MELATONIN SUPPLEMENTATION IS MORE EFFECTIVE THAN ERT OR EVEN A COMBINATION OF THE TWO IN AMELIORATING OVARIECTOMY INDUCED TISSUE OXIDATIVE STRESS, HEPATIC AND RENAL DYSFUNCTIONING AND SERUM CORTICOSTERONE LEVEL

Oxidative stress plays a pivotal role in pathogenesis of various diseases including atherosclerosis, diabetes, neurodegerative disorders, cancer and aging (Strehlow *et al.*, 2003; Tomás-Zapico and Coto-Montes, 2007). Thus free radicals generated lead to alterations in various enzymes, cellular signaling proteins and of cell membrane components (Tomás-Zapico and Coto-Montes,2007). Estrogen deficiency, under 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 defense resulting in pathogenesis of several alterations that commonly affect menopausal women (Kumru *et al.*, 2005; Kireev *et al.*, 2008).

Gonadal hormones and oxidative stress share an interesting relationship, well established by some recent studies (Kaya *et al.*, 2004; Prediger *et al.*, 2004). With reference to oxidative stress induced cardiovascular impairment in post menopausal women there are reports suggesting a significant decrease in oxidative protein damage under HRT (Behl *et al.*, 2002; Bhavnani, 2003). Still other studies have reported inhibition of formation of lipid peroxides in liver tissue *in vitro* by estrogen supplementation in bilaterally ovariectomized rats (Huh *et al.*, 1994). However, recent *in vitro* studies on antioxidant potentials of estrogen are not

fully supported by *in vivo* models that tend to provide conflicting results (Ozgonul *et al.*, 2003).

Melatonin is known to be a powerful free radical scavenger that can act at two levels; either scavenging the free radicals generated directly, similar to the action of antioxidants or, induce expression and activity of major antioxidant enzymes (Tomás-Zapico and Coto-Montes, 2007). Due to its highly acclaimed antioxidant potentials, melatonin has found wide application in various age related diseases like Alzheimers, Parkinson`s, Diabetes mellitus, Rheumatoid arthritis etc (Tomás-Zapico and Coto-Montes, 2007). Melatonin is evidenced to be an anti-aging hormone and an actual decline in melatonin secretion in association with menopause is reported (Sandyk *et al.*, 1992). There are unjustified reports on melatonin as a replacement agent in post-menopausal women implicating the need of focused studies (Brzezinski, 1998; Wakatsuki *et al.*, 2001) .

In view of the lacunae on studies justifying the role of melatonin in alleviating oxidative stress in surgical menopause and the absence of reports employing a combinational replacement approach of melatonin and estrogen, the present study is designed to evaluate the role of melatonin supplementation on a dose dependent basis individually and in combination with estrogen on various oxidative stress markers in three major organs, markers of hepatic and renal dysfunction and serum Corticosterone levels.

Results

Serum Corticosterone (Cort): (Table and fig 8)

There was significant increment ($p<0.001$) in serum Cort titre in OVX rats compared to sham operated control rats. The Cort level was fully restored to normally (even better) in MH supplemented OVX rats found to be better than even E_2 in recovering the Cort level. All other treatments (ML, E_2+ML, E_2+MH) were all equally effective but lesser in degree compared to MH.

Lipid peroxidation: (Table 1 and Fig 1)

Ovariectomized animals showed significant increase in levels of lipid peroxidation of all the three tissues compared to sham operated rats. Hepatic lipid peroxidation was decreased maximally in animals subjected to a combination of MH supplementation and E_2 replacement. Next in effectiveness to reduce lipid peroxidation were the groups supplemented with MH alone or subjected to a combination of ML and E_2. The muscle LPO level of OVX animals were restored fully by both MH supplementation and MH+E_2 combination schedule. Renal LPO was however reduced maximally by MH alone in comparison to other treatment groups bringing it even below the sham operated control level.

Tissue non enzymatic anti oxidant status: (Tables 3,5 and Figs 3,5)

Glutathione and Ascorbic acid

Both GSH and AA contents of all the three organs were significantly decreased in OVX rats. However, the depletion in GSH content was more prominent than that of AA. Replacement of GSH contents in general was equally effective in E_2 replaced, MH supplemented as well as MH+E_2 supplemented groups of OVX rats. In general, AA depletion compared to GSH

subsequent to OVX was not significant except for kidney. The increase in tissue AA content registered in various OVX rats supplemented/replaced with M and or E_2 was in general in the order MH and MH+E_2>ML+E_2>ML and E_2.

Tissue enzymatic antioxidant status: (Tables 2, 4, 6 and Figs 2, 4 , 6)

Activities of GPx, CAT and SOD

The activities of all the three enzymes in all the three organs were significantly decreased in OVX animals. As a point of pristine interest, muscle was found to have 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 seen best in MH supplemented OVX rats followed by MH+E_2 and ML+E_2. Both ML and E_2 were least but equally effective.

Serum markers of renal dysfunction: (Table and fig 8)

Serum levels of urea and creatinine as markers of renal dysfunction were significantly increased in ovariectomized rats as compared to the sham operated rats. Both E2 and P4 had similar effects on the levels of both these markers and could reduce it to a smaller extent while the most significant normalizing action was observed in the OVX+E2+MH group followed by OVX+MH, OVX+E2+ML and OVX+ML respectively.

Markers of hepatic dysfunction: (Table and Fig 7)

As a result of ovariectomy there was a marked increment in the activities of all the marker enzymes SGPT, SGOT, ALP and ACP to a significant extent. Treatments with either estrogen, progesterone or melatonin low dose showed a decrease in these marker enzymes but the decrease was significant only in the melatonin high dose and combinational groups of estrogen and melatonin

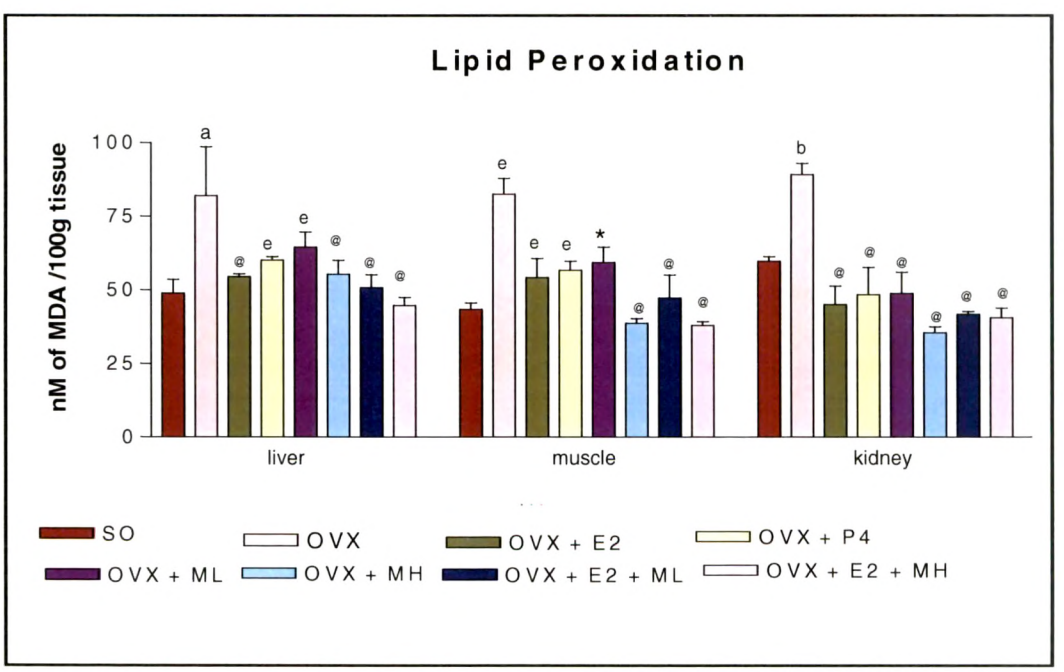
treatments in OVX animals showing almost a near normal value for these enzymes.

Table 1: Levels of lipid peroxidation (MDA) in liver, muscle and kidney of control and experimental rats.

LIPID PEROXIDATION (nM of MDA /100g tissue)			
GROUPS	LIVER	MUSCLE	KIDNEY
SO	48.86±4.7	43.33±2.2	59.71±1.6
OVX	81.97± 16.58[a]	82.54± 5.28[e]	89.16± 3.80[b]
OVX +E_2	54.40 ±0.98[@]	54.20± 6.36[e]	44.90± 6.24[@]
OVX +P_4	60.10± 1.2[e]	56.73± 2.9[e]	48.26± 9.3[@]
OVX +ML	64.45± 5.1[e]	59.31± 5.17[*]	48.78± 7.16[@]
OVX +MH	55.30± 4.79[@]	38.60± 1.65[@]	35.30± 2.05[@]
OVX +E_2+ML	50.67 ± 4.5[@]	47.18±7.7[@]	41.59± 1.0[@]
OVX +E_2+MH	44.74± 2.6[@]	37.96± 1.3[@]	40.55± 3.3[@]

Data are expressed as Mean±SE
[b]p<0.01when compared to sham operated control and [*]p<0.05, [e]p<0.01, [@]p<0.001 when compared to ovariectomized animals.

Fig 1: Levels of lipid peroxidation (MDA) in liver, muscle and kidney of control and experimental rats.



Data are expressed as Mean ± SE
[b]p<0.01when compared to sham operated control and [*]p<0.05, [e]p<0.01, [@]p<0.001 when compared to ovariectomized animals.

Table 2: Glutathione peroxidase (GPx) activity in liver, muscle and kidney of control and experimental rats.

GLUTATHIONE PEROXIDASE (μg GSH/min/mg protein)			
GROUPS	LIVER	MUSCLE	KIDNEY
SO	4.21 ± 0.174	12.75± 0.33	3.40± 0.208
OVX	2.94± 0.419^{c}	10.00± 0.105^{c}	2.07± 0.045^{c}
OVX +E_2	4.92± 0.159$^{@b}$	13.04± 0.089$^{@}$	3.22± 0.154$^{@}$
OVX +P_4	4.39 ± 0.271$^{@}$	12.75± 0.137$^{@}$	3.10± 0.29^{e}
OVX +ML	3.99± 0.072*	11.85± 0.627	3.14 ±.084^{e}
OVX +MH	4.80 ± 0.17$^{@b}$	14.07± 0.093$^{@c}$	4.25± 0.164$^{@b}$
OVX +E_2+ML	4.58± 0.196$^{@}$	12.66± 0.226$^{@}$	3.53 ± 0.176$^{@}$
OVX +E_2+MH	5.08 ± 0.1$^{@b}$	14.77 ± 0.2$^{@c}$	4.86± 0.2$^{@b}$

Data are expressed as Mean±SE
^{b}p<0.01, ^{c}p<0.001 when compared to sham operated control and ^{e}p<0.01, $^{@}$p<0.001 when compared to ovariectomized animals.

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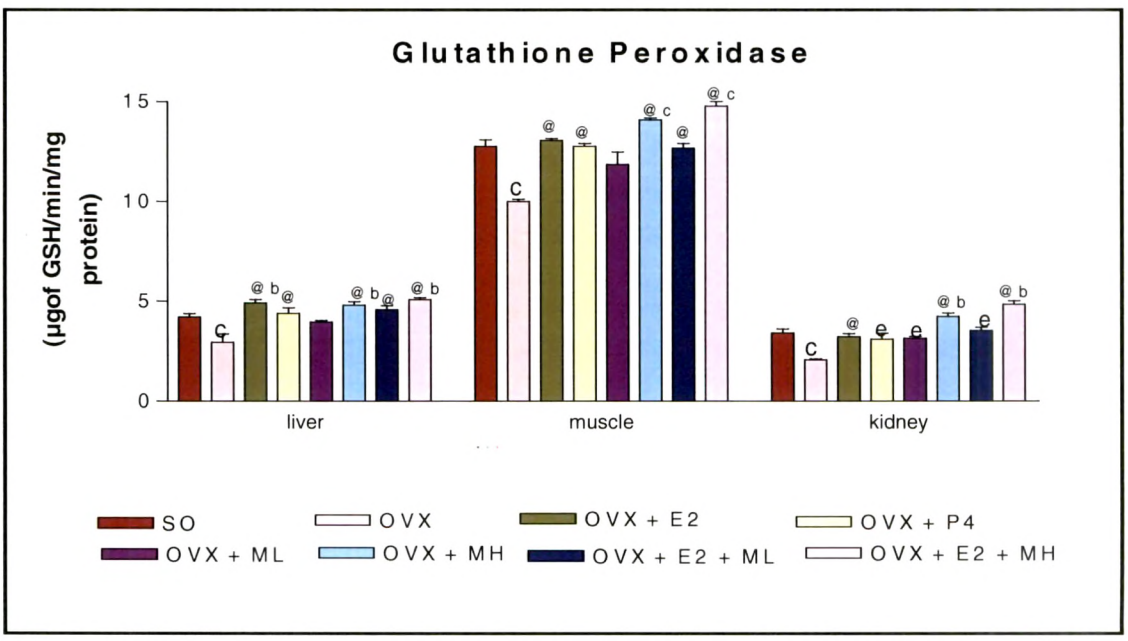


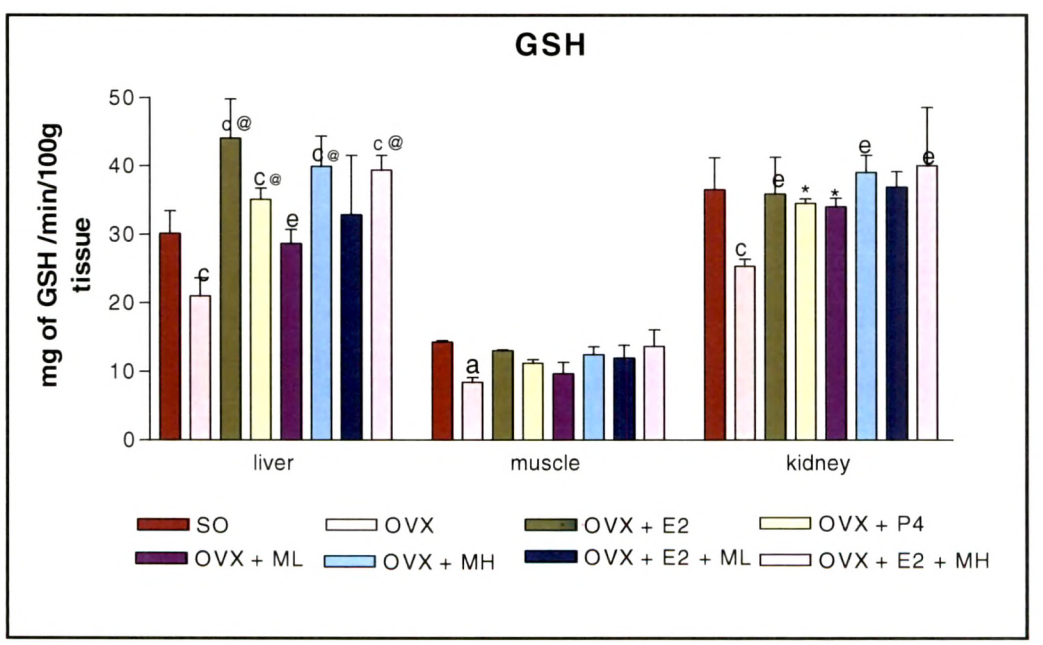
Data are expressed as Mean±SE
^{b}p<0.01, ^{c}p<0.001 when compared to sham operated control and * ^{e}p<0.01, $^{@}$p<0.001 when compared to ovariectomized animals.

Table 3: Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats

REDUCED GLUTATHIONNE CONTENT mg of GSH /min/100g tissue			
GROUPS	LIVER	MUSCLE	KIDNEY
SO	30.15 ± 3.27	14.27± 0.210	36.50± 4.68
OVX	21.00± 2.649^{c}	8.40± 0.680^{a}	25.30± 1.05^{c}
OVX +E_2	44.03± 5.732$^{c@}$	13.04± 0.107	35.89± 5.380^{e}
OVX +P_4	35.11± 1.642$^{c@}$	11.19± 0.526	34.53± 0.679*
OVX +ML	28.67± 02.029^{e}	9.67± 1.660	34.02± 1.259*
OVX +MH	39.91 ±4.417$^{c@}$	12.45 ±1.142	39.02± 2.52^{e}
OVX +E_2+ML	32.82± 8.709	11.96± 1.867	36.85± 2.327
OVX +E_2+MH	39.38± 2.132$^{c@}$	13.69± 2.385	40.00± 8.514^{e}

Data are expressed as Mean±SE
$^{a}p<0.05$, $^{c}p<0.001$ when compared to sham operated control and $^{*}p<0.05$, $^{e}p<0.01$, $^{@}p<0.001$ when compared to ovariectomized animals.

Fig 3: Reduced glutathione (GSH) content in liver, muscle and kidney of control and experimental rats



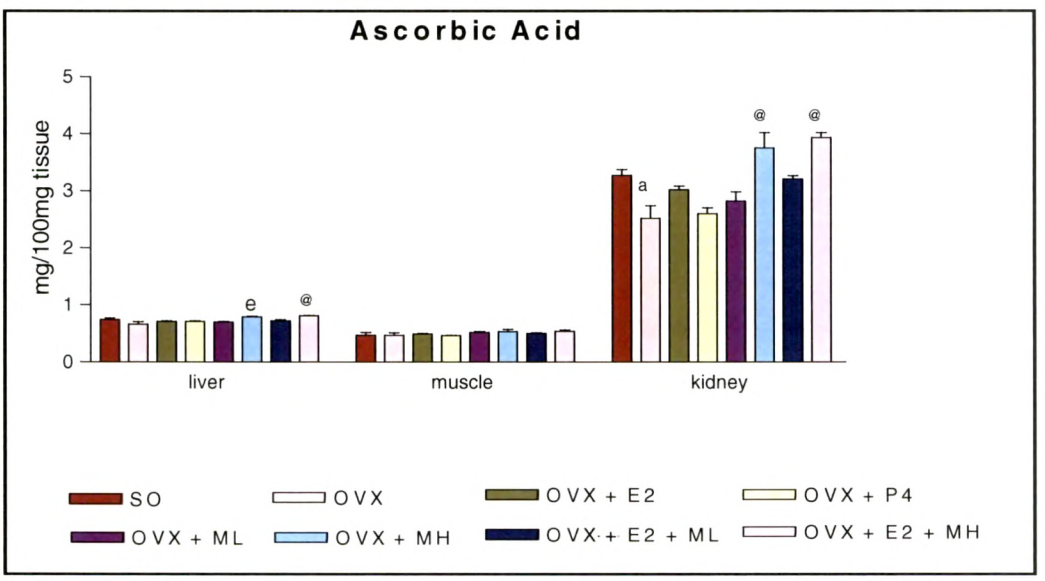
Data are expressed as Mean±SE
$^{a}p<0.05$, $^{c}p<0.001$ when compared to sham operated control and $^{*}p<0.05$, $^{e}p<0.01$, $^{@}p<0.001$ when compared to ovariectomized animals.

Table 5: Ascorbic acid (AA) content in liver, muscle and kidney of control and experimental animals.

GROUPS	LIVER	MUSCLE	KIDNEY
SO	0.74± 0.03	0.47± 0.05	3.27± 0.100
OVX	0.66 ±0.040	0.47± 0.04	2.52± 0.220[a]
OVX +E_2	0.71± 0.01	0.49± 0.01	3.02± 0.060
OVX +P_4	0.71± 0.010	0.46 ±0.010	2.60± 0.100
OVX +ML	0.70 ±0.010	0.52± 0.010	2.82 ±0.160
OVX +MH	0.79± 0.010[e]	0.53± 0.040	3.75± 0.270[@]
OVX +E_2+ML	0.72± 0.020	0.50± 0.010	3.20 ±0.060
OVX +E_2+MH	0.81± 0.010[@]	0.54± 0.020	3.93± 0.090[@]

Data are expressed as Mean±SE
[a]p<0.05when compared to sham operated control and [@]p<0.001 when compared to ovariectomized animals.

Fig 5: Ascorbic acid (AA) content in liver, muscle and kidney of control and experimental animals.



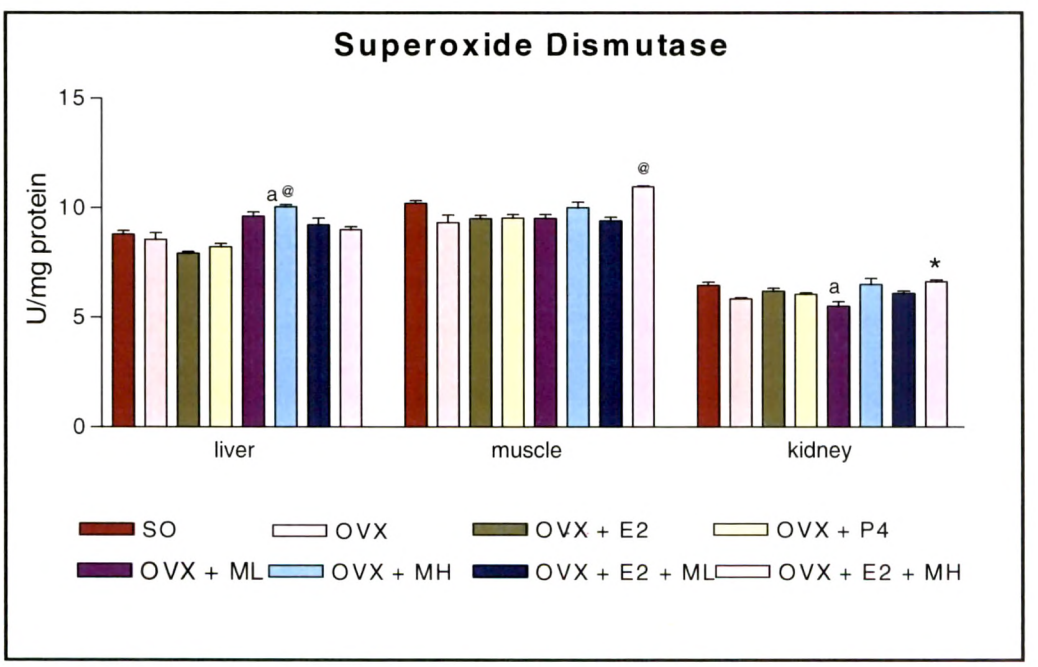
Data are expressed as Mean±SE
[a]p<0.05when compared to sham operated control and [@]p<0.001 when compared to ovariectomized animals.

Table 6: Superoxide dismutase (SOD) activity in liver, muscle and kidney of control and experimental rats.

GROUPS	LIVER	MUSCLE	KIDNEY
SO	8.79 ±0.180	10.21± 0.120	6.47± 0.150
OVX	8.56± 0.310	9.33± 0.350	5.85± 0.060
OVX +E_2	7.93± 0.090	9.50± 0.170	6.20± 0.150
OVX +P_4	8.23± 0.150	9.53± 0.180	6.06± 0.070
OVX +ML	9.62 ±0.200	9.52 ±0.190	5.52± 0.200[a]
OVX +MH	10.05± 0.100[@a]	10.02± 0.25	6.50± 0.280
OVX +E_2+ML	9.23± 0.320	9.42± 0.160	6.11 ±0.110
OVX +E_2+MH	9.00 ±0.150	10.96± 0.040[@]	6.63± 0.090[*]

Data are expressed as Mean±SE
[a]p<0.05when compared to sham operated control and [*]p<0.05, [@]p<0.001 when compared to ovariectomized animals.

Fig 6: Superoxide dismutase (SOD) activity in liver, muscle and kidney of control and experimental rats.



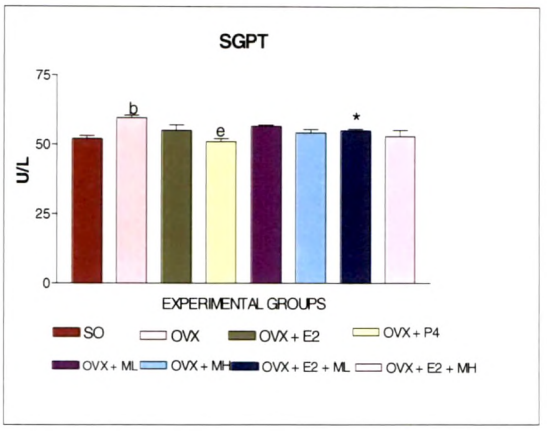
Data are expressed as Mean±SE
[a]p<0.05when compared to sham operated control and [*]p<0.05, [@]p<0.001 when compared to ovariectomized animals.

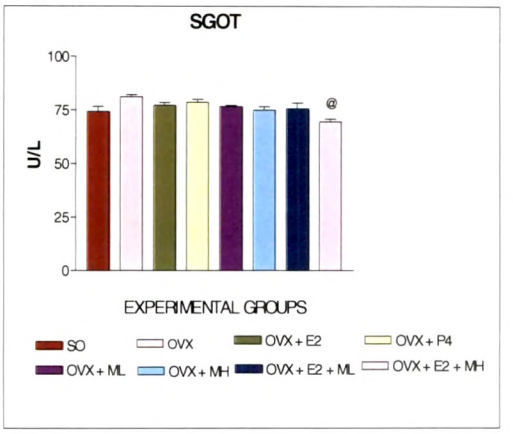
Table 7: Serum markers of hepatic dysfunction in control and experimental groups.

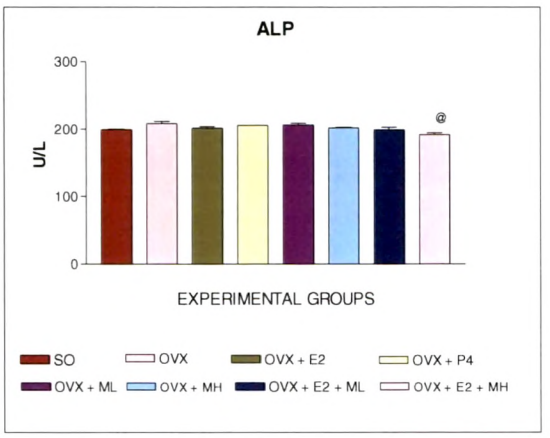
GROUPS	SGPT U/L	SGOT U/L	ALP U/L	ACP U/L
SO	52.00±1.15	74.33±2.32	199.33±0.88	9.33±0.24
OVX	59.67±0.88^{b}	81.33±0.88	208.33±3.27	11.67±0.88^{b}
OVX +E_2	55.00±2.07	77.33±1.20	201.67±2.32	10.85±0.16
OVX +P_4	51.00±1.15^{e}	78.67±1.45	205.33±0.33	11.36±0.38^{b}
OVX +ML	56.67±0.33	76.67±0.66	206.00±2.51	11.33±1.20
OVX +MH	54.33±1.20	75.00±1.72	202.00±0.57	7.93±0.63*
OVX +E_2+ML	55.00±0.57	75.67±2.72	198.67±4.04	10.23±0.19
OVX +E_2+MH	53.00±2.30*	69.67±1.20$^{@}$	191.67±2.59$^{@}$	8.52±0.44*

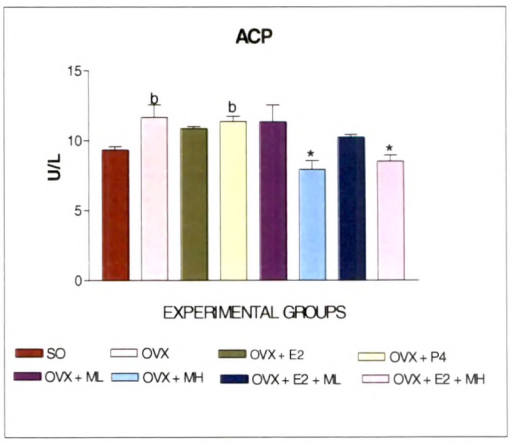
Data are expressed as Mean±SE
$^{b}p<0.01$when compared to sham operated control and $^{*}p<0.05$, $^{c}p<0.01$, $^{@}p<0.001$ when compared to ovariectomized animals.

Figure 7: Serum markers of hepatic dysfunction in control and experimental groups.









Data are expressed as Mean±SE

[a]p<0.05 when compared to sham operated control and [*]p<0.05, [e]p<0.01, [@]p<0.001 when compared to ovariectomized animals.

Table 8: Serum levels of Corticosterone, urea and creatinine in control and experimental rats.

GROUPS	CORTICOSTERONE pg/ml	UREA mg/dl	CREATININE mg/dl
SO	8.36±0.30	37.33±1.20	0.44±0.02
OVX	12.62±0.26^{c}	46.00±1.16^{c}	0.54±0.02^{a}
OVX +E_2	10.11±0.11$^{b@}$	42.78±0.33	0.44±0.03
OVX +P_4	9.70±0.42$^{a@}$	42.33±2.03	0.46±0.02
OVX +ML	10.59±0.29$^{c@}$	41.67±0.88	0.46±0.01
OVX +MH	8.11±0.08$^{c@}$	39.33±0.88^{e}	0.41±0.01$^{@}$
OVX +E_2+ML	10.13±0.18$^{@}$	42.90±1.49	0.41±0.02$^{@}$
OVX +E_2+MH	10.63±0.35$^{@}$	38.67±0.88^{e}	0.39±0.02$^{@}$

Data are expressed as Mean ± SE
$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to sham operated control and $^{e}p<0.01$, $^{@}p<0.001$ when compared to ovariectomized animals.

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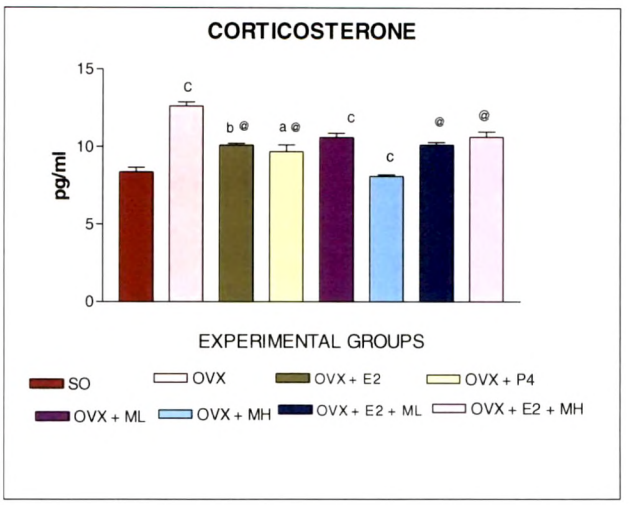

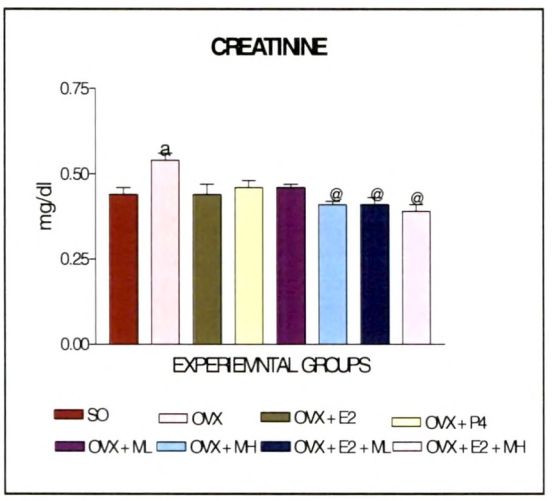

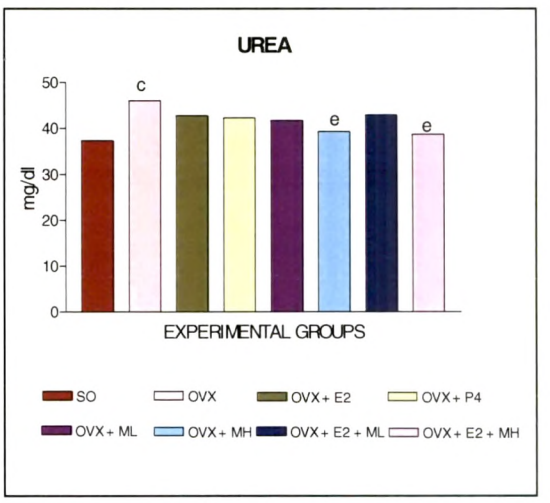

Data are expressed as Mean ± SE
[a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to sham operated control and [e]p<0.01, [@]p<0.001 when compared to ovariectomized animals.

DISCUSSION:

Ovariectomized animals are used as appropriate in vivo models to mimic post menopausal pathophysiological alterations in women (Luine *et al.*, 1998; Sato *et al.*, 2003). Menopause with the associated estrogen deficiency accounts for remarkable metabolic alterations and oxidative stress which can lead to various organic illness including cardiovascular disorders, bone loss (Basu *et al.*, 2001; Feng *et al.*, 2005). Hypoestrogenic status as in OVX animals contribute to body weight gain, adiposity, metabolic alteration affecting both carbohydrates and lipids, as well as predisposition to diabetes due to rendered insulin sensitivity etc (Basu *et al.*, 2001; Chapter 1). Many of the organic disorders in estrogen deficient post menopausal women can be accredited to an increased oxidative stress as E_2 is reported to have antioxidant potentials. The most obvious need in the postmenopausal phase in this context would be to contain the escalated oxidative stress as precaution to prevent the various serious predictations/predispositions. The most obvious recourse to be thought of would be HRT which has obvious beneficial effects even on neurobehavioral aspects (McEwen *et al.*, 2001; Genazzani *et al.*, 2006). However, role of E_2 as an anti-oxidant *in vivo* is beset with many contradictory findings and remains at best controversial (Stampfer *et al.*, 1991; Grady *et al.*, 1992; Hemminki and McPherson, 1997; McManus *et al.*, 1997; Santanam *et al.*, 1998; Bureau *et al.*, 2002). Moreover, a glaring pharmacological disadvantage of E_2 as an antioxidant is its inability to pass through blood-brain barrier (Gilgun-Sherki *et al.*, 2001). Critical studies on ERT have cautioned on long term use of E_2 and has suggested short term

treatment of menopausal women with ERT (Geerlings *et al.*, 2003; Wassertheil-Smoller *et al.*, 2005). In lieu of these, it is rather pertinent to look for alternatives for safer treatment of post-menopausal women.

Melatonin fits the bill as an efficient natural antioxidant with obvious advantages such as solubility in both lipids and water and ability to cross blood-brain barrier, qualifying it as unique amongst antioxidants (Reiter *et al.*, 1999). Its competence to contain toxicant induced oxidative stress and associated neuro-degenerative disorders, cardiovascular diseases and diabetes have found serious consideration in recent times (Mukherjee, 2007; Banerjee, 2009; Singh *et al.*, 2010). The present study on melatonin supplementation therapy (MST) and its comparison with Estrogen supplementation therapy (EST) and a combination of MST and EST in containing hepatic, muscle and renal oxidative stress post-ovariectomy is in this behest a continuation of our previous evaluation on diabetogenic alterations in OVX rats (Chapter 1). Our present observations clearly show that MST is very potent and effective in reversing OVX induced tissue oxidative stress. In keeping with the tricky nature of melatonin in terms of dosage and time of administration gleaned from studies from our laboratory, two doses (low and high) of melatonin have been employed along with a combination with E_2 to compare the effects with ERT.

Estrogen deficient OVX rats show significant increase in LPO in liver, muscle and kidney portraying a generalized increase in whole body oxidative stress. Being an important marker of oxidative stress, the elevated LPO levels seen herein in OVX rats are suggestive of the modulating role of E_2 in

countering pro-oxidant changes in the body. There are reports suggesting increased lipid peroxidation in various tissues following ovariectomy (Hernandez *et al.*, 2000; Baltaci *et al.*, 2004; Prediger *et al.*, 2004). Estrogen is also reported to protect liver against ischemic/reperfusion injury (Eckhoff *et al.*, 2002). Decline in ovarian steroid output as, a consequence of aging, has also been shown to increase LPO (Sagara, 1989). Circulating estradiol concentration below physiological limits (< 50 pg/ml) has also been implicated in elevating in vivo LPO. Our data clearly indicates a near doubled increase in LPO in liver, muscle and kidney. All the three tissues depicted an identical level of LPO in ovary intact as well as OVX rats.

Increased oxidative stress marked by increased LPO in OVX rats stands substantiated by the observed decrease in endogenous anti oxidants. Relative content of GSH and AA in the three tissues tend to suggest higher GSH content than AA and further, muscle has the least amount compared to liver and kidney. Depletion in GSH as a consequence of OVX is to the tune of 30% in liver and muscle while muscle shows 41% depletion. Maximal depletion in AA contents seems characteristics of renal tissue (23%) compared to the hepatic tissue (11%) with muscle being totally resistant to any depletion. The observations of Feng and Zhang (2005) of 50% reduction in brain mitochondrial GSH content and of Oztekin *et al.* (2007) of 25% reduction in hepatic GSH content lend support to our observations. In fact, depletion in both GSH and AA seen in our study clearly suggest a prominent role for female sex steroids in resisting setting in of oxidative stress. These changes in non-enzymatic antioxidants are paralleled by significant

decrement in enzymatic antioxidants. The decrement in GPx and CAT far outweighs that of SOD and in fact decrement in SOD activity is minimal and of equal intensity in all three tissues. Interestingly, muscle has the highest level of activity of all the three enzymes compared to liver and kidney. Muscle seems to have greater resistance to oxidative stress as can be gleaned from the higher levels of enzymatic antioxidants. Rat liver and bone have been shown to come under greater oxidative stress by way of decreased SOD and GPx after ovariectomy (Muthusami *et al.*, 2005). A decrease in CAT activity as well in uterus of OVX rats has found mention in literature (Gomez-Zubeldia *et al.*, 2000). Studies in late menopausal women have observed significantly increased GPx activity in blood compared to pre-menopausal women (Gurdol *et al.*, 1997; Massafra *et al.*, 2000; Sontakke *et al.*, 2002). Further, Sontakke and Tare (2002) recorded significant decrement in GPx and SOD activity in osteoporotic postmenopausal women as has also been observed by Maggio *et al.* (2003). Decreased CAT 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 (Feng and Zhang, 2005). It is likely that superoxide anion concentration would take an upswing and which can be further compounded by the observed decrease in SOD activity. Evidence for inhibition of CAT by superoxide radicals is available (Kono and Fridovich, 1982) 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, Muthusamy *et al.* (2005) have reported an increase in H_2O_2 concentration in bones of OVX animals. The set of changes

delineated herein suggests the creation of a vicious circle leading to an increased load of ROS in tissues of OVX rats leading to greater oxidative stress as long term consequences.

Compromised antioxidant status with a tilt towards increased prooxidant to antioxidant ratio in liver and kidney is also likely to erode into their functional competence and cause certain degree of dysfunctioning. Increased levels of SGOT, SGPT, ALP and ACP (Serum markers of hepatic dysfunction) and of urea and creatinine (serum markers of renal dysfunction) observed in the course of the present study is suggestive of the same. There are hardly any studies which have evaluated the possible effects of estrogen deficiency in hepatic and renal functions in females. We have shown recently a parallel correlation between oxidative stress and hepatic and renal functional impairment in diabetic animals (Singh *et al.*, 2010). It is pertinent to note that, reports have appeared in literature demonstrating an age dependent increase in cytokines production and alleviated hepatic structure and function (Le Couteur and Mclean, 1998; Cao *et al.*, 2001; Zeeh, 2001; Gupta *et al.*, 2003; An and Xiao, 2007). Apart from an age dependent increase, even OVX and menopause have also been shown to result in increased cytokine production (Yasui *et al.*, 2007; Kireev *et al.*, 2008). A mutualistic protective inter-relationship between ROS mediated oxidative stress and pro-inflammatory cytokines is inferable from the many reports in this context (Schulze-Osthoff *et al.*, 1993; Ozgonul *et al.*, 2003; Parlakpinar *et al.*, 2003; Oztekin *et al.*, 2007) and as such a parallel increase in both has been demonstrated (Kireev *et al.*, 2008). Though there are no reports on

ROS-cytokine inter-relationship in the renal tissue, similar set of changes can be inferred as a distinct possibility in serum deducible from the elevation in serum mixture of renal function observed herein. Apparently in the light of the reports cited above and the present observations of increased serum markers of hepatic and renal function, it will not be far-fetched in assuming relatively greater stress on hepatic and renal structure and functions in females due to OVX or menopause induced E_2 deficiencies.

A major marker of chronic stress in animals and humans is the adrenal steroid Corticosterone/Cortisol. The elevated serum Cort Level recorded in OVX rats in the current study confirms the prevalence of heightened stress. Elevated serum Cort levels are characteristic of aged rats (Landfield *et al.*, 1978; Oxenkrug *et al.*, 1984) and human subjects (Friedman *et al.*, 1969). Central disturbances in HPA feedback system are found to be responsible for age-associated increase in Cort titre (Landfield *et al.*, 1978; Dilman *et al.*, 1979; Oxenkrug *et al.*, 1983; Oxenkrug *et al.*, 1984). This defect in HPA axis has been related with decreased sensitivity of hypothalamic glucocorticoid receptors and failure to suppress ACTH secretion, contributing thereby to un-regulated Cort secretion from the adrenals (Dilman *et al.*, 1979; Roth, 1979; Missale *et al.*, 1983). Accordingly, aging related elevation in Cort levels due to altered negative feedback on the HPA axis, has been noted in both animals (Bradbury *et al.*, 1991; Jacobson and Sapolsky, 1991) and humans (Dodt *et al.*, 1991; Seeman *et al.*, 1994; Cagnacci *et al.*, 1995) with, such changes being more pronounced in females than in males (Haleem *et al.*, 1988; Lesniewska *et al.*, 1990; Ahima *et al.*, 1992; Heuser *et al.*, 1994). Predictably,

Cort can be implicated in a cause or effect relationship with ROS and cytokines in contributing to heightened oxidative stress under conditions of estrogen deficiency.

Redressal of sex steroid deficiency in OVX rats by either ERT or P_4 replacement has revealed a mitigating effect on Cort level to the same extent though not fully brought down to ovary intact level. The fact that both E_2 and P_4 could lower serum Cort level to the same degree suggests a similarity of action of both sex steroids by improving negative feedback on the HPA axis. Support to this contention can be sought in the reports of E_2 interference on the activity of neurotransmitters that regulate CRF secretion (Cintra *et al.*, 1991; Yen, 1991) and of E_2 up regulating hippocampal adrenocorticoid steroid receptors (Ferrini *et al.*, 1991; Redei *et al.*, 1994). Both ERT and P_4 replacement have also shown reversible effects on both the studies of endogenous antioxidants (non-enzymatic and enzymatic) and oxidative stress as denoted by LPO though ERT is found to be more effective than P_4 replacement. Conceivably, E_2 is more dominant a sex steroid in improving anti-oxidant status than P_4. Though there is significant improvement in endogenous antioxidant status and levels of LPO in all the three tissues of OVX rats, ERT is apparently not able to fully recover the status to pre ovariectomy level. Some other workers have also reported the ability of sex steroid replacement to redress the compromised antioxidant system and LPO (Liu *et al.*, 2002; Oztekin *et al.*, 2007). Higher replacement doses of E_2 seem to be needed to bring about full reversal as can be deduced from a couple of previous studies (Feng and Zhang, 2005; Oztekin *et al.*, 2007). This is

prohibitive in the light of known negative consequence of ERT with a higher dose or a long term application as discussed at the beginning.

Dose dependent melatonin supplementation attempts 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 OVX on all fronts and at times creating a status even better than in ovary intact animals. A comparison of all schedules employed clearly portray, melatonin alone at a higher dose to be fully effective than E_2 and ML. Whereas the combination of E_2+ML depict equal effectiveness next to MH and E_2+MH with ML being the least effective. A possible reason for the inability of ERT to completely nullify the OVX induced changes could also be related with a concurrent decrease in Mel level in OVX animals as E_2 is known to bring about secretion of melatonin and even modulate Mel production throughout the Estrous cycle (White *et al.*, 1997; Feng and Zhang, 2005). As Mel is a powerful antioxidant (Cabrer *et al.*, 2001; Parlakpinar *et al.*, 2002; Parlakpinar *et al.*, 2003) with modulating influence on endogenous antioxidant system, a decrease in melatonin titre subsequent to OVX 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 LPO in various tissues of rat (Reiter *et al.*, 1999; Sainz *et al.*, 2000; Baydas *et al.*, 2001) and a combination of Px and OVX further intensified the oxidative stress (Oztekin *et al.*, 2007). Concurrently, melatonin administration has been shown to decrease LPO and increase the levels of antioxidants (Baydas *et al.*,

2001; Cabrer *et al.*, 2001; Sener *et al.*, 2003). In this light, our current observations on full reversal of OVX induced changes by MH is very pertinent and provide compelling evidence for MST as an effective and better alternative to ERT in overcoming postmenopausal body oxidative stress and hepatic and renal dysfunctioning. Rectification of hepatic and renal functional impairments induced by OVX is also indicated by the recorded alterations in the levels of serum markers of hepatic and renal function. The dose dependent effect of melatonin as seen herein is substantiated by the observation of Feng and Zhang (2005) of a dose dependent efficacy of melatonin is further established by the reported lowering of LPO, ROS and RON species and cytokines in the liver of OVX rats by the administration of this indolamine hormone. Obviously, the present study provides evidence for MST as an efficient and safe alternative to ERT worthy of application to postmenopausal women.

The efficacy of Mel to overcome OVX induced oxidative stress, also relatable with the concomitant decrement in Cort titre to pre-OVX level, is supported by the reports of decreased Cort concomitant to age associated decrease in Mel and increased Cort after pinealectomy and age associated increase in Cort resistance to suppression by dexamethasone (Takahashi *et al.*, 1976; Oxenkrug *et al.*, 1983; Oxenkrug *et al.*, 1984). Further, melatonin has also been shown to inhibit ACTH secretion (Wetterberg *et al.*, 1983). Since even a combination of E_2+Mel is not more effective than melatonin alone, MST with a standardized dose is advocated in combating OVX/Post menopausal symptoms induced by estrogen deficiency with its many other

known favourable modulating effects on menopausal induced sleep disorders, reduced immune functions, cardio-vascular disorders, dementia and neuropathy etc (Feng and Zhang, 2005; Muthusami *et al.*, 2005), it can be surmised that, MST qualify as a potent and safe alternative to ERT in alleviating post menopausal symptoms and help maintain a healthy female outlook.