1. Background

Androgens play important roles in maintaining homeostasis of many metabolic processes. The growth and differentiation of prostate is governed by testosterone. Testosterone plays a significant role in metabolism of adipose tissue stores and is involved in the mobilization of free fatty acids. It regulates body composition, regulating body mass and insulin concentration. Many researchers reported that testosterone depletion led to the increase in total cholesterol, low-density lipoprotein, triglyceride levels and decrease high-density lipoprotein (Marin et al., 1992).

The development of BPH and later formation of PCa is androgen driven and hence modern therapy available targets AR. PCa is the second most frequently diagnosed cancers and the sixth leading cause of cancer death worldwide (Jemal et al., 2011). Flutamide is an antiandrogen drug used for the treatment of BPH and PCa. Long term androgen deprivation has been associated with an array of side effects. The benefit to harm ratio by use of ADT are very much debatable. However, androgen deprivation leads to several metabolic complications which are unavoidable and associated with the therapy. The decrease in androgen levels may lead to many age associated problems like decreased bone mineral density, increased oxidative stress, and decreased libido (Al-Shamsi et al., 2012, Walker and Robinson 2012, Shiota et al., 2011). The levels of testosterone decreases with advance in age, thus causes metabolic diseases (Starka 2012, McHenry 2012) hyperplasia of prostate and increase in oxidative stress (Shiota et al., 2009, Shiota et al., 2011).

Around half of the mortalities that occur in PCa patients is not related to cancer, but is associated with other etiologies, cardiovascular diseases (CVD’s) being one of the major cause (Satariamo et al., 1998, Lu-Yao et al., 2004). Recent reports suggest that there is decrease in number of deaths related to PCa, unfortunately the overall mortality figures remain the same due to the increase in number of non-cancer related deaths. According to the recent reports, there is an increase rate of deaths of PCa patients due to CVD’s rather than cancer (Sprenkle and Fisch 2007). Patients who are on long term androgen deprivation therapy are 20% more predisposed to cardiovascular morbidity (Saigal et al., 2007).
ECM plays an important role in maintaining the tissue architecture and its microenvironment. This in turn articulates the growth, differentiation of many organs like uterus, mammary glands, prostate and in ovulation etc. (Ohnishi et al., 2005, Fata et al., 2004, Wilson 1995) They also play an important role in angiogenesis (Galis and Khatri 2002, Lijnen 2001), tissue modeling, wound healing (Baum and Arpey 2005) and in the development of many pathologies in these organs (Zhang and Nothnick 2005). Therefore, modulating the level of these protease degrading enzymes keeps a check on these pathologies (Zhang and Nothnick 2005, Fata et al., 2004, Wilson, 1995).

ECM homeostasis is known to be mediated by the cooperative expression of a family of adhesion receptors called integrins and cell surface proteinases such as urokinase plasminogen activator receptor (uPAR) and MMPs (Sajani et al., 2005). Although the role of uPAR and MMP-9 in the process of invasion and metastasis is well demonstrated (Nagase et al., 1999), the role of androgens in the regulation of uPAR and MMP-9 is an active area of research. It has been well documented that with advancement in age there is a gradual decline in the level of testosterone. Bruni-Cardoso (2010) demonstrated that castration resulted in increased expression of MMPs (Bruni-Cardoso et al., 2010). It has been reported that response to wound healing in elderly people is delay; this was associated with the decreased testosterone levels which led to increase in secretion of type IV collagenases (MMP-2 and MMP-9) in the wounds (Ashcroft et al., 1997). Investigation into the regulation and expression of these genes/proteins would help in understanding the prostate growth in elderly.

Oxidative stress is another pathological condition that results due to the imbalance between pro-oxidants and antioxidants. It is much evident that exogenous and endogenous ROS is a major contributing factor for the cancer progression and metastasis (Bostwick et al., 2000, Oberley et al., 2000). ROS and NO govern the expression of a battery of genes orchestrating the angiogenesis function and are tightly linked to the development of BPH (Lucia and Lambert 2008, Fukumura et al., 2006).

Daidzein (3’, 4’, 5, 7-tetrahydroxyflavone) a naturally derived flavonoid found widely in soya. Daidzein was known for its antioxidant, anti-inflammatory and chemopreventive properties (Masilamani et al., 2012, Messina 2010, Mishra et al., 2009, Wijeratne et al., 2007). Moreover it
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is used as anti hypertension and in inflammatory diseases (Jackman et al., 2007). Dietary soy protein lowers blood lipid concentrations and reduces the incidence of cardiovascular diseases in animals and humans (Torres et al., 2006). Genistin and daidzin are isoflavones abundant in soybean.

Luteolin (3', 4', 5, 7-tetrahydroxyflavone) is commonly found in many fruits and vegetables. It is known for its antioxidant (Leung et al., 2006, Manju et al., 2005, Lien et al., 1999), and anti-inflammatory (Perwez Hussain and Harris 2007, Brody et al., 2006, Karin et al., 2006) properties. In Chinese traditional medicine, it is frequently used for hypertension, inflammatory diseases and cancer (Harborne and Williams 2000).

This study was conducted to elucidate the effect of daidzein and luteolin on long term androgen deprivation by flutamide administration and to see its effects on ECM degradation genes (Mmp-9 and uPAR), lipid profile and oxidative stress on ventral prostate of Wistar rats and correlated with changes in tissue architecture.

2. Treatment regimen

Rats were divided into eight groups with 8 animals in each group. Rats given 0.5% Carboxymethyl cellulose served as vehicle control (group I). Flutamide was give at the dose of 30 mg/kg b.wt in 0.5% Carboxymethyl cellulose (group II). In group III and IV daidzein was administered at 20 mg/kg b.wt, 60 mg/kg b.wt respectively after 3 hours of flutamide administration. Luteolin 0.2 mg/kg b.wt. and 0.4 mg/kg b.wt. was given to group VI and VI respectively after 3 hours of flutamide administration. Group VII received only daidzein 60 mg/kg b.wt and only luteolin 0.4 mg/kg b.wt was administered to group VIII. All the animals were sacrificed after 60 days. Prostate tissue was excised and 10% homogenate was prepared in phosphate buffer. Some tissue was also stored in 10% buffered formalin and stored for histopathology and other parameters.
2.1 Material and methods

AR protein expression (IHC) and expression of \( Ar, uPAR, Mmp-9 \) were studied by real time RT-PCR. The level of antioxidant enzymes (Catalase, GSH, LPO, SOD, NO) were studied in rat ventral prostate. We also checked the serum lipid profile of androgen deprived rats. For detail methodology please refer to chapter no II.

3. Results

3.1 Expression of \( Ar, uPAR \) and \( Mmp \ 9 \) in androgen deprived rat VP and its restoration by daidzein and luteolin

Quantitative real-time PCR was used to measure the relative abundance of mRNA levels of \( Ar, uPAR \) and \( Mmp-9 \) (Figure 1). Relative mRNA levels of \( Ar \) in the VP of androgen deprived
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Jamia Hamdard, Department of Medical Elementlogy and Toxicology

(group II) rats were sevenfold (p < 0.05) lower than levels found in control rats (group I). On the other hand relative mRNA levels of uPAR and Mmp-9 in the VP of androgen deprived (group II) rats were 4 fold and 12 fold (p < 0.001) higher than levels found in control rats (group I), respectively. Daidzein significantly restored the transcript levels of Ar group III and IV (p < 0.001) as compared with levels in androgen deprived (group II) rats by 1.2-fold and 2.3 fold respectively. Whereas luteolin administration significantly restored the transcript levels of Ar group V and VI (p < 0.001 respectively) as compared with levels in androgen deprived (group II) rats by 6.5-fold (p < 0.05) and 1.5-fold (p < 0.01) respectively. The expression of uPAR and Mmp-9 were significantly decreased (p < 0.001) as compared with levels in androgen deprived (group II) rats by -2.8 fold (group III), -1.2 fold (group IV) p < 0.001 and -1.0 fold (group III), -2.0 fold (Group IV) (p < 0.001) respectively by daidzein administration. Whereas luteolin administration down regulated the mRNA expression of uPAR and Mmp-9 rats by -2.9 fold (group V), -4.0 fold (group VI) [p < 0.001] and -1.15 fold (group V), -3.7 fold (Group VI) [p < 0.001] respectively.

3.2 Immunohistochemical staining of androgen receptor

Flutamide administration resulted in decreased expression of AR (16.8 ± 1.3%) [group II] when compared with control (group I), while daidzein administration restored the AR expression to normal 58.7 ± 2.0 %, 67.4 ± 1.7 % in group III and IV respectively. Whereas luteolin administration restored the AR expression to normal 70.5 ± 1.6%, 75.6 ± 2.4% in group V and VI respectively. (Figure 2A and B)

3.3 Effect of daidzein on serum cholesterol, HDL and LDL levels.

Androgen deprivation increase total cholesterol, LDL, TG in group II (p < 0.05, 0.01 and 0.05 respectively) as compared to control group I and it was decreased by daidzein significantly and dose dependently in group III and IV (p < 0.001, 0.01 and 0.05 respectively). Whereas there was a decrease in HDL levels in androgen deprived group (p < 0.01). Daidzein administration group III and IV resulted in significant increase in HDL levels (p < 0.05). We found no significant changes in lipid profile by luteolin administration (Figure 3).
3.4 Effect of daidzein and luteolin on alterations of antioxidant enzymes in androgen deprived rats’ ventral prostate.

Flutamide administration resulted in the depletion of antioxidant GSH level (p < 0.001), GPx (p < 0.001), GR (p < 0.001), and Catalase activity (p < 0.001) as compared to vehicle control (group I). Daidzein treatment at two different doses (group III and IV) restores GR and Catalase activity to normal (p < 0.001), whereas only higher dose (group IV) (p < 0.001) increased the activity of GPx better than lower dose (group III) (p < 0.05). But only higher dose (group IV) was effective in restoring the GSH levels (p < 0.001). Luteolin treatment at two different doses (group V and VI) restores GR and catalase activity to normal (p < 0.001), whereas only higher dose (group VI) (p < 0.001) significantly increased the activity of GPx. Both the doses of luteolin (group V and VI) were effective in restoring the GSH levels to normal when compared with group II (p < 0.001) (Table 1).

![Figure 1: Effect of daidzein and luteolin on real time RT PCR expression of Ar, uPAR and Mmp-9 in androgen deprived rat VP](image)

Figure 1: Effect of daidzein and luteolin on real time RT PCR expression of Ar, uPAR and Mmp-9 in androgen deprived rat VP: Daidzein and luteolin at both the doses down regulated flutamide induced uPAR, Mmp-9 and restored Ar expression to normal. The fold change values are expressed in mean ± S.D. (n = 8). Significant differences are indicated by #p < 0.001 when compared with control animals (Group I), and *p < 0.001 when compared with flutamide treated animals (Group II).
Figure 2A: Effect of daidzein and luteolin on AR Immunohistochemical staining of AR protein in androgen deprived rat VP: Photomicrograph shows 40x and 100x resolution of AR of prostate of Wistar rats fed on different treatment regimen as discussed in methodology. Group I showed normal expression of AR positive nuclei (■) and AR negative cells (▲). Group II (flutamide) showed decreased expression of AR positive nuclei as compared to group I. In daidzein treated group III and IV there is a marked increase in AR positive nuclei. Whereas luteolin treated groups V and VI also resulted in increased expression of AR when compared to group II.
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Figure 2B: Effect of daidzein and luteolin on AR positive cell counting: Effect of daidzein and luteolin on AR positive cell counting. Flutamide administration decreased AR expression by 16%, while daidzein restored the AR expression by 67.4% in group IV. Whereas luteolin restored the AR expression to normal by 75.6% in group VI. Values are mean ± S.E.M (n = 6). Significant differences are indicated by #p < 0.001 when compared with control animals (Group I), and *p < 0.001 when compared with Group II.

Figure 3: Effect of daidzein on serum lipid profile in androgen deprived rat VP: Flutamide (Group II) administration resulted in increased TC, LDL and decreased HDL. Daidzein administration restored the levels to normal (Group III and IV). Values are mean ± S.E.M (n = 8). Significant differences are indicated by #p < 0.05, ##p < 0.01, and ### p < 0.001 when compared with control animals (Group I), and *p < 0.05, **p < 0.01 and *** p < 0.001 when compared with flutamide treated animals (Group II).
Table 1: Effect of daidzein and luteolin on alterations of antioxidant enzymes in androgen deprived rats VP:

<table>
<thead>
<tr>
<th>Anti oxidant enzymes</th>
<th>GR</th>
<th>GPx</th>
<th>CAT</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>294.80±2.30</td>
<td>99.01±2.60</td>
<td>166.31±2.30</td>
<td>382.40±3.52</td>
</tr>
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<td>Group II</td>
<td>45.43±3.18###</td>
<td>13.25±2.60###</td>
<td>67.04±1.16###</td>
<td>246.32±6.26###</td>
</tr>
<tr>
<td>Group III</td>
<td>285.11±3.7***</td>
<td>25.29±2.3*</td>
<td>125.31±5.8***</td>
<td>268.3±6.6</td>
</tr>
<tr>
<td>Group IV</td>
<td>313.2±4.0***</td>
<td>72.44±2.3***</td>
<td>199.66±3.50***</td>
<td>316.2±6.4***</td>
</tr>
<tr>
<td>Group V</td>
<td>265.1±6.10***</td>
<td>23.66±3.95</td>
<td>134.11±3.30***</td>
<td>330.76±11.80***</td>
</tr>
<tr>
<td>Group VI</td>
<td>283.62±8.91***</td>
<td>74.0±4.00***</td>
<td>156.74±4.10***</td>
<td>346.49±8.10***</td>
</tr>
<tr>
<td>Group VII</td>
<td>295.0±0.8</td>
<td>113.14±1.6#</td>
<td>171.96±3.8</td>
<td>392.36±9.2#</td>
</tr>
<tr>
<td>Group VIII</td>
<td>302.10±4.31#</td>
<td>110.27±3.31#</td>
<td>159.07±4.90</td>
<td>380.65±13.20</td>
</tr>
</tbody>
</table>

Flutamide administration resulted in a marked decrease in GR, GPx, Catalase and GSH levels and their levels were restored to normal, dose dependently after luteolin administration Values are means ± S.E.M. (n =8). GR as nmol NADPH oxidized/min/mg protein, GPx as nmol NADPH oxidized/min/mg protein, Catalase as nmol H2O2 consumed/min/mg protein, GSH is expressed as nmol GSH/gm tissue. Significant differences are indicated by #p < 0.05, ##p < 0.01, and ### p < 0.001 when compared with control animals (Group I), and *p < 0.05, **p < 0.01 and *** p < 0.001 when compared with Flu-treated animals (Group II). Flu = Flutamide 30 mg/kg b.wt; Group III = Daidzein 20 mg/kg b.Wt + Flutamide 30 mg/kg b.wt; Group IV = Daidzein 60 mg/kg b.wt+ Flutamide 30 mg/kg b.wt; Group V = Luteolin 0.2 mg/kg b.Wt + Flutamide 30 mg/kg b.wt; Group VI = Luteolin 0.4 mg/kg b.wt.; Group VI= Daidzein 60 mg/kg b.wt.; Group VI= Luteolin 0.4 mg/kg b.wt.
3.5 Effect of daidzein and luteolin on androgen deprived lipid peroxidation, SOD, NO in rat ventral prostate

Androgen deprivation induced a marked increase in TBARS in group II (p < 0.001) as compared to control group I and it was significantly restored by daidzein at both the doses group III and IV (p < 0.05 and 0.001 respectively). Luteolin treatment significantly restored the levels to normal at both the doses group V and VI (p < 0.001), (Figure 4A). NO generation was increased in group II (p < 0.001) as compared with group I. Daidzein administration inhibited NO generation significantly at both the doses group III and IV (p < 0.01 and 0.001 respectively) and Luteolin administration also inhibited NO generation significantly at both the doses group V and VI (p < 0.001), (Figure 4B). The levels of SOD was decreased significantly (p < 0.001) in group II as compared to group I and its levels were restored to normal by daidzein at both the doses group III and IV (p < 0.01 and 0.001 respectively) whereas its levels were restored to normal by only higher dose of luteolin administration group IV (p < 0.05), (Figure 4C). Both daidzein and luteolin treatment did not show any significant affect on LPO, NO and SOD as compared with control (group I). (Figure 4)

3.6 Effect of daidzein and luteolin on androgen deprived histological alterations in rat ventral prostate

The H&E staining of the rat VP reveals that there is prostatic atrophy and degenerative changes, loss in prostate architecture, the disintegrated in stroma of VP is clearly seen in the androgen deprived group II. Daidzein and luteolin treated groups (III, IV and V, VI respectively) showed a restoration in prostate architecture and acini of VP dose dependently. Secondly there is minimal damage seen to the stroma of VP. There was not any difference in the control group I and the only daidzein and only luteolin treated group VII & VIII (figure not shown). (Figure 5)
Figure 4: Effect of luteolin on lipid peroxidation (4A), NO (4B) SOD (4C) in androgen deprived rat VP: Flutamide administration increased nitric oxide, lipid peroxides generation and depleted the levels of SOD. These antioxidant levels were restored to normal after daidzein administration (Group III and IV). We found a dose dependent restoration to normal by luteolin in administration (Group V and VI). Values are mean ± S.E.M (n = 8). Significant differences are indicated by #p < 0.05, ##p < 0.01, and ### p < 0.001 when compared with control animals (Group I), and *p < 0.05, **p < 0.01 and *** p < 0.001 when compared with flutamide treated animals (Group II). Group I vehicle control, Group II flutamide 30 mg/kg b.Wt; Group III daidzein 20 mg/kg b.Wt + flutamide 30 mg/kg b.wt; Group IV daidzein 60 mg/kg b.wt + flutamide 30 mg/kg b.wt; Group V Luteolin 0.2 mg/kg b.wt + flutamide 30 mg/kg b.wt; Group VI Luteolin 0.4 mg/kg b.wt + flutamide 30 mg/kg b.wt; Group VII Only daidzein 60 mg/kg b.wt; Group VIII only Luteolin 0.4 mg/kg b.wt
Figure 5: Effect of daidzein and luteolin on androgen deprived histological alterations in rat VP: Group I 40x VP section of rat control group showing acini and normal prostate architecture. Group II (flutamide) treated group showing atrophy of prostate acini with disruption of stroma (→), secretory cells (→), and degenerate prostate gland. Group III daidzein at a dose of 20 mg/kg b.wt + flutamide 30 mg/kg b.Wt showed a lesser degree of atrophy and degeneration of prostate gland. Group IV daidzein 60 mg/kg b.wt + flutamide 30 mg/kg b.Wt showed almost normal architecture (40x). Group V luteolin 0.2mg/kg b.wt + flutamide 30 mg/kg b.Wt. Group VI luteolin 0.4mg/kg b.wt + flutamide 30 mg/kg b.Wt. Luteolin higher (Group VI) dose showed better restoration of prostate architecture to normal.
4. Discussion

Testosterone not only governs the growth and differentiation of prostate gland, but it also plays an important role in other physiological processes. Testosterone plays a significant role in metabolism of adipose tissue stores. Studies suggest that testosterone is directly involved in mobilization of free fatty acids. There is growing evidence that androgens may influence the predominant site of body fat deposition and muscle morphology. The effects of androgens on lipid profile may be sex dependent i.e hormonal replacement may be beneficial or may show adverse effects (Zgliczynski et al., 1996). On the other hand antiandrogen drugs used to treat prostate diseases have many ill effects, disturbing the normal homeostasis of many physiological processes. Many researchers have reported that there is a decrease in testosterone levels with the increase in age (Starka 2012). ADT results in hypogonadism which leads to unfavorable influence on lipid profile. Hence, patients who are on ADT are highly predisposed to cardiovascular disease (Braga-Basaria et al., 2006), further adding up to the mortality burden of PCa related deaths. Sub-chronic androgen deprivation by flutamide resulted in increased levels of LDL, TC and TG which were significantly restored by daidzein administration at both the doses. Our results are in accordance with (Smith et al., 2006, Yannucci et al., 2006) who reported an increase in TC, LDL and TG and decrease in HDL levels. In the present study, we studied the preventive role of daidzein and luteolin on long term flutamide induced androgen deprivation, its role in ECM degradation, serum lipid profile and oxidative stress in VP of Wistar rats.

ECM serves an important role in maintaining the tissue architecture and its microenvironment. Secretion of proteases such as uPAR and MMPs disrupts ECM resulting in altered tissue structure. Although, the role of MMPs in PCa and their importance in metastasis is well studied, the association of MMPs, plasmin degrading enzymes and their relationship with ROS in prostate gland of androgen deprived rats is not well understood. This study was designed to understand the effect of daidzein and luteolin on sub-chronic flutamide administered rat VP, ECM degrading genes uPAR and Mmp-9.

Rats were treated with flutamide, a known androgen blocker, alone or in combination with daidzein or luteolin and at two different doses. The expression of AR was remarkably reduced and expression was restored by the administration of daidzein and luteolin dose dependently this
was further strengthened by Immunohistochemistry of AR. Chiu and Lin (2008) also reported similar findings in androgen dependent and androgen independent human prostate cancer cell lines.

The major component of cellular microenvironment is ECM, which is a very dynamic structure. ECM is under the control of many factors like ROS, MMPs family of genes and other transcription factors, which maintains the tissue homeostasis. Any discrepancies in ECM homeostasis may lead to severe pathologies. Accumulating evidence within the last two decades demonstrated the proteolytic degradation and modification of ECM proteins and proteolytic modeling of stromal tissues play an important role in cell invasion and metastasis (Sajani et al., 2005).

We studied the matrix degrading proteases that were activated during remodeling, MMP-9 and uPAR a serine proteases involved in the activation of plasminogen to plasmin. Our result suggests that, androgen deprivation upregulated uPAR expression resulting plasmin formation, which activates MMP-9 leading to ECM degradation. Both daidzein and luteolin treatments resulted in decreased in ECM degradation by restoring the uPAR and MMP-9 expression. Our results support the previous findings that the serene protease, plasmin formed by the up regulation of uPAR acts directly on matrix proteins such as laminin and fibrin and degrade them. It also activates pro-MMP-9 to MMP-9 (Dass et al., 2008, Lijnen et al., 2001). The serine protease plasmin, uPAR and MMP-9 are implicated in the matrix degradation and have been reported to be secreted by neoplastic, non-neoplastic and stromal cells (Bugge et al., 1998, Dane et al., 1993).

We found an increase in generation of NO in androgen deprivation group, which was significantly decreased by both the modulator treatments. The NO released due to oxidative stress further activates MMP-9 and results in the formation of plasmin (McCarthy et al., 2008). Higher levels of NO facilitate neoangiogenesis by activating VEGF transcription factor (Benassayag et al., 2002, Dulak et al., 2000, Ziche et al., 1997). Daidzein and luteolin administration resulted in the decrease NO release from the VP (Joussen et al., 2000, Ziche et al., 1997).
Generation of free radicals like superoxide, hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals occur constantly by mitochondrial respiration, a major source of ROS (Nohl et al., 2003). Excessive generation of ROS can cause damage to cellular DNA, protein and lipids. Cells are well equipped to counter this kind of phenomena by a cascade of antioxidant defense armatures. The first and the foremost defense is by enhance expression of superoxide dismutase converting superoxide radical to H$_2$O, which is further converted to H$_2$O by glutathione peroxidase and Catalase (Mates et al., 1999, Dickinson and Forman 2002). Other antioxidants like reduced glutathione serve directly as acceptors of electron and glutathione reductase help in replenishing the reducing power of the cell. Androgens are known to prevent cells from oxidative stress. Previous reports on orchitectomized mice showed a marked increase in oxidative stress which was restored to normal by testosterone supplementation (Shiota et al., 2009). We found that the complete blockage of the androgen receptor led to a marked increase in the reactive oxygen species generation (Shiota et al., 2011). The decrease SOD levels in flutamide treated group was due to increased expression of superoxides radical generation, this further led to the depletion of Catalase and glutathione dependent enzymes. The antioxidant status was restored to normal significantly by the administration of daidzein and luteolin groups at both the doses; similar antioxidative effect of daidzein and luteolin was reported by others (Leung et al., 2006, Ross et al., 2002, Manju et al., 2005). The ROS species generated by flutamide induced lipid peroxidation causing the depletion of antioxidant activities of Catalase, Glutathione and dependent enzymes GPx and GR. The increase in lipid peroxidation may also be due to the disruption of basement membrane which may be attributed to the increased expression of uPAR. Our results are in accordance support the previous findings (Marnett 2000, Parola et al., 1999, Winrow et al., 1993). Our study was further supported by histological evaluation of H & E of rat VP, which clearly reveled prostate atrophy with degenerative changes and it was restored to normal by both modulators treatment. This may be due to the antioxidant, anti-inflammatory and chemopreventive properties of daidzein and luteolin. It can be inferred that sub-chronic flutamide administration resulted in marked increased in oxidative stress and increased ECM degrading proteins. On the other hand rats treated with daidzein and luteolin in combination with flutamide showed a marked decrease in ECM degrading proteins and restoration of antioxidant status and tissue architecture to normal.