4.1. *Andrographis paniculata*

*Andrographis paniculata* Wall. ex Nees also known as *nemone chinensi* is a herb belonging to the family Acanthaceae family (XJ, 2006). It belongs to the tribe *Andrographideae* of the subfamily *Acanthoideae*. All the parts of *Andrographis paniculata* are used extensively for medicines. Some of the active constituents of *Andrographis paniculata* are diterpene lactone compounds that are present in the aerial parts of the plant. The plant is known to alleviate inflammation by inhibiting iNOS, TNF-α, IL-1b, IL-6, and IL-12 expression and NO production by down-regulation of p38MAPKs signaling pathways (Puri et al., 1993; Melchior et al., 1997; Muluye et al., 2014).

It is an annual branched herb around 1/2 to 1 meter in height. Its leaves and stems are generally used to extract the active phytochemicals. It is known to grow abundantly in the countries of Southern East Asia such as India, Sri Lanka, Pakistan and Indonesia. It is one of the extensively cultivated plant in China and Thailand (Sandberg, 1994), the East and West Indies, and Mauritius (Gupta et al., 1990). Because of its hardness it is widely distributed, it grows in soil types in particular serpentine soils where a relatively high in aluminum, copper and zinc.

It has been reported that *Andrographis paniculata* is known to possess antioxidant, antipyretic, antibacterial, antiviral, anticancer, hypoglycemic, hepatoprotective, gastroprotective, immunoprotective, and cardioprotective effect (Pai Kotebagilu et al., 2014). The leaves of *Andrographis paniculatam*, popularly used in Scandinavia as a remedy for cold and influenza, used traditionally in Ayurvedic, Thai and Chinese medicine to treat fever associated with infectious diseases. It is used in the treatment and prevention of upper respiratory tract infections (Coon and Ernst, 2004).
The most medicinally active phytochemical in the *Andrographis paniculata* is andrographolide which is present in highest amount in leaves (2.39%) and lowest amount in seeds (Sharma et al., 1992). Andrographolide is very bitter in taste, colorless crystalline in appearance and is called a diterpene lactone (Siripong et al., 1992). Research studies showed that *Andrographis paniculata* has been surprisingly used for a broad range of pharmacological effects that are extremely beneficial. Some of these include; abortifacient (for aborting the pregnancy), acrid, analgesic (for killing the pain), anti-inflammatory (for reducing the swelling), antibacterial, antiperiodic (for periodic diseases such as malaria), antipyretic (for reducing the fever), antithrombotic (for preventing the blood clot), antiviral, anticancerous, cardioprotective, choleretic, depurative (purifies the blood), digestive (for promoting the digestion), expectorant (for promoting the mucus discharge from the respiratory system), hepatoprotective, hypoglycemic (reduces the blood sugar), immune enhancer, laxative, sedative and vermicidal.

**4.2. Male reproductive system**

Human male reproductive system is a dynamic phenomenon. Evidences from previous studies showed that wildlife species and humans are known to be exposed to several ubiquitous endocrine disrupting chemicals through environmental and lifestyle factors (Skakkebaek et al., 2001; Sharpe, 2010). These substances can act through many mechanisms, including agonistic or antagonistic action at the hormone receptor or post-receptor level or by interference with the synthesis, transport and metabolism of certain hormones (Zoeller et al., 2012). These endocrine disrupting chemicals are identified based on the three criteria: endocrine activity, adversity of effects and a plausible link between endocrine activity and the adverse effect.
Male reproductive system function starts with the signal provided by the hypothalamic peptide Kisspeptin which triggers the release of Gonadotropin-Releasing hormone pulses from the hypothalamus which further stimulates the secretion of gonadotropins such as Follicle-Stimulating hormone and Luteinizing hormone by the anterior pituitary (Matsumoto AM and Bremner WJ, 2011). Gonadotropins, Follicle-Stimulating hormone and Luteinizing hormone act at the testicular level to induce sperm maturation and steroid hormone production at the tubular and interstitial compartment. Testis produces more than 95% of the male androgen hormones and testosterone is known to be the main product of the Leydig cell, with a daily normal output of 6-7 mg (Nieschlag E and HM, 2010).

Testosterone is known to play a pivotal role in spermatogenesis and it is a key in triggering the male sexual characteristics such as the development of genital organs, muscle and bone mass, and body hair growth (Bhasin et al., 2010). Spermatogenesis on the other hand is a process of male germ cell proliferation and differentiation. It starts with the division of diploid spermatogonial stem cells and continues with the processes such as sequential cell division of spermatogonia and meiosis of spermatocytes to form round spermatids (de Kretser et al., 1998; Ogawa, 2001). Differentiation of round spermatids into the complex structure of the spermatozoon is known as spermiogenesis (Huleihel et al., 2007).

Some of the important organs of the male reproductive system include; testis, excurrent ducts (efferent ducts and epididymis), accessory sex glands (prostate, seminal vesicle, coagulating gland, bulbourethral gland). The testis comprises of compactly arrayed convoluted loops of seminiferous tubules separated by an interstitium containing Leydig (interstitial) cells, vasculature, macrophages, a protein and testosterone-rich ultrafiltrate, and supporting stroma. The seminiferous epithelium is
formed by basally located Sertoli cells and spermatogonia, spermatocytes, round spermatids, and elongating spermatids (Boorman G. A. et al., 1990). The germ cells present in testis are distinct in morphology and susceptibility to harmful influences such as exposure to certain chemical and pharmaceutical compounds. Sertoli cells are the large, post-proliferative cells essential for spermatogenesis (Y., 1990). Leydig cells are the endocrine cells that are present outside of the protective blood–testis barrier.

The male excurrent duct system contains the rete testis, efferent ducts, epididymis, and vas deferens (Foley, 2001). The rete testis, efferent ducts, and epididymis form an integrated system that transports sperm from the testis to the vas deferens. Epididymis is on the other hand plays a primary role in sperm maturation, sperm transport, and sperm storage as well as ejaculation. Efferent ducts link the rete testis to the epididymis. The epididymis develops from the Wolffian duct under the influence of testicular testosterone (Rodriguez C. M. et al., 2002). In mammals, the male accessory sex glands include the prostate, coagulating gland (anterior prostate), seminal vesicle, ampullary gland, the bulbourethral gland, the urethral gland, and the preputial gland.

Several reports showed that the male reproduction has steadily deteriorated over the last six decades (Carlsen et al., 1992; Swan et al., 1997; Aitken et al., 2004). Over several years, male reproductive disorders like decrease in sperm count and quality, incidences of testicular cancer have been reported to be on rise in human population (Carlsen et al., 1992; Auger et al., 1995; Sharpe and Irvine, 2004; Jorgensen et al., 2011). Changes in life style and exposure to various chemicals are found to be some of the reasons for the increase in male reproductive disorders. Usage of herbal extracts for medicinal use has been a general phenomenon from the ancient ages to modern world. So far, studies have dealt the effects of herbal extracts on the male reproductive system.
individually. One such herbal plant is *Andrographis paniculata* which has been used as a medicine from the ancient ages to modern world. The present study was carried out to evaluate the effect of *Andrographis paniculata* leaf extract and Andrographolide on the reproductive toxicity in male Wistar rats.

### 4. 3. Effect of *Andrographis paniculata* leaf extract on the male reproductive system

In the present study, adult male Wistar rats were divided into five groups, with each group containing eight rats. Rats were administered low (20mg/kg body weight), medium (200mg/kg body weight) and high (1000mg/kg body weight) doses of ethanolic extract of *A. paniculata* orally for 65 days daily. Subjecting the rats to graded doses of ethanolic extract of *A. paniculata* did not show any clinical signs of toxicity such as salination, eye size - dilation/shrinking, urination - increase/decrease, loss of fur, vocalization, head flicking, circling and walking backwards and redness around eyes, mouth, nose. Rats did not show any significant difference in the final body weights, food and water intake indicating the absence of overall general toxicity. These results are in accordance with the previous investigations on the effect of ethanolic extract of *A. paniculata* on the body weight, reproductive and other internal organs weight changes in male rats (M.S. et al., 2013).

The tissue indices of the liver showed no altered significant difference in the rats administered with low, medium and high doses of ELAP indicating that the metabolic activity of liver is normal at all the graded doses of ELAP. These results confirm the previous investigations that *A. paniculata* is a hepatoprotective herb (Trivedi and Rawal, 2001). The tissue indices of the spleen showed no altered significant difference in the rats administered with low, medium and high doses of ELAP indicating that the metabolic activity of spleen is normal at all the graded doses of ELAP. Previous report on the aqueous extract (whole plant) of *A. paniculata* showed that it exhibits a
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significant renoprotective effect in gentamicin-induced nephrotoxicity in male Wistar albino rats (Singh et al., 2009). The tissue indices of the kidney showed no altered significant difference in the rats administered with low and medium doses of ELAP whereas it showed a significant decrease in the tissue indices at the high dose of ELAP indicating that the metabolic activity of kidney is normal only at low and medium doses of ELAP. Previous report on the oxidative stress in the brain of nicotine-induced toxicity showed the protective role of *Andrographis paniculata* (Das et al., 2009). Results from the tissue indices of the brain showed no altered significant difference in the rats administered with low and medium doses of ELAP whereas it showed a significant decrease in the tissue indices at the high dose of ELAP indicating that the metabolic activity of brain is normal at low and medium doses of ELAP.

Previously several studies were done on the assessment of A. paniculata and A. paniculata derivatives on the reproductive functions (Burgos et al., 1997; Allan et al., 2009; Sattayasai et al., 2010). To evaluate these study results, the mean tissue indices of testis were calculated. The tissue indices of the testis showed no altered significant difference in the rats administered with low dose of ELAP whereas it showed a significant decrease at the medium and high doses of ELAP indicating that the metabolic activity of testis is normal at low dose of ELAP. The decrease in weight of testis in rats subjected to low and medium doses of ELAP may be due to degeneration of germinal epithelium by direct action on the spermatogenic compartment or an indirect effect through inhibition of testosterone production. The tissue indices of the epididymis showed no altered significant difference in the rats administered with low, medium and high doses of ELAP indicating that the metabolic activity of epididymis is normal at the graded doses of ELAP. The tissue indices of the vas deferens, seminal vesicle and prostate gland penis showed no altered significant difference in the rats administered with low, medium and high doses of ELAP indicating that the metabolic
activity in these organs is normal at the graded doses of ELAP. Results from the tissue indices of the penis showed no altered significant difference in the rats administered with low dose of ELAP whereas it showed a significant decrease at the medium and high doses of ELAP indicating that the metabolic activity of penis is apparently normal at low dose of ELAP. The observed reduction in the weights of testes reflects reduced bioavailability and/or production of androgen in the rats administered with graded doses of ELAP. The reduction in the weight of testis and accessory organs in rats administered with graded doses of ELAP delved the author to undertake studies related to sperm production and maturation in experimental rats.

For a successful fertilization, the production of healthy spermatozoa is a prerequisite. Testicular sperm are able to swim directionally or to recognize and fertilize an egg. These capabilities are achieved progressively as sperm mature during their passage through the epididymis, an androgen-dependent organ and also the primary site of sperm storage in mammals (Robaire et al., 2006). The development and maintenance of fertile spermatozoa depends on the cell- and region-specific gene expression, as well as transport, secretion, and absorption of luminal fluid components by epididymal epithelial cells, and the remarkable sequential changes in the composition of luminal fluid throughout epididymal length (Turner, 1991; Dacheux et al., 2003; Shum et al., 2009). The epididymal structure and its functions are maintained by a complex interplay of endocrine, neuronal and paracrine factors, among which androgens are the most important components (Robaire et al., 2006).

In the present study, the sperm endpoints such as daily sperm production, epididymal sperm count, sperm viability and sperm motility were significantly decreased in rats administered with all the graded doses of ELAP compared to the control rats. Further, the decrease in the percent number of tail-coiled sperms compared
to the control rats as evidenced by hypoosmotic swelling test in all the rats administered with the graded doses of ELAP suggests that membrane integrity of the sperm is deteriorated. Although an analysis of spermatozoon chromatin integrity was not performed in the present study, the possibility of altered chromatin in the sperm of rat administered with ELAP cannot be ruled out. The present findings are in agreement with the earlier report in which exposure to Andrographis paniculata leaf extract resulted in a significant decrease in the quality and quantity of sperm (K.Sathiyaraj et al., 2011a).

It is known that the production of spermatozoa able to fertilize and to develop a normal progeny results, at least in part, from normal sperm maturation in the epididymis. The composition of the internal epididymal milieu, responsible for sperm maturation, is under androgen control. In rats, an androgen-binding protein secreted by Sertoli cells into the lumen of seminiferous tubules under FSH stimulation is transported to the epididymis, where it accumulates at concentrations higher than those found in the testes. This leads to a high local concentration of androgens, essential for maturation of epididymal spermatozoa (Grover et al., 2005). The reduced weight of testis and accessory organs and reduction in spermatogenesis in rats administered with ELAP warrant the author to look in to steroidogenesis and testosterone levels in circulation, since bioavailability of testosterone is not only important for the maintenance of structural integrity of testis and accessory sex organs and also essential for maintenance of spermatogenesis (Mann, 1974). Results from the present study showed a reduced serum testosterone levels in rats administered with the gradient doses of ELAP. The decreased circulatory testosterone level in experimental rats might have led to the degenerative changes in the testis, since continuous androgenic stimulation is required for the normal growth and function of testis (Klinefelter and Hess, 1998). Histological examination of testes revealed severe effects on spermatogenic cells and degeneration
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of seminiferous tubules and lumen devoid of spermatozoa in rats administered with the gradient doses of ELAP.

In the present study, rats administered with the gradient doses of ELAP are characterized by a significant increase in the levels of lipid peroxidation in the testes and different epididymal regions as compared to control rats. Epididymal sperm count, sperm viability and motility were also significantly decreased in the rats administered with the gradient doses of ELAP compared to control rats. The deterioration in the selected sperm parameters might be due to increased oxidative stress during immobilization. Sperm plasma membrane, being rich in polyunsaturated fatty acids (PUFA), is highly susceptible to reactive oxygen species (ROS) attack. Several studies suggest the correlation between increased ROS production and decreased sperm motility (Armstrong et al., 1999; Aitken and Baker, 2006). The mechanism of ROS induced altered sperm motility is still unclear. However, it is hypothesized that H$_2$O$_2$ one of the peroxidation products, might diffuse across the membrane and affect the vital enzymes in the sperm (Makker et al., 2009) thereby results in decreased sperm motility.

Production and maturation of the sperm are very sensitive to pro-oxidant/anti-oxidant balance. In the biological system, SOD is the first line of defense against deleterious effects of oxy-radicals in the cell (oxidative stress) and plays a pivotal role in dismutation of superoxide anions to hydrogen peroxide (Hassan and Schellhorn, 1988). Catalase neutralizes hydrogen peroxides to molecular oxygen and water (Inal et al., 2001). Hydrogen peroxide is responsible for the structural damage, loss of motility, decline in metabolic activity, and release of intracellular enzymes and cell death (Tosic and Walton, 1950). Because spermatozoa have a high content of polyunsaturated fatty acids in their membranes, they are highly susceptible to lipid peroxidation by reactive oxygen species (ROS). To negate the harmful effects of ROS, reproductive tissues are
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equipped with a powerful antioxidant defense system (Vernet et al., 2004). Thus, balance of this enzyme system may be essential to eliminate superoxide and peroxide radicals generated in the tissues. It has been reported that, oxidative stress and a failure of antioxidant defense system induces several sperm abnormalities and results in infertility (Makker et al., 2009).

Spermatozoa formed in the testis enter the caput epididymis, progress to the corpus epididymis, and finally reach the cauda epididymal region. In the caput and corpus epididymal regions, the sperm are structurally compacted and acquire motility and complete maturation in cauda epididymis. The levels of MDA increased significantly in the testis and epididymis of rats administered with graded doses of ELAP as compared to the controls. This is may be due to the increased levels of free radicals. Administration of graded doses of ELAP caused a significant decrease in the activity levels of catalase and SOD both in the testis and epididymis. Increased lipid peroxidation in testis and different epididymal parts may indicate an increased generation of ROS, which can cause damage to sperm and other cytoplasmic organelle membrane structures through peroxidation of lipids, proteins and nucleotides, thereby altering sperm motility (Aitken et al., 1989).

A number of studies have shown that lipid peroxidation affects the sperm concentration, motility and morphology (Gomez et al., 1998; Huang et al., 2000) and higher levels of ROS could accelerate the process of germ cell apoptosis, leading to decline in sperm counts and associated with male infertility (Agarwal and Allamaneni, 2004). Sperm, which are incomplete in maturation and with altered motility, may lead to infertility due to the failure of sperm to reach the site of fertilization as well as their ability to penetrate zona pellucida. Several studies suggest the correlation between increased ROS production and decreased sperm motility (Armstrong et al., 1999).
Decreased motility of sperm by ROS might be due to the diffusion of lipid peroxidation products across the membrane and affect the vital enzymes in the sperm (Makker et al., 2009). Poor sperm quality caused by the administration of graded doses of ELAP is due to generation of ROS has been reported to result in infertility (Aitken and Baker, 2006). The reduction in activities of antioxidant enzymes shows the failure of primary antioxidant defense system of testis and epididymis. In agreement with the present findings, (Koksal et al., 2003) confirmed that the severe pathological changes in testicular tissue are associated with a high level of lipid peroxidation.

The presence of copulatory plugs or sperm in vaginal washings in females mated with males administered with graded doses of ELAP suggests that sexual behavior is not compromised in these males. Females mated with control males had normal implantations. The fetal loss may occur both before and after implantation. Decrease in number of implantations and increase in pre- and post-implantation losses observed in females mated with immobilized males suggest compromised sperm fertility. The reduced fertilization capacity of sperm from rats administered with graded doses of ELAP is probably due to decrease in sperm quality, such as motility, viability and membrane structural integrity.

In conclusion, this study provides compelling evidence of altered reproductive functions, including spermatogenesis, steroidogenesis and fertility in adult male rats that were administered with graded doses of ELAP.

4.4. Effect of Andrographolide on the male reproductive system

In the present study, adult male Wistar rats in the fifth group were administered with 25mg/kg body weight of Andrographolide orally for 65 days once daily. Administration of rats with Andrographolide did not show any clinical signs of toxicity.
such as salination, eye size - dilation/shrinking, urination - increase/decrease, loss of fur, vocalization, head flicking, circling and walking backwards and redness around eyes, mouth, nose. Rats did not show any significant difference in the final body weights, food and water intake indicating the absence of overall general toxicity. These results are in accordance with the previous investigations on antidiabetic and antihyperlipidemic effect of *Andrographis paniculata* and andrographolide in high-fructose-fat-fed rats (Nugroho et al., 2012).

The tissue indices of the liver showed a significant decrease in the rats administered with Andrographolide indicating that the metabolic activity of liver is changed. These results are in accordance with the previous investigations that Andrographolide induces autophagic cell death in human liver cancer cells through cyclophilin D-mediated mitochondrial permeability transition pore (Chen et al., 2012). The tissue indices of the spleen showed no altered significant difference in the rats administered with Andrographolide indicating that the metabolic activity of spleen is normal. The tissue indices of the kidney showed a significant decrease in the rats administered with Andrographolide compared to the control rats indicating a decrease in metabolic activity of kidney. These results are in accordance with the previous report that Andrographolide induces acute kidney injury (Zhang et al., 2014). Results from the tissue indices of the brain showed no altered significant difference in the rats administered with Andrographolide indicating that the metabolic activity of brain is normal.

Previously few studies were reported on the assessment of *A.paniculata* derivative Andrographolide on the reproductive functions (Sattayasai et al., 2010). To evaluate these study results, the mean tissue indices of testis were calculated. The tissue indices of the testis showed a significant decrease in the rats administered with
Andrographolide. The decrease in weight of testis in the rats administered with Andrographolide may be due to degeneration of germinal epithelium by direct action on the spermatogenic compartment or an indirect effect through inhibition of testosterone production. The tissue indices of the epididymis showed no altered significant difference in the rats administered with Andrographolide indicating that the metabolic activity of epididymis is normal. The tissue indices of the vas deferens and prostate gland showed no altered significant difference in the rats administered with Andrographolide indicating that the metabolic activity in these organs is normal.

Results from the tissue indices of the seminal vesicle and penis showed a significant decrease in the rats administered with Andrographolide. The observed reduction in the weights of testes reflects reduced bioavailability and/or production of androgen in the rats administered with Andrographolide. The reduction in the weight of testis and accessory organs in rats administered with Andrographolide delved the author to undertake studies related to sperm production and maturation in experimental rats.

Production of healthy spermatozoa is a pre-requisite for successful fertilization. Testicular sperm swim directionally to recognize and fertilize an egg. In the present study, the sperm endpoints such as daily sperm production, epididymal sperm count, sperm viability and sperm motility were significantly decreased in rats administered with Andrographolide compared to the control rats. Further, the decrease in the percent number of tail-coiled sperms compared to the control rats as evidenced by hypoosmotic swelling test in all the rats administered with Andrographolide suggests that membrane integrity of the sperm is deteriorated. Although an analysis of spermatozoon chromatin integrity was not performed in the present study, the possibility of altered chromatin in the sperm of rat administered with Andrographolide cannot be ruled out. The present findings are in agreement with the earlier report on the effect of andrographolide on
sexual functions, vascular reactivity and serum testosterone level in rodents and aspects of the male reproductive toxicity/male antifertility property of andrographolide in albino rats (Akbarsha and Murugaian, 2000; Sattayasai et al., 2010).

The reduced weight of testis and accessory organs and reduction in spermatogenesis in rats administered with Andrographolide warrant the author to look into steroidogenesis and testosterone levels in circulation, since bioavailability of testosterone is not only important for the maintenance of structural integrity of testis and accessory sex organs and also essential for maintenance of spermatogenesis (Mann, 1974). Results from the present study showed a reduced serum testosterone levels in rats administered with Andrographolide. The decreased circulatory testosterone level in the rats administered with Andrographolide might have led to the degenerative changes in the testis, since continuous androgenic stimulation is required for the normal growth and function of testis (Klinefelter and Hess, 1998). Histological examination of testes revealed severe effects on spermatogenic cells and degeneration of seminiferous tubules and lumen devoid of spermatozoa in rats administered with Andrographolide.

In the present study, rats administered with Andrographolide are characterized by a significant increase in the levels of lipid peroxidation in the testes and different epididymal regions as compared to control rats. Epididymal sperm count, sperm viability and motility were also significantly decreased in the rats administered with Andrographolide compared to control rats. The deterioration in the selected sperm parameters might be due to increased oxidative stress during immobilization. Sperm plasma membrane, being rich in PUFA, is highly susceptible to ROS attack.

Spermatozoa that are formed in the testis enter the caput epididymis, progress to the corpus epididymis, and finally reach the cauda epididymal region. In the caput and corpus epididymal regions, the sperm are structurally compacted and acquire motility
and complete maturation in cauda epididymis. The levels of MDA increased significantly in the testis and epididymis of rats administered with Andrographolide as compared to the controls. This is may be due to the increased levels of free radicals. Administration of graded doses of ELAP caused a significant decrease in the activity levels of catalase and SOD both in the testis and epididymis. Increased lipid peroxidation in testis and different epididymal parts may indicate an increased generation of ROS, which can cause damage to sperm and other cytoplasmic organelle membrane structures through peroxidation of lipids, proteins and nucleotides, thereby altering sperm motility (Aitken et al., 1989).

A number of studies have shown that lipid peroxidation affects the sperm concentration, motility and morphology (Gomez et al., 1998; Huang et al., 2000) and higher levels of ROS could accelerate the process of germ cell apoptosis, leading to decline in sperm counts and associated with male infertility (Agarwal and Allamaneni, 2004). Sperm, which are incomplete in maturation and with altered motility, may lead to infertility due to the failure of sperm to reach the site of fertilization as well as their ability to penetrate zona pellucida. Several studies suggest the correlation between increased ROS production and decreased sperm motility (Armstrong et al., 1999). Decreased motility of sperm by ROS might be due to the diffusion of lipid peroxidation products across the membrane and affect the vital enzymes in the sperm (Makker et al., 2009). Poor sperm quality caused by the administration of graded doses of ELAP is due to generation of ROS has been reported to result in infertility (Aitken and Baker, 2006). The reduction in activities of antioxidant enzymes shows the failure of primary antioxidant defense system of testis and epididymis. In agreement with the present findings, (Koksal et al., 2003) confirmed that the severe pathological changes in testicular tissue are associated with a high level of lipid peroxidation.
The presence of copulatory plugs or sperm in vaginal washings in females mated with males administered with Andrographolide suggests that sexual behavior is not compromised in these males. Females mated with control males had normal implantations. The fetal loss may occur both before and after implantation. Decrease in number of implantations and increase in pre- and post-implantation losses observed in females mated with immobilized males suggest compromised sperm fertility. The reduced fertilization capacity of sperm from rats administered with Andrographolide is probably due to decrease in sperm quality, such as motility, viability and membrane structural integrity.

In conclusion, this study provides compelling evidence of altered reproductive functions, including spermatogenesis, steroidogenesis and fertility in adult male rats that were administered with Andrographolide.

4.5. Reproductive toxicity

The experimental data presented in various locations show the effect of *Andrographis paniculata* and Andrographolide as judged by their action on a wide spectrum of reproductive end points of the organism, ranging from the reduction in the weights of reproductive organs, through spermatogenesis and steroidogenesis to deterioration of testicular architecture and induction of tissue oxidative stress. The various sectors of reproduction may be seen to the reader to be influenced by the administration of graded doses of *Andrographis paniculata* and Andrographolide at different end points. The critical examination of the data given at various earlier locations indicates that reduced testicular steroidogenesis and elevated tissue oxidative stress are responsible for the reduction in sperm reserves and deterioration in sperm quality. Decreased daily sperm production and epididymal sperm reserves might be responsible for the reduction in weights of reproductive tissues and suppressed fertility.
The conclusions drawn from this study, are mainly based on the histological, biochemical, sperm analysis, circulatory reproductive hormones and fertility data.

The significant finding of the present study was that the graded doses of *Andrographis paniculata* and Andrographolide potentiated the induced reproductive toxicity in rats. The suppression in steroidogenesis, spermatogenesis and fertility was observed in the rats administered with graded doses of *Andrographis paniculata* and Andrographolide. Therefore, determination of safety levels of herbs and herbal extracts in treatment has to be re-evaluated by taking these parameters into consideration.
Chapter V

Summary and Conclusion
In the present study, the effects of *Andrographis paniculata* and Andrographolide on male reproductive health in rats were studied. Exposure to graded doses of *Andrographis paniculata* or Andrographolide did not affect the body weight gain. The weights of liver and kidneys were not significantly changed in rats administered with low and medium doses of *A. paniculata* when compared to the control rats indicating that the metabolic activity in these organs is not affected. In contrast, rats administrated with high dose of *A. paniculata* and Andrographolide resulted in a significant decrease in the body weights and indices of liver and kidney, whereas the relative weight of brain is comparable among all groups of rats indicating that the selected doses and time schedule of treatment may not be effective to induce neurotoxicity. A significant decrease was observed in the weights of accessory sex organs such as seminal vesicles and penis were observed in rats administered with medium, high doses of *A. paniculata* and Andrographolide, suggesting that these reproductive organs are the vulnerable targets of toxicity. A significant decrease was also observed in the weights of testes in all the experimental rats except in the rats administered with low dose of *A. paniculata* when compared to controls. Since the relative weights of testes decreased in all the experimental rats, in the present study, the author delved in to analyze the testicular (daily) sperm production, sperm reserves in epididymis and the quality of mature sperm in rats administered with graded doses of *A. paniculata* and Andrographolide, which were used as important markers for the determination of male fertility.

Significant decrease in daily sperm production, sperm reserves, sperm motility, sperm viability and deterioration in sperm membrane integrity (HOS test) was observed in rats administered with graded doses of *A. paniculata* and Andrographolide when compared to the control rats. The data clearly indicates that *A. paniculata* and Andrographolide affect both sperm production and maturation. Since growth and functioning of accessory sex organs and secondary sexual characters, production and
maturation of sperm is under the control of androgens, the author ventured on determining the steroidogenesis and testosterone levels in circulation in control and experimental rats.

As demonstrated above, administration of graded doses of *A. paniculata* and Andrographolide is clearly associated with suppressed steroidogenesis and abnormal spermatogenesis in rats. Therefore, the author investigated whether testes from experimental rats had any change in the number of spermatocytes and spermatids when compared with testes from control animals, using histological characteristics. Histological observations of the transverse section of testis of the control rats have showed that seminiferous tubules contain all stages of spermatogenesis and interstitial cells. The transverse section of testis of experimental rats has showed a decrease in the number of spermatocytes, spermatids and sperm in the lumen of seminiferous tubules. The deterioration in the testicular architecture of rats administered with graded doses of *A. paniculata* or Andrographolide is generally ascribed to insufficient availability of testosterone or may be due to increased oxidative damage.

The results in the preceding chapter clearly indicated that administration of graded doses of *A. paniculata* or Andrographolide induced oxidative stress in the testes of experimental rats. It is well established that imbalance in pro-oxidant and antioxidant status leads to several sperm abnormalities including infertility (Makker et al., 2009). Excess generation of ROS, results in lipid peroxidation which can cause damage to sperm and other cytoplasmic organelle membrane structures through peroxidation of lipids, proteins and nucleotides resulting in production of inferior sperm quality (Aitken et al., 1989; Armstrong et al., 1999; Agarwal and Allamaneni, 2004) and leading to infertility (Aitken and Baker, 2006).
The final reproductive endpoint that was tested was the male’s ability to sire offspring in a fixed time period. Fertility related studies provide valuable information about reproductive behavior and attitude and will help to analyze the relationship, if any, with testicular sperm quantity and quality and/or sexual desire of the animal. Observations that all males in experimental groups sired pups, but with reduced number, indicate that treatment used in this study was effective in inducing suppressed fertility in males. The presence of copulatory plugs or presence of sperm in vaginal washings in females mated with the experimental males suggests that sexual behavior is not compromised, whereas additional observations of fewer pups per litter and higher pre- and post-implantation loss in females mated with experimental males suggest compromised sperm fertility. In conclusion, the present study provides compelling evidence of altered reproductive functions, including levels of testosterone, density, motility and viability of sperm and fertility in adult rats administered with graded doses of *A. paniculata* or Andrographolide. The decreased serum testosterone levels might be responsible for the decreased sperm density and poor sperm quality in rats. The lower testosterone level observed in rats administered with graded doses of *A. paniculata* and Andrographolide alone probably resulted from an effect directly on Leydig cells. The inferior sperm quality and low sperm density might be responsible for suppressed reproductive performance in the male rats.

**Conclusions**

1. Administration of graded doses of *A. paniculata* or Andrographolide reduced the weights of reproductive organs indicating that they are the vulnerable targets for physical or chemical toxicity. In addition, administration of high dose of *A. paniculata* and Andrographolide resulted in reduction in the weights of liver and kidney.
2. Administration of graded doses of *A. paniculata* or Andrographolide decreased the daily sperm production, sperm reserves, motile and viable sperm and HOS tail coiled sperm, indicating spermato-toxic effects of *A. paniculata* and Andrographolide.

3. Administration of graded doses of *A. paniculata* or Andrographolide induced lipid peroxidation levels as evidenced by elevated tissue MDA levels, with a significant decrease in the SOD and catalase activity levels in the testes and epididymis, indicating the disturbance in pro- and anti-oxidant machinery in these tissues.

6. Many histological alterations are observed in the testis of rats administered with graded doses of *A. paniculata* or Andrographolide, which includes large lumen with reduced sperm tails, few degenerating spermatocytes and empty lumen devoid of sperm indicating deterioration in testicular architecture.

7. A decrease in fertility index, number of implantations and live pups and increases in pre- and post-implantation loss observed in rats mated with males administered with graded doses of *A. paniculata* or Andrographolide indicates suppressed fertility out in experimental rats.

8. The significant finding of the present study is that administration of graded doses of *A. paniculata* or Andrographolide potentiated the reproductive toxicity in rats. The suppression in steroidogenesis, spermatogenesis and fertility was observed in the experimental rats.


Discussion


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Author’s Publications
Research Publications
