules employed clearly portrays MH to be more effective than the others. A possible reason for the inability of ERT to nullify completely the ovariectomy-induced changes could be a concurrent decrease in the melatonin level in ovariectomized animals as estradiol reportedly brings about a secretion of melatonin and even modulates its production throughout the estrous cycle[22,58]. As melatonin is a powerful antioxidant[59] with a modulating influence on the endogenous antioxidant system, a decrease in melatonin titer subsequent to ovariectomy is likely to have an additive effect and hence the need for a higher dose of ERT for offsetting the changes induced by a deficiency in both the hormones. In this context, pinealectomy is shown to increase lipid peroxidation in various tissues of rat[32], while a combination of pinealectomy and ovariectomy further

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intensifies the oxidative stress[37]. Concurrently, melatonin administration decreases lipid peroxidation and increases the levels of antioxidants[59]. In this light, our current observations on full reversal of ovariectomy-induced changes by MH is very pertinent and provides compelling evidence for MST as an effective and better alternative to ERT in overcoming postmenopausal whole-body oxidative stress and hepatic and renal dysfunction. The recorded reversal of the serum levels of markers of hepatic and renal dysfunction due to MST in ovariectomized animals further suggests rectification of hepatic and renal functional impairments due to E2 deficiency. The dose-dependent effect of melatonin as seen herein is substantiated by the observation of Feng and Zhang[22] of a dose-dependent efficacy of melatonin in lowering lipid peroxidation, ROS and RON species and cytokines in the liver of ovariectomized rats. Obviously, the present study provides evidence for the usage of a high dose of MST as an efficient and safe alternative to ERT in postmenopausal women. There is ample justification available for the use of high doses of melatonin as no known pharmacological effects have ever been found[60,61].

The efficacy of melatonin in overcoming ovariectomy-induced oxidative stress, also related to the concomitant decrement in cortisol titer to the pre-ovariectomy level, receives support from many studies[62]. Further, melatonin also exhibits an inhibitory influence on ACTH secretion[63]. In conclusion, it can be said that MST with a standardized dose is more potent in combating ovariectomy/postmenopausal estrogen deficiency symptoms and has many other known favorable effects on menopause-induced sleep disorders, reduced immune functions, cardiovascular disorders, dementia and neuropathy[22,43]. Apparently, MST qualifies as a potent and safe alternative to ERT in alleviating postmenopausal symptoms to help maintain a healthy outlook for women.

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