Results
3. Results

3.1. General effects of acute inflammation in rat

The dose response study with bacterial lipopolysaccharide (Fig. 11) showed that the rats exhibit symptoms of illness like lethargy, ruffled fur, shivering, hypothermia (Fig. 12 and 13) with less mortality rate i.e. 20% at 5mg/kg body weight LPS treatment. The posture, gait, respiration and behavior patterns were also affected due to LPS administration. The mortality may be due to endotoxic shock and hypothermia developing after LPS treatment. Though the values are not significant, the body weight (Fig. 14) was decreased by 16 and 20% by 24 h and 72 h respectively after LPS treatment, which might be due to the reduced food and water intake.

Fig. 11. Dose response study with LPS

Rats were injected with LPS intraperitoneally at the indicated doses and the % mortality was calculated based on the number of rats died (six male Wistar rats were treated per dose).
Fig. 12. Effect of LPS on general activity of rats

Rats were injected intraperitoneally with either 500 µl Saline (A) or LPS (5 mg/kg b.w.) dissolved in saline (B).

The LPS treated rats appears to be inactive and lethargic.
**Results**

**Fig. 13. Effect of LPS treatment on the body temperature**

*Rats were injected with 5mg/kg b.w. LPS and the rectal temperatures were monitored at the indicated time points. Each group consists of 6 rats.*

**Fig. 14. Effect of LPS treatment on body weight of rats**

*Rats were injected with LPS (5mg/kg b.w.) intraperitoneally and the body weights were monitored at the indicated time points. Each group consists of 6 rats.*
Results

3.2. LPS induced acute inflammation - Effects on the testicular functions

The effects of LPS induced acute inflammation on the testicular functions such as steroidogenesis and spermatogenesis were studied at various time periods from 3 h to 72 h after LPS administration.

3.2.1. Effect on testicular interstitial fluid volume

The interstitial fluid volume is the index of the total extra cellular and extra tubular fluid volume of the testis (reflects the vascularity of testis) and reflects the changes in the interstitial hormonal environment (Sharpe and Cooper, 1983).

Fig. 15. Effect of LPS treatment on testicular interstitial fluid volume

* indicates significant difference over control. p < 0.05.

The interstitial fluid volume is decreased by 60% of control levels by 6 h, and then returned to normal levels by 72 h indicating that the vascularity of the testis is affected (Fig. 15).
Results

3.2.2. Effect on steroidogenesis

The luteinizing hormone released by anterior pituitary binds to the receptors on the Leydig cells and stimulates androgenesis. The serum LH levels (Fig. 16A.) were significantly decreased by 58% and 48% by 6 and 12 h after LPS treatment respectively, indicating the damage to the pituitary-testicular axis. The testosterone levels (Fig. 16B.) were also significantly decreased by 40%, 43%, 66% and 46% by 3 h, 6h, 12h and 24 h after LPS treatment respectively, suggesting impaired androgenesis as a result of LPS treatment. The decreased activity of the 3β-hydroxysteroid dehydrogenase also supports such a possibility (Fig. 19B, pg. no. 55). The decreased expression of StAR protein (Fig. 17A), which plays a central role in cholesterol uptake by mitochondria, also supports such a possibility. This decrease in StAR protein as shown by immunoblot analysis was time dependent with maximum decrease being at 12 h after LPS treatment. The levels of StAR protein, however, reached normal levels by 24 h after LPS administration. There was no change in the expression of StAR gene at transcriptional level as shown by RT-PCR analysis (Fig. 17B), thereby indicating the effect of acute inflammation is post transcriptional. Thus, the changes observed in LH, testosterone and StAR protein levels in response to LPS treatment were of a reversible nature as each parameter reached normal level by 72 h.
Fig. 16. Effect of LPS treatment on serum Luteinizing hormone and testosterone levels

A.

Levels of serum LH (A) and testosterone (B) (ng/ml) in the saline control (□) and LPS-treated (▲) rats. Rats were injected intraperitoneally with LPS (5mg/kg) or saline (0.5ml) and sacrificed at times indicated. Serum testosterone levels were measured by testosterone EIA. Significant differences between control and LPS-treated responses at each time point are indicated (*, p< 0.05).
Results

Fig. 17. Effect of LPS on expression analysis of StAR

A. Representative Western blot of the time course of StAR protein changes in the testis of rats treated with LPS. Rats were injected intraperitoneally with LPS (5 mg/kg) or saline vehicle (0.5 ml) and sacrificed at times indicated. The testes were removed and mitochondrial fraction isolated and lysed for immunoblot analysis. The results were repeated twice with different animals.

B. RT-PCR analysis of StAR mRNA, showing a product of 274 bp as estimated from comparison to molecular size markers (M). The specificity of the RT-PCR reaction is indicated by the lack of a band in the -ve RT control. GAPDH (302 bp) is shown as amplification control.
3.2.3. Effect on spermatogenesis

3.2.3.1. Testicular marker enzyme assays

The biochemical and histological studies in rodents have identified several enzymes associated with specific cell types of testis referred to as marker enzymes (Gupta et al., 1997), which have been used in studying the type of defects induced in the spermatogenic process by antispermatogenic agents (Shen and Lee, 1984). In view of this earlier literature, the effect of LPS induced inflammation was studied on the activities of testicular marker enzymes. The activity of the glucose-6 phosphate dehydrogenase (Fig. 18 A), which is a marker for premeiotic germ cells, decreased significantly within 6 h after LPS treatment and remained more or less at the same levels upto 72 h, which might result in decreased proliferation of spermatogonia and primary
Results

spermatocytes. Lactate dehydrogenase, a marker for mature germ cells, showed no significant changes in response to LPS treatment at all the time periods (Fig. 18 B).

![Fig. 18 B. Effect of LPS treatment on testicular marker enzymes](image)

**Fig. 18 B. Effect of LPS treatment on testicular marker enzymes**

*Time course of testicular lactate dehydrogenase activity in rats (mean±S.E.M, n=6 rats/group) treated with LPS (5mg/kg i.p.). * - indicates a significant difference from Control (saline treated) rats (p<0.05). Rats were dissected, testes isolated, 10% homogenates prepared and the enzyme activities measured as described in materials and methods.*

The activity of γ-glutamyl transpeptidase (Fig. 19 A), which is a marker of Sertoli cells (nursing cells for germinal epithelium), showed non-significant changes at all time periods after LPS treatment. This enzyme catalyzes the transfer of γ-glutamyl group between peptides and amino acids. The activity of this enzyme parallels the pattern of Sertoli cell maturation and replication (Sherin, 1976). These results suggest that sertoli cells are not affected by LPS treatment upto 72 hours. The activity of the 3β-HSD decreased significantly by 39% and 41% by 6 h and 12 h respectively after LPS administration.
Results

(Fig.19 B). 3β-hydroxysteroid dehydrogenase is a key steroidogenic enzyme, which acts on the C-19 and C-21 steroids by specifically acting on 3β-hydroxy groups. This enzyme converts pregnenolone to progesterone.

A.

![Graph A](image)

B.

![Graph B](image)

**Fig. 19. Effect of LPS treatment on testicular marker enzymes**

*Time course of testicular γ-glutamyl transpeptidase activity (A) and 3β-hydroxysteroid dehydrogenase activity (B) in rats (mean±S.E.M, n=6 rats/group) treated with LPS (5mg/kg i.p.). * - indicates a significant difference from control (saline treated) rats (p<0.05). Rats were dissected, testes isolated, 10% homogenates prepared and activities were measured as described in materials and methods.*
The present decrease in the activity of 3β-hydroxy steroid dehydrogenase might be due to impaired synthesis/denaturation of the enzyme. This decreased 3β-HSD activity in the testis of LPS treated rats, suggest impaired steroidogenesis.

3.2.3.2. Histopathological observations (Fig. 20)

In order to study the histopathological integrity of testes after LPS treatment, rats were killed after the indicated time periods (3 h, 6 h, 12 h, 24 h, and 72 h). The testes were fixed in Bouin’s fluid, embedded in paraffin wax,

Fig. 20. Light microscopic pictures of HE-stained testicular sections of control and LPS- treated rats.

Histological examination of rat testes. A, Cross section of Saline-injected control testis showing normal spermatogenesis in the seminiferous epithelium. B, Seminiferous tubule cross-section showing the accumulation of immature germ cells in the lumen 24 h after LPS treatment. C & D, LPS treated testis showing large number of immature germ cells in the lumen and increased inter cellular gaps due to disruption of cell-cell contacts (indicated by short arrows) in the seminiferous epithelium. Original magnification-100X.
sliced into sections, stained with haematoxylin-eosin and observed by light microscopy. The photomicrographs of the testis tissue of saline treated rats showed normal spermatogenesis pattern. In the LPS (5 mg/kg) treated rats, the organization of the seminiferous tubules at 3 and 6h after treatment was not different from that in saline-injected control rats. Thereafter, at 12, 24, and 72 h of treatment there was increasing evidence of degeneration of seminiferous epithelium. However, as shown in Figure 20, the effects were more prominent at 24 and 72 h. By 24 and 72 h after treatment, numerous round germ cells were observed, indicative of their sloughing from the seminiferous epithelium. Also, the disordered seminiferous epithelium observed was suggestive of disruption of cell-cell contacts and loss of germ cells.

**3.2.3.3. Ultrastructural changes by Transmission electron microscopy**

In the light of changes observed under light microscope, transmission electron microscopic studies were performed on testes of control and experimental rats in order to study any organellar deformities. In the rats treated with LPS (5mg/kg), clear ultrastuctural deformities were observed compared to that in saline-injected control rats (Fig. 21 A & B). Accumulation of vacuoles within a Sertoli cell process where various stages of germ cell development takes place was observed. Formation of multinucleate cells is observed which normally occurs during acute stress conditions. Chromatin condensation, a hallmark of apoptosis was also observed. Mitochondrial swelling and formation of vacuoles within took place which indicates damage to the mitochondrial membrane integrity leading to disruption of
Results

mitochondrial membrane potential (Fig. 21. C-F). This might cause decreased StAR protein import into the mitochondria.

Fig. 21. Transmission Electron Microscopy

Transmission electron micrograph of testes of rats. A. Testes from saline injected rat showing normal basal lamina (2400 X) and Sertoli cell nucleus and various stages of germ cells (5400 X) (B). Testes of rats administered with LPS, sacrificed 72 h after treatment showing C. formation of multinucleate cell (6300 X), D. chromatin condensation (9000 X), E. formation of vacuoles within a Sertoli cell (Sc) (5400 X) and, F. mitochondrial swelling (22000 X). Basal lamina (BL).
3.3. Acute inflammation induced impairment in steroidogenesis and spermatogenesis: Relative contribution of inflammatory mediators, oxidative stress and cell death mediators

3.3.1. Role of stress response proteins

HSP-60 is important for mitochondrial protein import and high levels of HSP-60 in germ cell stages with mitotic activity suggest a very active mitochondrial protein import and protein assembly machinery that generates further mitochondria for the dividing cells (Meinhardt et al., 1995). Intraperitoneal administration of LPS, in the present study, resulted in the induction of HSP-60 as early as 3 h and remained increased upto 72 h as shown by immunoblot analysis (Fig. 22 A). Also, the expression of the other

Fig. 22. Immunoblot analysis of HSP-60 (A), HMG-1 (B), and HMG-2 (C) in the testicular whole cell lysates from saline and lipopolysaccharide (LPS) injected rats at 3, 6, 12, 24 and 72 h. Rats were injected with either saline or LPS (5mg/kg) intraperitoneally and sacrificed at the times indicated. Data is representative of one of three separate experiments. The molecular sizes of the bands shown on right of the figure were determined by markers run on the same gel. D. β-actin in the immunoblot is shown as a loading control.
Results

stress response proteins like HMG-1 and HMG-2 followed the same pattern of expression in the testes of the rats upon LPS treatment (Fig. 22 B and C). Thus, expression of HSP-60, HMG-1 and HMG-2 increased in the LPS treated rat testes indicating that the testicular tissue is under stress.

3.3.2. Increased expression of inflammatory mediators

During the course of infection, it is known that production of cytokines and iNOS are increased with neutrophil infiltration (Uchida et al., 1989; Wheeler et al., 1997; 1999). RT PCR analyses using gene specific primers of IL-1β (Fig. 23 A), iNOS (Fig. 23 B), COX-2 (Fig. 23 C) and GAPDH (Fig. 23 D) mRNAs in the testes of rats following LPS treatment are shown in figure 23. There was marked induction of all these proinflammatory genes by 3 h following intraperitoneal injection of LPS. The level of induction of these genes was maximum at 12 h after LPS administration, with a decline at later periods. The testes of saline treated animals also showed mild expression of these inflammatory mediators because the animals used in this study were not held under specific pathogen free conditions and constitutive expression of inflammatory mediators might take place in any population, including humans (Tovey et al., 1988).
**Results**

**Fig. 23:** Expression analysis of inflammatory mediators in the testes of rats by RT-PCR

A representative RT-PCR analysis of IL-1β (A), iNOS (B) and COX-2 (C) mRNA in saline treated control testis (saline) and at different time periods after LPS injection. An IL-1β cDNA of 544 bp; iNOS cDNA of 326 bp and COX-2 cDNA of 335 bp, as estimated from comparison to molecular size markers (M,) were detected at the indicated time periods. The specificity of the RT-PCR reaction is indicated by the lack of a band in the –ve controls. The results were repeated twice with different animals. GAPDH (302 bp) is shown as amplification control (D).
3.3.3. Induction of lipid peroxidation during acute inflammation induced testicular damage

The cellular damage induced by ROS was estimated by monitoring the lipid peroxidation level, a well-known indicator of cellular damage from oxidative stress (Favier, 1995). Lipid peroxidation is a mechanism of non-specific cell injury leading to the production of lipid peroxides and their by-products such as TBARS and HNE.

Administration of LPS caused significant increase in the testicular levels of TBARS. The TBARS levels in the testes increased significantly at 6 h to 24 h after LPS treatment with maximum increase observed at 12 h after LPS treatment (Fig. 24 A). This time dependent increase in TBARS levels could be due to the systemic activation of endothelial cells, platelets, macrophage-monocytes and neutrophils to produce various endogenous mediators, including reactive oxygen intermediates, collectively known as the septic cascade. The apparent increase in the level of the TBARS indicates damage to cellular membranes.

3.3.4. Glutathione levels

Reduced form of glutathione (GSH) is an important constituent of intracellular protective mechanism against a number of noxious stimuli. It scavenges $O_2^-$ and protects protein thiol groups from oxidation. Upon reaction with xenobiotics, GSH gets converted to oxidized form of glutathione (GSSG). The ratio of GSH to GSSG (GSH/GSSG) is an index of the protective capacity of glutathione metabolism of a system against toxicant induced stress. In the present study, the GSH/GSSG decreased significantly by
Results

6 h and remained at low levels up to 24 h in the testes of LPS treated rats (Fig. 24 B), indicating depletion of tissue GSH levels due to production of ROS. However, the GSH/GSSG reached to normal levels by 72 h after LPS treatment.

A.

![Graph A](image)

B.

![Graph B](image)

**Fig. 24.** Time course of testicular lipid peroxidation (A) and GSH/GSSG (B) in rats (mean±S.E.M, n = 6 rats/group) treated with LPS (5mg/kg i.p.). * - indicates a significant difference from control (saline treated) rats, (p< 0.05).
Results

3.3.5. Immunohistochemical detection of 4-hydroxynonenal-modified proteins

Fig. 25 shows the staining of 4-hydroxynonenal-modified proteins (brown) in representative testicular sections from rats treated with LPS. The immunostaining was very much apparent at 24 h and 72 h after LPS administration. The testicular sections from saline treated rats did not show any staining. No positive staining was detected in isotypic controls, indicating that nonspecific binding of secondary antibody did not occur under these conditions.

Fig. 25. Immunohistochemistry of HNE-modified proteins in the testes of rats during LPS induced acute inflammation

Effect of LPS induced acute inflammation on accumulation of 4-hydroxynonenal in testes. Immunohistochemistry using antibodies against 4-hydroxynonenal adducts is described under materials and methods. Representative photomicrographs (100X original magnification) depicting immunohistochemistry of 4-hydroxynonenal-modified proteins (brown) in the testicular cross sections at (C) 24 h and (D) 72 h post LPS treatment in rats. (A) Saline, (B) LPS treated rat testes (without HNE antibody) showed no staining for HNE.
3.3.6. Decreased activities of antioxidant enzymes

The antioxidant systems play an effective role in protecting tissue beyond a critical threshold of ROS, thus preventing testicular dysfunction (Oschsendorff, 1999). Data on the activities of antioxidant enzymes measured in testes of LPS treated rats is presented in Table 3. Significant perturbations in antioxidant enzyme activities were evident suggesting acute endotoxemia induced oxidative stress in the testis. There was significant decrease in the activities of catalase (53% and 57%), superoxide dismutase (49% and 40%), glutathione peroxidase (37% and 33%) by 6 h and 12 h after LPS treatment. The glutathione reductase (41% and 37%) and glutathione-S-transferase (40% and 29%) activities also decreased by 12 h and 24 h.

Table 3. Effect of LPS induced acute inflammation on antioxidant enzymes of rat testis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>3 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>30.34±0.937</td>
<td>26.57±0.678</td>
<td>15.29±0.897</td>
<td>17.99±0.789</td>
<td>23.88±0.966</td>
<td>26.28±0.987</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.86±0.069</td>
<td>0.79±0.084</td>
<td>0.40±0.068</td>
<td>0.37±0.089</td>
<td>0.68±0.092</td>
<td>0.62±0.089</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>0.93±0.320</td>
<td>37.0±3.3</td>
<td>27.1±2.8</td>
<td>28.6±3.2</td>
<td>38.4±4.6</td>
<td>36.6±3.76</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>0.54±0.400</td>
<td>42.8±0.76</td>
<td>36.6±0.63</td>
<td>27.0±0.83</td>
<td>28.4±0.64</td>
<td>30.6±0.57</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>1.12±0.035</td>
<td>0.59±0.043</td>
<td>0.58±0.024</td>
<td>0.67±0.047</td>
<td>0.80±0.038</td>
<td>0.94±0.034</td>
</tr>
</tbody>
</table>

Adult male rats were injected with LPS (5 mg/kg, bw) intraperitoneally. Testes were isolated from saline and LPS treated animals at 3 h, 6 h, 12 h, 24 h, and 72 h after treatment and antioxidant enzyme activities were estimated.

- a - nmol pyrogallol oxidized/min/mg protein; b - µmol H_{2}O_{2} consumed/min/mg protein; c - nmol NADPH oxidized/min/mg protein; d - µmol of S-2, 4-dinitrophenyl glutathione formed/min/mg protein; Data were expressed as mean±SEM of three or more independent experiments. ∗ p < 0.001 when compared with controls. % decrease in the activities are also mentioned in parenthesis.
The activities of all these antioxidant enzymes of testes, however, showed insignificant alteration below 6 h and beyond 24 h after LPS treatment compared to saline treated rats.

### 3.4. Role of cell death mediators

The objective to study the major apoptotic mediators in the rat testis was to document if the expression of these proteins is altered when degenerative apoptotic changes are induced during acute testicular inflammation induced by LPS. Following LPS treatment, significant changes in the protein expression levels of cytochrome c, Bcl-2, Bax, p53 and Apaf-1 were observed, as shown by immunoblot analysis.

One of the major apoptotic pathways is activated by the release of apoptogenic protein, cytochrome c, from mitochondria into cytosol. To specify the molecular basis of apoptosis, the release of cytochrome c into the cytosol of the testicular cells of LPS treated rats was measured by immunoblot analysis employing rabbit polyclonal antibodies. As shown in figure. 26 A, testicular cytoplasm of saline treated rats showed less amount of cytochrome c whereas that of LPS treated rats showed increasing levels in a time-dependent manner.

The Bcl-2 family members are thought to regulate apoptosis by formation of hetero- and homodimers in the mitochondrial membrane and the prevailing outcome depends on the ratio of protector (Bcl-2) to the promoter (Bax) of apoptosis. The anti-apoptotic Bcl-2 levels decreased (Fig. 27 A) in the testes of all the rats treated with LPS up to 72 h whereas the pro-apoptotic Bax protein levels increased (Fig. 27 B) in a time dependent manner,
Results

compared to saline treated control rats. Thus, LPS treatment caused decrease in Bcl-2/Bax ratio significantly. The expression of tumor suppressor p53 increased in a time dependent manner in the testes of the rats treated with LPS (Fig. 27 C). Further the apoptotic protease activating factor-1 (Apaf-1) levels were studied in the light of earlier release of cytochrome c into cytosol which might activate intrinsic pathway of apoptosis. The Apaf-1 expression increased in the testes upon LPS treatment (Fig. 26 B).

**Fig. 26. Immunoblot analysis of apoptotic mediators – cyt c and Apaf-1**

**A. Time course of accumulation of mitochondrial cytochrome c in cytosol.** Immunoblot analysis of cytosolic fraction of testicular lysate from saline and Lipopolysaccharide (LPS) injected rats sacrificed at 3, 6, 12, 24 and 72 h after LPS treatment.

**B. Immunoblot analysis of Apaf-1 in the testicular whole tissue lysates from saline and lipopolysaccharide (LPS) injected rats at 3, 6, 12, 24 and 72 h.** Rats were injected with either saline or LPS (5mg/kg) intraperitoneally and killed at the times indicated. Data are representative of one animal at each time point from one of three separate experiments. The molecular sizes of the bands shown on right of the figure were determined by markers run on the same gel. β-actin in the immunoblot is shown as a loading control.
Results

Fig. 27. Immunoblot analysis of apoptotic mediators – Bcl-2, Bax, p53

**Immunoblot analysis of Bcl-2 (A), Bax (B), and p53 (C) in the testicular whole cell lysates from saline and lipopolysaccharide (LPS) injected rats at 3, 6, 12, 24 and 72 h. Rats were injected with either saline or LPS (5mg/kg) intraperitoneally and killed at the times indicated. Data are representative of one animal at each time point from one of three separate experiments. The molecular sizes of the bands shown on right of the figure were determined by markers run on the same gel.** D. β-actin in the immunoblot is shown as a loading control.

Further in order to know if the activation of effector caspase, which involves in the cleavage of various cellular proteins, the caspase-3 activity was studied using a non-fluorescent substrate Ac-DEVD-AFC (Ac-Asp-Glu-Val-Asp-AFC), that forms a fluorescent product AFC (7-Amino-4-trifluoromethyl coumarin) upon cleavage with caspase-3. The caspase-3 activity was markedly increased in all the LPS treated rat testes, the increase being maximum at 72 h (Fig. 28).
Results

Fig. 28. Activation of testicular caspase-3

DEVD-ase activity of caspase-3 was measured by fluorimetric detection of cleavage of the artificial substrate Ac-DEVD-AFC. Testes were isolated, protein was estimated and 50 µg of lysates were used for fluorimetric assay. The excitation and emission of the cleaved fluorimetric product are 400 and 505 nm respectively and wavelength scan was performed in the range of 420-580 nm. Data are representative of one animal at each time point from one of three separate experiments.

The polyADP ribosyl polymerase (PARP) is an enzyme involved in the DNA repair and is a substrate for caspase-3. During apoptotic conditions, caspase-3 gets activated and cleaves the native 116 kDa form of PARP to 89 and 24 kDa fragments (Tewari et al., 1995). During necrotic conditions release of various lysosomal proteases and calpains takes place due to disruption of lysosomal membranes and activation of uncontrolled inflammatory cascade and results in the cleavage of native 116 kDa PARP into 60 kDa, 54 kDa, and 44 kDa along with 89 and 24 kDa fragments, where the former fragments predominate (Gobiel et al., 2001). In the present study the immunoblot
analysis of the PARP using a rabbit polyclonal antibody against PARP and its subunits showed both necrotic and apoptotic pattern of PARP cleavage (Fig. 29). The 60 kDa, 54 kDa, and 44 kDa fragments predominated during 3 h to 24 h indicating the necrotic condition of the testicular cells during early time periods. However, 72 h after LPS treatment 24 kDa fragment predominated, indicating the apoptotic state of the testicular cells during later time periods in the rats treated with LPS.

**Fig. 29. Immunoblot analysis of PARP**

Time course expression of poly (ADP-ribose) polymerase (PARP) protein during apoptotic (A) and necrotic (N) pattern of cleavage. Immunoblot of testicular whole cell lysates from saline and Lipopolysaccharide (LPS) injected (i.p.) rats sacrificed at 3, 6, 12, 24 and 72 h after LPS treatment. Results show increased cleavage of PARP only after LPS treatment. Testes were isolated and whole cell lysates were used for immunoblot analysis. Data are representative of one animal at each time point from one of three separate experiments. The molecular sizes of the bands shown on right of the figure were determined by markers run on the same gel. β-actin in the immunoblot is shown as a loading control.