Discussion
Organophosphates (OP) are among the most extensively used synthetic pesticides. The wide spread use of organophosphates has enthused research into the possible extended of effects related with their reproductive toxic activity (Joshi et al., 2007). There have been increasing concerns about the effects of various OP insecticides on humans and animals. These include cholinergic and non-cholinergic biological disturbances. Due to maintaining the modern life a significant amounts of pesticides are unlikely enter into the mammalian system through their food chain. Thus the pesticides affect the overall dynamism of development, metabolism and reproduction in the mammalian model. Several research studies have been indicated that the pesticides have profound toxic effect in male reproductive system (Kniewald et al., 2000; Whorton et al., 1979; Rani et al., 2007; Hess, 1998; Pant et al., 1995; Joshi et al., 2007; Sayym, 2007; Choudhary et al., 2003; Meltem et al., 2007). Chlorpyrifos is an effective board- spectrum organophosphate pesticides widely used throughout the world. It has been reported that it is linked to male and female genital deformities (ENDS, 1999).OP pesticides exert their biological effect mainly through electrophilic attack of cellular constituents with simultaneous generation of reactive oxygen species. It may be involved in the toxicity of various pesticides (Dwivedi et al, 1998).OP is also found to damage the semen producing structure in the testis of rats and human (Mikhail,1979; sherman,1995). All those our study has been demonstrated various degree of toxicological effects of pesticidal exposure in mammalian reproductive system, as well as also demonstrated that the remedial success of herbal material.

It is known that pesticides affect the body weight and reproductive organ weight in mammalian system. It is proved that the reproductive organs weights are the criteria used for evaluation of reproductive toxicity (Zidan, 2009). In general toxicity studies, it is well known that the alterations in body and organ weights are sensitive indicators of the detection of potentially toxic chemicals. In this experiment, during the chlorpyrifos exposure (CPF 7mg and 12mg/kg/d body weight for 15 and 30 days treatment), toxic symptoms were observed. The body and organ weight (both absolute and relative weight) decreased during chlorpyrifos exposure (fig- 3, 40). It is studied that the weight of reproductive organs decreases significantly at various dose levels (7.5, 12.5 and 17.5 mg/kg bw/day) of CPF for 30 days exposure (Joshi et al., 2007). According to the EL-Kashoury et al., (2010), significant reduction of testis weight
are observed due to chlorpyrifos exposure during 28 days of treatment. Similar findings were observed in mancozeb treated rats for 30 days of exposure (Joshi et al., 2005), profenofos treated rats @23.14 mg/kg body weight for 60 days exposure (EL-Kashoury, 2009). Kanga et al., (2004), reported a decrease in body weight of CPF 250 mg/kg administrated rats and also Chitra et al., (1999), shows a significant reduction in body weight and testicular organ weight in endosulfan treated rats. In this study, body as well as reproductive organs (testis, seminal vesicle and epididymis, both absolute and relative) weight were decreased which is dose and day dependent (fig-4, 41). Maximum decreasing of body weight was found in higher dose level (12 mg CPF/kg/d) than lower dose (7 mg CPF/kg/d) of CPF treatment, during 15 and 30 days of exposure. Similar finding was demonstrated by Olorunshola et al., (2011), they reveal that chlorpyrifos (16.3 and 23.6 mg/kg body weight) decreased the body and organ weight for 21 days of exposure, and this decline in body weight was dose dependent manner. Similar results were recorded by Chodhuary and Joshi (2003), who reported a significant reduction in the rat testis weight after exposure of endosulfan for 15 and 30 days, at the dose levels of 5, 10 and 15 mg/kg bw/day. This decreasing testicular weight is indicating impairment of testicular functions and androgenesis. Testicular steroidogenesis is regulated by hypothalamo-pitutary axis, which might be troubled by toxic inputs (Singh and Pandey, 1989). The epididymis and seminal vesicles both are androgen-dependent organs. Testosterone is more essential for their growth and function and a reduction in their weights may reflect a decline in bioavailability and production of androgens. A similar type of decrease was found in body weight and reproductive organs weight of adult male rat during 90 days treatment of pirimiphos-methyl exposure (Ngoula et al. 2007). Our results showed that weight of testis and epididymis were significantly lower in the pesticide (CPF) treated rats than in the control. This type of results also found in case of profenofos treated rats. The decrease in testicular weight in pesticides treated rats may be due to reduction of tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of Leydig cells (Sujatatha et al., 2001; Kaur and Mangat, 1980). According to Zidan, (2009), the reproductive organ weights (testis, epididymis and seminal vesicle) of male rats were decreased depending on the rising of dose, and the reproductive organs weight were significantly lowered at the dose level of 5 and 50 ppm of chlorpyrifos-methyl, diazinon and profenofos treatment for 65 days. It is well known that the weight of testis and accessory sex organs are primary indicator of testicular androgen
production (Price and Williams-Ashman, 1961; Rind et al., 1963). The decrease of reproductive organ weight is due to the disruption in functions of testicular androgen production. The reduction of organs weight may be due to pesticides exposure by affecting their hypothalamus, pituitary or both (Okazaki et al. 2001). Aziz et al., (1994) found that diazinon treatment decreased the weight of most genital organs at two different doses of 1.5 and 3mg/kg body weight in male rats for 65 consecutive days. Significant decrease in testicular weight may be a cause of decrease in the number of spermatogenic elements and spermatozoa (Sherins and Hawards, 1974; Takihara et al., 1987). Another study revel that, the decrease in testicular weights is said to be due to reduced in tubular size which were found to degeneration and atrophy of seminiferous tubules, another factor implicated in the reduction of testicular weight is the decrease in thyroid hormone levels on administration of chlorpyrifos which is essential to the development of the testis (El-Kashoury and El-Far, 2004). Variation in dose of CPF, affect the body and organs weight of treated animals and it was significantly lower in both G1 and G2 group for 15 and 30 days with respect to control (Table 3, 4, 18, 19) group. The higher dose (12 mg CPF/kg body weight) exhibit a maximum decreased in body and organ (both absolute and relative) weight than in lower dose (7 mg CPF/kg body weight).

In the present study, the body and organs weight was reduced by the administration of chlorpyrifos which was ameliorated by the oral administration of aqueous extract of Emblica officinalis (fig-3, 4, 18, 19).

When amla was fed with CPF, there was a significant recovery seen in body and organ weight (both absolute and relative) than only treated with chlorpyrifos for 15 and 30 days. It was found that 20 mg/kg/d of amla exposur shows maximum ameliorating effect for 7 mg CPF/kg/d treated group. Same type of study was found to be ameliorated by vitamin C in case of CPF treated rat. Olorunshola et al., 2011 shows that the chlorpyrifos induced dose dependent decreased in body and organ weight was ameliorate through the vitamin C (Ambali et al., 2011). It may be due to recovery of organ or tissue injury or due to revitalization of androgen secretion. It is well known that vitamin C fight against widespread environmental pollutant including carbon monoxide, hydrocarbons, heavy metal and pesticides etc by stimulating enzyme (Rajkumar et al., 2011). Emblica officinalis is a rich source of vitamin C, minerals, steroids, amino acid, gallic acid, tannoids and also contain a wide variety of phenolic compounds etc and this compounds have been reported as powerful
antioxidants so they scavenge the toxic substance in mammalian system. According to this study, when *Emblica* was singly administered, the body weight was more or less similar to the control level in 15 days of exposure but in 30 days of exposure it was significantly increased than the control level. However, the weight of reproductive organs (testis, epididymis and seminal vesicle) was found to be similar in control level in both 15 and 30 days of only *Emblica* exposure.

Decline in testis and epididymis weight could be an indicator of sperm toxicity due to CPF treatment. Parallely sperm cell degeneration is an essential indicator of sperm toxicity and the sperm count is one of the most sensitive tests for spermatogenesis and it's highly related with male fertility. Due to CPF exposure in 7 mg and 12 mg treated group, shows a significant increment in sperm morphological abnormalities and simultaneously a decrease value is presented in sperm count, sperm motility and sperm density for 15 and 30 days of CPF exposure (fig-5-7, 28-33, 42-44, 75-80). In this experiment higher dose level shows maximum toxicological abnormalities in 15 and 30 days of CPF exposure than the lower dose. Similar type of dose dependent decrease in sperm motility and sperm count was found in 16.3 and 32.6mg/kg/d orally chlorpyrifos treated rats for 21 days treatment (Olorunshola et al., 2011). El-Kashoury and El-Din, (2010) also reported that the pesticidal exposure reduces the sperm motility. This was suggested to be due to accumulation of proteins in the testes and epididymis secondary to lack of androgen resulting in increased abnormal spermatozoa (Rao and Chinoy, 1983). Same type of result was found in 5.4 and 12.8mg/kg/d chlorpyrifos treated rats, during 90 days treatment. According to Choudhary and Joshi, (2003), the decrease of sperm motility and density after oral treatment of chlorpyrifos may be due to inadequacy of androgen secretion. Disrupting of testicular functions through the altering activities of enzymes is causative for spermatogenesis (Siha et al. 1995; Reuber, 1981). Similar type of variation was found in 23.14 mg/kg body weight profenofos treated rats for 60 days (EL-Kashoury, 2009). The decrease of sperm density in the epididymis is one of the indicators of reduction in spermatogenesis (Poon et al., 2004). According to Narayana et al., (2006), the sperm density of adult male rats was decrease due to various dose of methyl parathion exposure. Due to histological observation (fig-28-33,75-80) we found a dose dependent severely decrease sperm concentration in epididymis lumen. Decline in sperm density may be due to direct spermicidal effects shows on chlorpyrifos treated rats epididymis. Zidan, (2009) established this fact that
percentage of sperm motility and sperm count significantly decrease and simultaneously total sperm abnormalities are significantly increase in both the three pesticides (chlorpyrifos methyl, dizinon and profenofos,) treated groups. According to the result thick coil tail, tapered head and without head were the selected parameters for sperm abnormalities studies, without head abnormalities showing maximum percentages in both G1 and G2 group (fig-7, 44) during 15 and 30 days of CPF exposure, and these anomalies are considered as a better discriminator between fertile and infertile males (Guzik et al., 2001). It is well known that the sperm morphology is considered as a better discriminator between fertility and infertility and motility is a useful marker of toxic damage. Two main regulatory processes control the spermatogenesis, one of is endocrine regulation via the gonadotropin hormones and another is local regulation via inter-cellular communication (Holdcraft and Braun, 2004). The similar result presented by Abd EI-Aziz et al., (1994), who revealed that diazinion treated rats show decreased sperm motility associated with an increment of dead sperm percentage. Prior epidemiologic work on Chinese pesticide factory workers showed that OP (organophosphates) exposure was associated with decreased sperm concentration and motility (Padungtod et al., 2000). It is established that, sperm motility is an important functional measurement to anticipate sperm fertilizing capacity (Aikten et al., 1984). Any negative impact on motility would seriously affect the fertilizing ability of the organism (Murugavel et al., 1989). It is a fact that sperm motility is seriously affected by low level of ATP content and it may be affected by alteration of the enzymatic activities of oxidative phosphorolytic process. Similarly oxidative phosphorolytic process is required for ATP production, so a source of energy is essential for the alleviated movement of spermatozoa (Joshi et al., 2007). The full ATP pool is crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility, which is one of the major causes of infertility (Poon et al., 2004).

CPF with combination of 20 mg Emblica officinalis treated group (G4 and G5 group) for 15 and 30 days exposure, significantly increases the sperm count, sperm motility, sperm density and normal sperm morphology and simultaneously abnormal sperm morphology was significantly decreased than the CPF treated group G1 and G2 for 15 and 30 days treatment. According to Chakraborty and Verma (2009), oral administration of aqueous extract of Emblica officinalis along with ochratoxin for 45 days exposure, significantly mitigates ochratoxin-induced alterations in reproductive
parameters. In recovery aspect, the herbal product, *Emblica officinalis*, find similar light in our study, where there was a significant increment found in sperm count, normal sperm morphology and sperm motility. This shows the ameliorative effect of *Emblica officinalis*, which might be due to the presence of bioactive compounds, namely: emblicanin A, emblicanin B, punigluconin and pedunculagin which are known to provide protection against free oxygen radicals in various *in vitro* studies (Bhattacharya et al., 1999). Similar type of result was found in Ali et al., (2011). They revel that, during 35 days treatment of 100 mg and 150 mg/kg/d aqueous extract of *Emblica officinalis* mitigate the toxicological effects of endosulfan, and they restore the sperm count and sperm motility. According to Ali et al., (2011), it is shows that the higher dose of amla (150mg/kg.b.w/d) gives a better result than the lower dose (100mg/kg.b.w/d). Olorunshola et al., (2011), established that vitamin C treated rats was significantly increased epididymal sperm concentration and motility than CPF treated groups. 

Similar decreased motility was noticed in the study by Faraga et al., (2010) principally caused by defective spermatozoa. Sperm DNA damage occurred secondary to oxidative stress exacerbated by a decline in local anti-oxidant protection especially during epididymal maturation resulting in a variety of adverse clinical outcomes (Aitken and Iuliis, 2004). It produced defective spermiogenesis with cells characterized by retention of excess residual cytoplasm, persistent nuclear histones, poor zona binding, disrupted chaperone content and referred to as dysmature cells. Elevated levels of Malondialdehyde levels (induction of a lipid peroxidation process) and decrease in superoxide dismutase reaction corroborated their findings.

Chlorpyrifos also induces biochemical changes in testis, epididymis and seminal vesicle. This study reveals that the protein content was significantly elevated in male reproductive organs due to chlorpyrifos exposure for 15 and 30 days treatment and this elevation was dose and day dependent. Maximum protein value was found in testis @12mg CPF treated group for 30 days treatment. According to Joshi et al., (2007), the protein content of testis was significantly increased in chlorpyrifos treatment during 30 days exposure. Similar results showed the same trend for the elevation of protein content caused by several pesticides, at different exposure levels and different concentrations, as reported by EI-Kashoury and Tag El-Din (2010); EI-Kashoury (2009); Shivanandappa and Krishna Kumar (1981);
Bulusu and Chakravarty (1992); Joshi et al., (2003) and Ngoula et al., (2007). Puga et al., (1974) demonstrated that the elevation of protein content may be due to the stimulation of growth proteins and RNA synthesis. Dikshith and Dutta (1972), Gupta et al., (1981) and Singh and Pandey (1989) showed that an elevation in the testicular protein may be due to the hepatic detoxification activities which resulted in the inhibitory effect on the activities of enzymes which is involved in the androgen biotransformation. Rao and Chinoy (1983), suggested that the accumulation of protein occurred in testis and epididymis due to androgen deprivation to target organs and this deprivation effect also led to a reduction in testicular and cauda epididymal sperm population, loss of motility in the latter and an increase in the number of abnormal spermatozoa.

Aqueous extract of Emblica officinalis reflect a remedial measure, when it fed with CPF. In this study CPF exposure elevated the protein content (testis, seminal vesicle and epididymis), and it significantly decrease during amla treatment (G4 and G5 group for 15 and 30 days treatment). This decrease is showing a dose and day dependent normalization. Chakrawarti et al., (2010) reported an earlier and faster recovery shows in Emblica officinalis treated groups. A significant increase in the number of ribosome may be occurring due to their increase mobilization from endoplasmic reticulum (ER) and this leads to the augmented protein synthesis (Mukerjee and Goldfeder, 1974). Through radical scavenging activity Emblica scavenge the free radicals and they mitigates the toxicological substance, and normalished the protein content which is up graded due to CPF exposure.

During this study chlorpyrifos exposure significantly increased the uric acid level in blood serum. This increase is maximum in 12 mg CPF treated group during 30 days of treatment. Our result associate with Prashanthi et al., (2006); Narayana et al., (2006), they revel that the various doses of methyl parathion treated rats significantly increase their uric acid level. Similar type of increment in uric acid level was found in 28 mg/kg/d malathion treated group for 28 days treatment (Uzun and Klender, 2010). This may be due to stress induced toxicity leading to increase the uric acid level in blood serum. It is well known that the elevation of uric acid level is strongly associated with hypertension and renal disease and those have a negative effect in reproduction. The changes were less severe in Emblica officinalis treated group suggesting a protection against pesticides. Emblica officinalis is one of the
richest sources of vitamin C and it mitigates the uric acid level in blood serum through their antioxidant activity.

In present study, a significant reduction was found in ACP and ALP level of testis, seminal vesicle and epididymis tissue of chlorpyrifos treated rats for 15 and 30 days exposure. During experimental period a dose dependent variation are present in rat’s reproductive organ, reflecting suppression in testicular function (Johnson et al., 1970) and indicating a nonfunctional spermatogenesis. Our result is supported by the finding of Prashanthi et al., (2006) and Narayana et al., (2006). They revealed that the ACP level was significantly decreased in methyl parathion induced rat’s epididymis. Simultaneously a significant day and dose dependent decreased was found in ALP level of testis and seminal vesicle of CPF treated rats but other side ALP activity of epididymis significantly increase than the control animals. According to El-Kashoury, (2009), the ACP and ALP level was significantly decreased in profenofos treated testicular tissue of male rats. Chlorpyrifos induced damage cells are started to release the ACP and ALP into the blood stream, hence reducing its level in the reproductive tissue. This is similar to the findings of Abraham and Wilfred, (2000). Decline in ALP activity indicated that chlorpyrifos treatment created a state of decreased steroidogenesis where the intra- and inter-cellular transports were reduced and the metabolic reactions were disrupted and it become canalized the required inputs for steroidogenesis. (Yousef et al., 2001). Acid phosphatase is enzyme competent of hydrolyzing orthophosphoric acid esters in an acid medium. The testicular acid phosphatase gene is up-regulated by androgens and is down-regulated by estrogens (Yousef et al., 2001), when the androgen production is inferior, may be the ACP activity is sermonized. In remedial aspect when 20 mg/kg/d Emblica was administrated with CPF in G4 and G5 groups, shows a significant recovery in ACP and ALP level. Singly amla fed group shows the similar value of control. Due to antioxidant activity of Emblica officinalis, repaired the cell damage and it may be through their ameliorating activity, it increase the intercellular transport and androgen production.

In this study the hormonal regulation demonstrated a significant dose dependent decreased in serum testosterone level than the control group. However, the testosterone level of testis showed a decrease value in 7 mg and 12 mg/kg/d CPF treated group for 15 and 30 days than the control level, but the higher dose level increase the testis testosterone level than the lower dose group. Same finding was
demonstrated Mandal and Das, (2011), they revel that chlorpyrifos had a dual effect at both doses (5 and 10 mg/kg/30 days) on oxidative stress changes, but at higher doses, the cells we triggering its natural defense mechanism to combat the insult of lower doses of chlorpyrifos and become operative through corrective measure of antioxidant enzymes defense system and pituitary gonadotropins hormones feedback mechanism on testis. Due to chlorpyrifos exposure reduction in the serum testosterone level is demonstrated by Joshi et al., (2007). In this present study, serum and testis testosterone level were decreases in 7 and12 mg CPF treated rats during 15 and 30 days of exposure. Similar observation was noted by Zidan, (2009), who revealed that there is significant alteration found in chlorpyrifos methyl, diazinon and profenofos treated male rat testosterone level. The testosterone is the principal male hormone produced by the interstitial Leydig cells of testis. Thus testes are responsible for the synthesis of the male sex hormones; so the decrease in testosterone level might be due to an extensive damage of Leydig cells. Besides, disorders of male genital function (hypogonadism) are manifested by a decrease in plasma testosterone level. Hypogonadism may occur with faulty seminiferous tubular function or defective Leyding cell function and this leads to aridity through decreased production of spermatozoa (Zidan, 2009).

Hormonal estimation indicated that the values of Emblica officinalis treated groups were near the control values. And Emblica with CPF treated groups shows a significant increment in testis and serum testosterone level during 15 and 30 days of exposure than the CPF treated group G1 and G2. Emblica mitigate the toxicological activity of CPF and increase the testosterone level of testis and serum. Emblica officinalis extract has been shown to have antioxidant and antiperoxidant properties due to the presence of tanoids, mainly emblicanin-A, emblicanin-B, punigluconin, pedunculogin gallic acid (Bhattacharya et al., 1999) and also presence of steroid (Gupta et al., 2013). The in vitro antioxidant activity of tanoids was demonstrated by Ghosal et al., (1996). Emblica officinalis is the rich sources of vitamin C, minerals and amino acids and also contains a wide variety of phenolic compounds (Rajkumar et al., 2011), those are the excellent scavengers of oxygen free radicals within the cells where reactive metabolites are produced (Uzunhisarcikli et al., 2007) by chlorpyryfos toxicity. Steroids are present in water soluble Emblica officinalis extract (Gupta et al., 2013), that may mimic the normal function of testosterone which plays
an important role in reproductive development in mammals. But any of the other constituents of aqueous extract of *Emblica officinalis* may inhibit the secretion of testosterone when administrated singly amla as because *Emblica* increase the mitigated testosterone value of CPF but they cannot increase the testosterone level singly.

In addition sperm DNA damage are increasingly used to evaluate sperm function. The comet assay has been potentially useful tool to monitoring DNA damage in a number of different cell types (Singh *et al*., 1988; Olive and Banath, 1996), including human sperm (Duty *et al*., 2003). The comet assay relies on the electrophoretic movement of DNA out of single cells, in which low molecular fragments migrate away from the cell towards the positive electrode in a pattern resembling a comet tail. It is useful marker as an epidemiological end-point to measure of sperm DNA damage, which is predictive of fertilization and embryo cleavage rates (Sun *et al*., 1997).

Single-cell gel electrophoresis (SCG) is a simple, sensitive and useful technique. Being able to reflect quantitatively the genotoxicity of many hazardous agents, it is promising for application in environmental genotoxic monitoring and the study of carcinogenesis. In clinics, it can be able to evaluate the DNA repair ability and monitor DNA breaks (Yuquan *et al*., 1997). It has been found wide application in biomedical science, including epidemiology (Duthie *et al*., 1996), environmental pollution (Pandrangi *et al*., 1995), industrial hygiene (Plappert *et al*., 1995), biomonitoring (Tice and Strauss, 1995). Chlorpyrifos was found to be a toxicant in male fertility aspect which increased DNA single strand breaks (SSBs) in rat sperm cells (Yuquan *et al*., 1997). Comet assay shows the appearance of the damage DNA after electrophorasis, is based on the observation of single cells and gives quantitative data mainly showing a DNA single-strand (including some double-strand) breaks by means of micro electrophoresis. The degree of DNA damage can be well reflected through measuring the parameters such as cell tail length as a biomarker to judge the genotoxicity of the exogenous and endogenous (Delaney *et al*., 1993) hazardous agents in the human body; it has many advantages and some limitations of its own.
In this study, it was revealed that during low dose and high dose exposure of CPF shows a dose dependent variation during 15 and 30 days treatment. According to the table 23, G2 group shows maximum DNA breakdown than the G1 group in comparison to controls. It’s presented a dose dependent toxicity during 15 days chlorpyrifos exposure. During 30 days treatment (Fig-34-39, 81-86) maximum DNA breakdown were shown in 12 mg CPF treated group G2 than the G1 group. Both the G1 and G2 group of 15 and 30 days CPF treatment shows a significant DNA alteration than the control group and all the variation are attempted dose and day dependent manner. Similar study was conducting Rahman et al., (2002); they clearly establish that significant DNA damage was observed in Chlorpyrifos at 24 and 48h post-treatment form of comet induction. And they establish the appropriateness of the comet assay to highlight the genotoxicity resulting from exposure to pesticides. Study indicated that Chlorpyrifos have the ability to damage DNA in a dose dependent manner. An in vivo study with fish erythrocytes showed a dose related increase in DNA stands breaks in OP pesticides using comet assay (Saleha Banu et al., 2001). In vitro studies on dose dependent induction of DNA damage with pesticides have also been reported. Investigations have shown that deltamethrin was able to induce DNA damage as revealed by comet assay with increasing dose in human peripheral blood leucocytes (Saleha Banu et al., 2001). It is fact that many scientists were reported that the comet assay is highly sensitive to detect DNA damage of pesticides (Villani et al., 1998; Villarini et al., 1998; Vignereux et al., 1998; Blasiak et al., 1999; Godard et al., 1999; DeMarco et al., 2000). A number of in vitro, animal and human studies have shown that insecticides may alter DNA integrity of different cell types. Carbaryl has been shown to induce unscheduled DNA synthesis, and presumably DNA damage, in cultured human fibroblasts (Ahmed et al., 1977a). In humans, a case study of eight patients exposed to chlorpyrifos following residential application found increased DNA damage (measured as chromosome alterations) compared with reference ranges, suggesting that chlorpyrifos may be associated with human genotoxicity (Lieberman et al., 1998/Z7). Carbamates and organophosphates, including carbaryl and chlorpyrifos or their metabolic intermediates, have been suspected to act as alkylating agents on DNA bases, directly or indirectly through protein alkylation (Wild, 1975; Ahmed et al., 1977a; Rahman et al., 2002). Due to remedial aspect when 20 mg/kg/d Emblica officinalis was administrated G4 group showed maximum recovery in 15 days treatment. In our study we established that Emblica shows a dose dependent
protection against Chlorpyrifos exposure. Because, in long term exposure showed maximum DNA breakage in sperm nucleus and higher dose represent the highest toxic damage in sperm nuclei. Due to ameliorating effect amla scavenges the toxic particle and mitigate the toxic DNA damages. This study revels that the amla interiority of toxic damage manner neutralized the sperm toxicity.

Another essential toxic indicator is metal deposition in essential organs. Copper, lead, chromium etc- metals are work as an activator of many enzymes and they play an important role in animal growth, development and reproduction (Underwood, 1977). Insufficiency or excess of any of those elements in animal tissue might lead to various diseases and even death (Rogers et al., 1985; Stemmer et al., 1985; Prohaska, 1987). Therefore, the concentration of these metals must be ever maintained at appropriate level. According to our study indicated a dose dependent increment in copper, lead, chromium, cadmium and nickel of CPF treated rat groups in testis, epididymis and seminal vesicle during 15 and 30 days of exposure. Maximum metal consumption was noticed in 12 mg CPF treated group of testis, seminal vesicle and epididymis than the control group of animals. Exposure to environmental contaminants has been suggested to play a role in the pathophysiology of adverse reproductive health effects including decreased semen quality, sub-fertility, change in birth sex ratio, and an increase in the prevalence of developmental abnormalities of the male reproductive tract (Carlsen et al., 1992; Colborn et al., 1993; Swan et al., 1997; Marcus et al., 1998; Allan et al., 1997; Swan et al., 2000). The trace element copper has been identified as a highly toxic element for sperm. Its affect the sperm count sperm motility and sperm viability in humans (Maryam et al., 2010). According to Aydemir et al., (2006), Copper may be mediator of the effect of oxidative damage and play an essential role in spermatogenesis and male infertility. It is plausible to consider that copper concentration in tissue as a good marker for evaluating reactive oxygen radicals, sperm metabolism, vitality, motility and relevant semen parameters. Therefore chlorpyrifos exposure determines the copper deposition in testis, seminal vesicle and epididymis, is recommended to the male infertility. In this experiment lead is maximally deposited in experimental tissue material due to CPF exposure. Lower dose of lead contamination significantly reduced the number of sperm within the epididymis, while the lead deposition is increased it can reduce both the sperm count and sperm motility and increase the sperm abnormalities within the epididymis simultaneously significantly decreased the epididymis and seminal vesicle
weight (Wadi and Ahmad, 1999). In another experiment Marouani et al., (2012), showed that the hexavalent chromium significantly decreased in testis weight, epididymal spermatozoa, sperm motility and serum testosterone level, simultaneously increased the sperm abnormal morphology of adult rats. In present experiment shows cadmium is present low concentration in testis epididymis and seminal vesicle. Some of the record indicated that the effects of cadmium exposure in laboratory animals shows renal tubular damage, placental and testicular necrosis, structural and functional liver damage, osteomalacia, testicular tumors, teratogenic malformations, anemia, hypertension, pulmonary edema and chronic pulmonary emphysema (Ragan and Mast, 1990). In another experiment when nickel sulphate administrated orally to adult male mice @ 5 and 10 mg/kg/ body weight during 35 days exposure, the absolute weight of testis, epididymis and seminal vesicle was significantly decrease, simultaneously normal sperm morphology, sperm count, sperm motility and testicular enzymatic activity was lowered. In this experiment histopathological change of testis, epididymis and seminal vesicle was noticed. These testicular and spermatotoxic changes may be responsible for infertility (Pandey et al., 1999). According to Massanyl et al., (2007), suggest that nickel has negative effect in spermatozoa production. In our study it is revel that may be chlorpyrifos disrupted the urinal function, some blockage are arise in urinal pathway through chlorpyrifos metabolism. Due to this urinary alteration may be heavy metal compounds (Cu, Pb, Cr, Cd, and Ni) are deposited the in male mammalian reproductive system (Testis, epididymis and seminal vesicle). It is known that those compounds have toxicological effect in male reproduction. Sperm morphological deformities, decrease of body and organ weight, hormonal ambiguity and histopathological changes are indication of this heavy metal deposition which is increase through CPF exposure. However, through scavenging and chelating activity Emblica scavenge those metallic compounds and mitigated the toxicological effect in mammalian reproductive system. When 20 mg/kg/d Emblica fed singly, those heavy metal compounds were found in normal amount. In recovery accept 20 mg/kg/d Emblica fed with 7 and 12 mg CPF /kg/d during 15 and 30 days exposure shows a significant reduction in deposition of heavy metal compounds. Through free radical scavenging activity amla can scavenge the toxic chemical, and some bio active component which is present in amla, they are help to mitigate the deposition of metal compound.
In histopathological observation sperm density in the epididymis is one of the indicators of reduction in spermatogenesis owing to the toxicity of any agent (Poon et al., 2004). Decline in sperm density may be due to direct spermicidal effects of chlorpyrifos treated rats. The obtained results are in accordance with Narayana et al. (2006), who revealed that the sperm density of adult male rats was decreased due to various dose of methyl parathion exposure. The photographic results indicates the same things, that, due to CPF exposure both the higher and lower dose exhibited moderately (++) and severely (+) decreased sperm density. Decreased sperm density attributed the negative fertility. According to Joshi et al., (2007), negative fertility may be attributed to lack of forward progression and leads to reduction in density of spermatozoa and altered biochemical limitation of cauda epididymis. Similarly in remedial aspect, singly amla (G3) and amla with CPF treated group (both G4 and G5) shows normal sperm density (++++), during 15 and 30 days of exposure.

According to histophotographical representation, changes were determined in low and high dose of CPF treated rat’s testis, epididymis and seminal vesicle during 15 and 30 days of exposure. Cellular edema, necrosis, germ cell disorganization, epithelial vacuolization, degenerative changes in seminiferous tubules and devoid of spermatozoa are present in testis. Similarly, fused inter epithelial cell and diffused stereocilia, cluster of spermatozoa in lumen, disrupted inter tubular stoma, fully empty lumen, devoid of sperm cell, sperm with cellular debris are present in epididymis and simultaneously maximum cellular lumen and minimum epithelial folds are present in CPF treated rats Seminal vesicle during course of experiment, both high and low dose of CPF treated rats. However, in control and Emblica treated rat’s shows, no pathological changes in testis, epididymis and seminal vesicle. During recovery aspects Emblica with both the CPF treated groups shows minimum degenerative changes in tested materials. methylparathion induced male rats showed lesser number of spermatogenic cells and necrosis in seminiferous tubules, edeme in interstitial tissue during 24h, 4 weeks and 7 weeks of experiments Uzunhisarcikli et al., (2007). However, in vitamin treated group and control group showed normal cellular architecture and vitamin + methylparathion treated group shows minimum cellular disruption during course of experiments. Another histopathological observation demonstrated by Joshi et al., (2007), 7.5, 12.5 and 17.5 mg CPF/ kg/d administrated rats was showing some degeneratives changes in testis during 30 days of exposure. However, low dose chlorpyrifos showed a slight change form normal
histological features and testicular section from the high dose groups showed severe degenerative changes. According to present experiments, higher dose (12mg CPF) of chlorpyrifos showed maximum toxicological alteration due to lower dose (7mg CPF) during 15 and 30 days exposure. It is fact that the pesticides have toxicological outcome to led significant histological changes in male reproductive organs like testis, epididymis and other related reproductive organs (Aziz et al., 2007 and 2008). The defective or so called inactive spermatozoa travel through the straight ducts of testis or reach to the epididymus for maturation under the influence of various enzymes and factors released by sertoli and epididymus cells, than they moved to the vas deferens till they are ejaculated along with prostatic and seminal vesicle secretion in the semen. Any ambiguity in the function of mucosal cell of epididymis and vas deferens may result in a defective maturation of spermatozoa and its leads to male infertility (Aziz et al., 2008). In another experiments Shittu et al., (2013), incorporated that 10.6 mg/kg CPF induced testicular toxicity showed some degenerative changes in male rat testis during 15 weeks exposure and vitamin C has been shown in this study to mitigate the reproductive toxicity evoked by chronic exposure to CPF in rats. Therefore, this vitamin may be used to mitigate male reproductive toxicity induced by CPF and by extension other OP insecticides.

*Emblica* can repair the cell or tissue injury through their free radical scavenging activity. In this study *Emblica* can mitigate the toxicological activity in to the toxic chemicals induced animal. Its can normalize the physiological and biochemical ambiguity of tissue.

Greater precautions must be taken in order to minimize the harmful side effects of organophosphorus compounds on all the surroundings to decrease the occurrence of environmental pollution. So we have to be aware of detrimental effects of chlorpyrifos on male reproductive system. And same time we encourage to used the herbal material like *Emblica officinalis*. 