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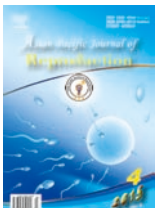

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Potential pathways of pesticide action on erectile function-a contributory factor in male infertility

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ABSTRACT

One of the important objectives of this manuscript is to focus on the place of erectile dysfunction as an important factor for infertility. The review is about correlating the indiscriminate use of pesticides and to find out and highlight the evidences for mechanism of action of these pesticides for erectile dysfunction and find out the most used and most dangerous pesticide from erectile dysfunction point of view. The review suggests that erectile dysfunction is having a significant place as a causal factor for infertility. Study infers that pesticides are having multiple mechanisms of action through which these cause erectile dysfunction. It also reflects that acetamiprid is having most devastating effect causing erectile dysfunction as it acts through multiple inhibitory pathways. The review successfully highlights the indiscriminate regional use of pesticides.

1. Introduction

Male fertility is reported to be declining day by day. According to recent estimates every year about 60-80 million couples all over world suffer from infertility, of which probably between 15-20 million are in India alone[1]. Researchers have shown that male factors account for 40%-50% of infertility in human[2, 3] and one of the major problems contributing towards male infertility is erectile dysfunction afflicting as much as 10% of the male population[4]. The data are more alarming above the age of 40 as nearly 52% of men are afflicted. There is enumerable number of factors like psychological factors, physiological, pathological, social, environmental, nutritional etc that play a major role in pathogenesis of erectile dysfunction. Today environment is laced with heavy metals, radioactivity, poisonous fumes of organic chemicals, pesticides that may attribute for erectile dysfunction.

Incidentally it has been reported that a number of birth defects and infertility problems are being faced in the pesticide afflicted areas in India and abroad[5]. Reports also depict that pesticides may cause erectile dysfunction [6]. A number of mechanisms have evolved for erectile dysfunction by various pesticide residues in the body. The purpose of this compilation is to put all the mechanisms of action of different pesticides at one platform so as to enable the researchers, physicians and regulatory authorities to design check points for dreaded chemicals being pumped in the environment. It will also give an idea about a pesticide that is having potential multiple toxic effects through different mechanisms.

2. Major causes of male infertility

Before going into details of different mechanism of actions, it is pertinent to enumerate some common factors that affect male fertility. Male infertility is commonly due to deficiencies in the semen and semen quality is used as a surrogate measure of male fecundity[7]. Some of the pre-testicular factors impede adequate support of the testes and include situations of poor hormonal support and poor general health including hypogonadism; drugs such as cimetidine that decrease follicle stimulating hormone (FSH) levels, and nitrofurantoin that decreases sperm motility; adopted life style (marijuana, cigarette smoking); and strenuous activities such as strenuous bicycle riding[8]. Testicular factors affect quality and quantity of semen produced by the testes and include age, genetic defects of the Y-chromosome (Klinefelter syndrome), neoplasm e.g.

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seminoma, cryptorchidism, varicocele which account for 14%[9], mumps viral infection[10] and may be idiopathic which accounts for 30% of male infertility[11]. USP 26 a peptidase enzyme expressed by USP 26 an X-linked gene in testis has been found to be defective in some cases of birth defects[12]. Besides this, there are some post-testicular factors that decrease male fertility due to conditions that affect the male genital system after testicular sperm production and include defects of the genital tract as well as problems in ejaculation: e.g. impotence, Vas deferens obstruction, lack of Vas deferens, infection e.g. prostitis, ejaculatory duct obstruction and hypospadias [13].

Other important factor are conditions that affect the hypothalamus and pituitary gland will eventually affect the gonadotropin releasing hormone (GnRh) and hence the levels of follicle stimulating hormone, luteinizing hormone and prolactin hormone. These conditions include Kallmann syndrome (isolated gonadotropin deficiency), hyperprolactenemia and hypopituitarism. Hyperprolactinemia may be due to diseases affecting the hypothalamus and pituitary gland or secondary to disease of other organs such as the liver, kidneys and thyroid[14]. Hyperprolactinemia may cause hypogonadism, erectile dysfunction, decreased libido and infertility[15].

The etiological importance of environmental factors in infertility has also been stressed[16]. The implication of toxins such as glues, volatile organic solvents, silicones, physical agents, chemical dusts and pesticides in infertility has already been established[17]. Radiations and excessive heat to the genitalia have damaging effect on the testicles. Hence individuals having direct contact with or exposure to such chemicals have high chances of having primary or secondary infertility as the case may be. Estrogen-like hormone-disrupting chemicals such as phthalates are of particular concern for infertility in men and for effects on offspring of women. Exposure to phthalates can occur via dietary consumption, dermal absorption or inhalation and has been linked with impaired spermatogenesis and increased sperm DNA damage[18, 19]. The mechanism for this is probably due to increase in the generation of reactive oxygen species (ROS) within the testis and a concomitant decrease in antioxidant levels, culminating in impaired spermatogenesis as observed in rats[20]. The contribution of tobacco smoking and alcohol intake to infertility has also been demonstrated. Tobacco smoking was observed to damage sperm DNA[21]. Though some of the damage is irreversible, but stopping smoking can prevent further damage [22]. It has been reported that smokers are 60% more likely to be infertile than non-smokers. Smoking reduces the chances of IVF producing a live birth by 34% and increases the risk of an IVF pregnancy miscarriage by 30%[23]. Smokers have decreased levels of antioxidants such as Vitamin E and Vitamin C, placing their spermatozoa at additional risk of oxidative damage.

Sexually transmitted diseases (STD) have also proved to be a leading cause of infertility. They are often asymptomatic but may display few symptoms, with the risk of failing to seek proper treatment in time to prevent decreased fertility[22]. Some of the identified STDs (such as syphilis, trichomoniasis, chancroid, chlamydia, gonorrhea, herpes simplex virus, human papilloma virus, lymphogranuloma venerum) are treatable while many are not and may eventually lead to death. Similarly the urinary tract has a relative anatomical association with the reproductive tract. Escherichia coli and Staphylococcus aureus are reported to be the most prevalent Gram negative and Gram positive organisms implicated in UTI respectively[24].

Obstructive azoospermia may result from previous vasectomy; epididymal, vassal, or ejaculatory duct pathology relating to genitourinary infection; iatrogenic injury during inguinal or scrotal surgery and congenital anomalies[25]. Azoospermia (low sperm counts), abnormal spermatozoa morphology (shape) and low sperm motility are usually asymptomatic conditions to most males but of great etiological importance. It is well recognized that sperm DNA can be damaged oxidatively by oxidative stress[26] and nonoxidatively by mechanisms such as aberrant apoptosis and incomplete sperm protamination[27].

3. Microorganisms and infertility

Microbial infections have been reported to reduce sperm viability. *Staphylococcus aureus* is the most prevalent gram positive organism, while *Escherichia coli* is the most prevalent gram negative organism isolated in the semen of males with primary infertility[28]. Chronic epididymitis secondary to *Chlamydia trachomatis* infection had been shown to blockage of the epididymis and thus obstructive azoospermia[29]. However, *Ureaplasma urealyticum* infections induce leukocytospermia and consequently lead to sperm damage, decrease sperm counts and invariably impaired sperm motility[30]. Herpes simplex virus (HSV) was reported to have been found in the semen of some infertile men and was related to low sperm count and poor motility[31]. Mumps viral infections in adolescent and adult males carry about 30% risk of developing orchitis or epididymitis, which can result in testicular atrophy and sterility[32].

4. Chemotherapy and infertility

Studies have shown that the antral follicle count decreases after the third series of chemotherapy, whereas follicle stimulating hormone (FSH) reaches menopausal levels after the fourth series; inhibin B and anti Mullerian hormone levels have been reported to decrease following chemotherapy[33]. Drugs with high risk of infertility include procarbazine, cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine; drugs like doxorubicin, cisplatin and carboplatin have medium risk while therapies with plant derivatives (such as vincristine and vinblastine), antibiotics

(such as bleomycin and dactinomycin) and antimetabolites (such as methotrexate, mercaptopurine and 5-fluoruracil) have low risk of gonadotoxicity[19].

5. Psychological/Physical/Behavioral problems

Several sexual problems exist that can affect male fertility. These problems are most often both psychological and physical in nature. It is difficult to separate the physiological and physical components. Stress can be an important reason for infertility. Ejaculatory incompetence is a rare psychological condition that prevents men from ejaculating during sexual intercourse even though they can ejaculate normally through masturbation. This condition sometimes responds well to behavioral therapy.

Above all one of the main upcoming reasons for male infertility is pesticides. Experimental evidence in the laboratory has linked the chemicals present in pesticides to reduced sperm quality, testicular cancer and reproductive abnormalities. The chemicals work by "blocking" the activity of hormones, known as androgens, which influence the development of the male reproductive system. Several studies have suggested that human semen quality and fecundity is declining[34–46]. A pesticide is "any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies". They fall into three major classes: insecticides, fungicides, and herbicides, Based upon the target organism classification these are also rodenticides, nematicides, molluscicides and acaricides[47]. Another classification categorizes pesticides according their chemical structure. Insecticides include organochlorines, organophosphates, and carbamates. Organophosphate and carbamates are less toxic and largely replaced organochlorines.

Pesticides may differ according to their chemical structure, their mechanism of action and the toxicity they exhibit, but typically each pesticide consists of one (or more) active ingredient, which exerts the pesticidal activity, and an inert ingredient, which is inactive and helps in handling the active ingredient. Several studies have shown that the inert ingredient is not as inactive as it was previously believed to be[47,48]. Over 700 active ingredients are in use worldwide as pesticides, each with distinct chemical and toxicological properties[49].

In addition to the desired effects of crop protection and pest management, pesticides have some recognized adverse impacts on human health and the environment. Humans have a great risk of exposure through several pathways in occupational, agricultural and household use. Inhalation, oral, dermal and ocular is four possible routes for pesticide exposure. Ingestion of food and water is thought to be the main routes of pesticide exposure in the general population, while dermal absorption is suspected to be the main source of occupational exposure[49]. Over 25% of fruits, vegetables, and cereals are known to contain detectable residues of at least two pesticides and more than 300 different pesticides are known to contaminate food products sold in the EU[50]. In the majority of cases, however, human exposure is unintentional and unintended [51,52]. Pesticides disrupt different mechanisms in the body.

6. Different mechanisms of action of pesticides causing erectile dysfunction

6.1. Pesticides can be the reason of erectile dysfunction

Penile erection is a complex neurovascular phenomenon. It involves the coordination of three hemodynamic events: increased arterial inflow, sinusoidal smooth muscle relaxation and decreased venous outflow. It also implies the interaction of the brain, nerves, neurotransmitters, smooth muscles and striated muscles. An alteration in any of these components may affect the response of the erectile tissue and cause erectile dysfunction[53–57]. The effect of the normal aging process on erectile function is unknown and the cause of age-related dysfunction is likely to be multifactorial in origin [58]. Pesticides act via different mechanism like oxidative stress, lowering testosterone levels, etc. [59–61]. Pesticides are responsible for decreasing testosterone concentration either by inhibiting release of FSH or LH [62]. Pesticides are also responsible for apoptosis of leydig cells and hence decreasing overall concentration testosterone. Pesticides also cause increase secretion of hypothalamic corticotrophin releasing hormone which stimulates release of adrenocorticotrophic hormone (ACTH) and so cortisol[63] which inhibits GnRH and so LH and testosterone decreases. So, all the above written pathways are responsible for decreasing testosterone concentration. Alterations in blood vessels, hormonal changes, neurologic dysfunction, medication and associated systemic diseases are the main causes of erectile dysfunction[64].

6.2. Pesticides inhibit acetylcholine esterase

An acetylcholine esterase inhibitor (AChEI) or anti-cholinesterase is a chemical that inhibits the acetylcholine esterase enzyme from breaking down acetylcholine (Figure 1), thereby increasing both the level and duration of action of the neurotransmitter acetylcholine. Existence of reversible, quasi-irreversible and irreversible inhibitors of ACh like chloropyrifos, Malathion has been reported[65]. Increase in acetylcholine inhibits the release of gonadotrophin releasing hormone (GnRH) and that inhibits release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). It results into

inhibition of gametogenesis and steroidogenesis[66, 67]. So, it is quiet likely that synthesis of testosterone being a steroid hormone may get hampered that could further result in erectile dysfunction. Pesticides like chlorpyrifos and carbofuran inhibit acetylcholine esterase[68, 69]. Hence increased acetylcholine suppresses the reproductive functions [53- 57].

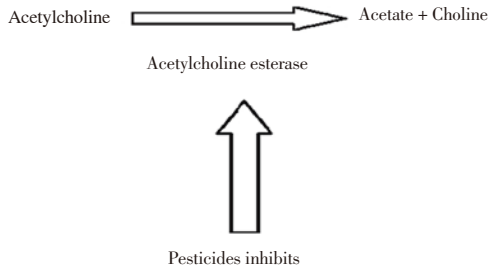

Figure 1. Inhibitory action of pesticides on Acetylcholine esterase

6.3. Oxidative stress

Oxidative stress occurs when cells are exposed to excessive levels of reactive oxygen species (ROS) as a result of an imbalance between pro-oxidants and the protective mechanisms conferred by antioxidants[70]. ROS are formed during regular metabolism due to the univalent reduction of oxygen molecule. Superoxide (O^{2-}) is the most important among the ROS. Organophosphate induces production of ROS and hence causes reproductive tissue damage[71]. Atrazine and imidachloroprid is also responsible for oxidative stress by reducing levels of glutathione[59, 60]. Hydrogen peroxide (H_2O_2), hypochlorous acid (HOCL), and peroxynitrite ($OONO^-$) are other important free radicals implicated in the pathophysiological mechanism of vascular disease. The vascularendothelium is the major source for these free radicals. Besides this, platelets and leukocytes are the other important sources of ROS[72]. Superoxide radicals are generated because of incomplete oxygen reduction in the electron transport system. Membrane bound enzymes, such as nicotinamide adenine dinucleotide hydrogenase–nicotinamide adenine dinucleotide phosphate hydrogenase oxidase, are the major source of superoxide radicals in activated phagocytic cells[73]. It has been reported that up regulation of these enzymes is associated with an increased risk of vascular disease[74, 75]. Superoxide dismutase (SOD) is an important enzyme that removes the superoxide radicals from the human body. There are 3 types of SOD isoenzymes: cytosolic Cu Zn-SOD, mitochondrial Mn SOD, and extracellular SOD. Extracellular SOD reportedly plays a critical role in maintaining the redox state of vascular interstitium and thereby prevents the pathophysiological effects of superoxide in the vasculature. Extracellular SOD converts superoxide to H_2O_2.

The interaction between NO and ROS is one of the important mechanisms implicated in the pathophysiological process of erectile dysfunction[76]. NO interacts with superoxide to form peroxynitrite, which has been reported to play a central role in atherogenesis[72]. The stability of peroxynitrite allows a greater opportunity for it to diffuse through a cell to find a target. The unusual stability of peroxynitrite is due to its being folded into the &-conformation (Figure 2), which cannot directly isomerize to the much more stable form, nitrate[77]. Peroxynitrite reacts with the tyrosyl residue of proteins, which inactivates superoxide dismutase and leads to increased amount of superoxide[78].This further increases the formation of peroxynitrite and reduces the available NO concentration. Peroxynitrite causes smooth-muscle relaxation and is less potent than NO. The effect of NO and peroxynitrite have been studied on stripped cavernosal tissue from rabbits[79]. They reported that relaxation induced by NO is short lived and immediate in onset compared with that due to peroxynitrite, which is prolonged and slow in onset. Moreover, the tissues returned to original tension immediately with NO, whereas with peroxynitrite, the tissues were unable to recover their original tension. These mechanisms ultimately produce an ineffective relaxation in cavernosal tissue, which produces erectile dysfunction shown in Figure 3.

6.4. Apoptosis and necrosis

The new findings are consistent with the well-known involvement Ca^{2+} in cell death from oxidative stress. Oxidative stress causes Ca^{2+}

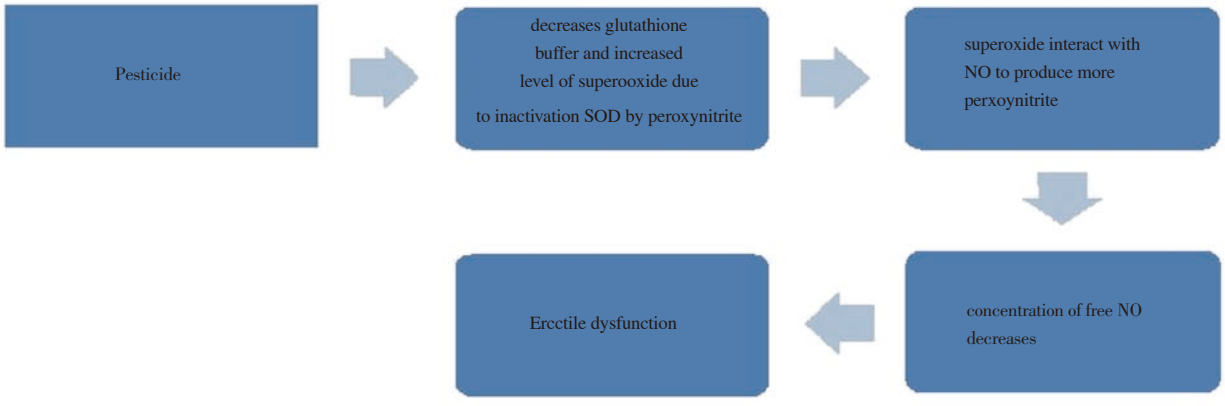

Figure 2. Pesticides causing oxidative stress.

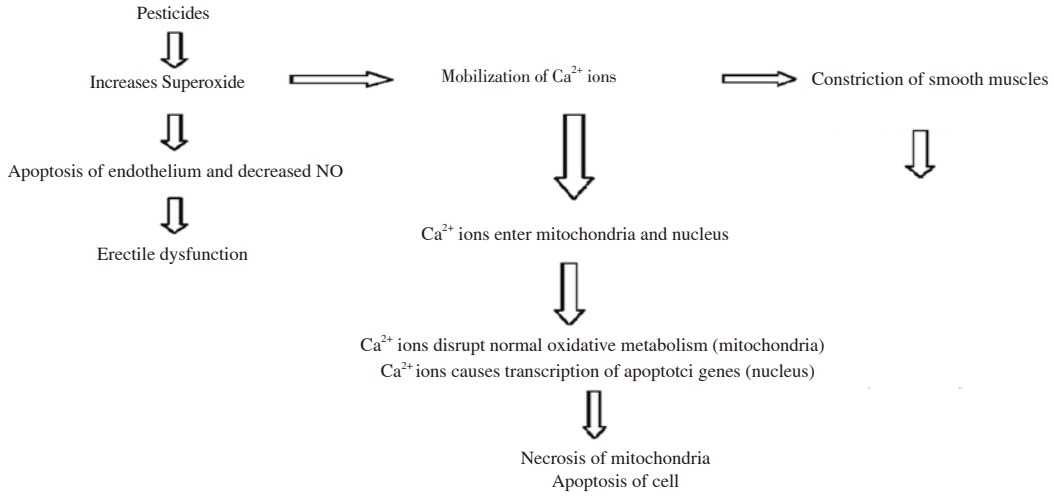

Figure 3. Apoptotic and necrotic action of pesticides.

influx into the cytoplasm from the extracellular environment and from the endoplasmic reticulum[80]. Rising Ca^{2+} concentration in the cytoplasm in turn causes Ca^{2+} influx into the mitochondria and nuclei. In the mitochondria, Ca^{2+} accelerates the disruption of normal oxidative metabolism leading to necrotic cell death. In nuclei, Ca^{2+} modulates gene transcription and nucleases that control apoptosis (programmed cell death that involves fragmentation of DNA). Insecticides and pesticides has been shown to act as reproductive toxicants in male rats and histologically induce severe focal necrosis of the germinal cells in seminiferous tubules associated with tubular atrophy (shown in Figure 3)[81–83]. NO interact with peroxide to form peroxynitrile. Peroxynitrite and superoxide have been reported to increase the incidence of apoptosis in the endothelium. This leads to denudation of endothelium and reduction of available NO[77, 79]. Currently, the following are considered biomarkers of vascular endothelial dysfunction: insulin resistance, homocysteinemia, lipoprotein (a), endogenous nitric oxide (NO) synthesis inhibitors, vasodilators (nitrites, nitrates, and 6-keto prostaglandin F1a), adhesion molecules (vascular adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], and P- and E-selectins), and thrombotic hemostatic factors[84]. Since, endothelium is made up of endothelial cells and these cells are responsible for NO production via eNOS. Superoxide is reported to have a direct vasoconstriction effect through mobilization of calcium ions[85]. This can potentially produce ED. According to the literature, the decreased availability of NO is the key pathophysiological process that leads to erectile dysfunction[86].

6.5. Endocrine disrupter

An endocrine disruptor was defined by the U.S. Environmental Protection Agency (EPA) as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction and developmental process[87]. The pesticide, Glyphosate is a known or likely potential cause of endocrine effects[88]. There is already evidence that Glyphosate may act as an endocrine disruptor for both males and females by altering aromatase activity, oestrogen regulated genes, and testosterone levels in rats[89] however Roundup has been reported to act via different mechanisms. Roundup exposure during pregnancy and lactation at a level that did not induce maternal toxicity in Wistar rats nevertheless induced adverse reproductive effects in male offspring, including decreased daily sperm production during adulthood, increase in abnormal sperms, and low testosterone serum level at puberty. In exposed female offspring, only a delay in vaginal canal opening was observed [90]. Pesticides disrupt level of different neurotransmitters and later these increased neurotransmitters effect the follicle stimulating hormone and luteinizing hormone by feedback inhibition[53– 57,66 67].

6.6. Effect on leydig cells

Leydig cells are responsible for production of testosterone. These cells are under influence of luteinizing hormone (LH). Luteinizing hormone acts on enzyme cholesterol demolase activity which results in testosterone synthesis from cholesterol. Round exposure damages testosterone producing Leydig cells from mature rat testis at concentrations a tenth of agricultural use and beginning 1 hour after exposure[61]. Insecticide like atrazine, carbaryl and methoxychlor effects leydig cells. Metabolic products of methoxychlor downregulates the Cytochrome P450 enzymes, the enzymes involved

in synthesis of testosterone[91].

6.7. Effect on testosterone and other hormones

Testosterone, the male hormone, is the major driver of male reproductive development and function. Suppression of its levels within the adult testis shuts down spermatogenesis the cause is unknown - and low sperm counts often show evidence of abnormal Leydig cells, which produce testosterone in the testis[92]. Dimethoate causes testicular damage, damage to sperm production & reduction in testosterone levels[93]. Glyphosate reduces production of testosterone[61]. At the very low, non-toxic concentration of 1 ppm, both Roundup and Glyphosate decreased testosterone level by 35%. It also inhibits production of other hormone[94]. Previous studies indicated that most insecticides inhibit the non-specific esterase activity in leydig cells that, in turn, result in reduced testosterone production[91, 95]. Organophosphate is responsible for decreasing total serum testosterone and estradiol levels[96]. In fact, IMI acts as a nicotine acetylcholine receptor agonist and somehow likely to interfere with the release of gonadotropin-release hormone from hypothalamus and/or with release of LH/FSH from the pituitary (Figure 4), resulting in the reduction of sperm production in the testes[97]. Pesticides like, carbamates, pyrethroids, organophosphatases, Thio-and dithiocarbamates, chlorphenoxy acids and chlormethylphosporic acids reduces testosterone concentrations in male after acute exposure during exposure season[98]. Pesticides may also be involved in erectile dysfunction by altering levels of testosterone which functions as activator of enzyme nitric oxide synthase, enzyme responsible for production of nitric oxide[99]. NOS are of two types iNOS and cNOS[100, 101]. cNOS is calcium dependent and it has two isoforms i.e. eNOS (endothelial) and nNOS (neuronal) [102]. Nitric oxide synthases are responsible for the synthesis of nitric oxide from L-arginine[103]. Experimental evidence suggests that the constitutive isoforms of NOS may be responsible for NO production in penile erection[104, 105]. Recently, several studies have revealed that nitric oxide (NO) is an important neural messenger which mediates penile erection[106–110]. Erection is mediated by the release of NO from non-adrenergic non-cholinergic nerve terminals, the endothelium of penile blood vessels, and corporal smooth muscle, producing smooth muscle relaxation and vasodilation[111]. NO stimulates the formation of guanylate cyclase in smooth muscle cells, converting GTP to 3'5'-cyclic GMP (cGMP) [111]. A cascade of cGMP-dependent intracellular events then leads to a decrease in intracellular calcium, ultimately causing smooth muscle relaxation, in part through changes in potassium conductance[111, 112]. A recent study also hypothesized that androgens maintained and facilitated male sexual potency through enhancement or maintenance of NOS activity in the corpus cavernous tissue in the penis[86]. Testosterone ensures penile erection through maintenance of nitric oxide synthase (NOS) activities in the peripheral nervous system and that deprivation of the androgen may reduce the number of corporal smooth muscle cells through apoptosis[113]. In addition, several studies have shown that androgen replacement facilitated neural activities in some areas of the brain which mediate sexual function[114, 115]. Neurogenic NO is an important mediator of penile erection[116]. Hence indirect effect of pesticides occurs as shown in Figure 5.

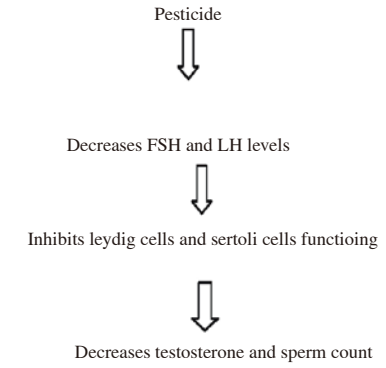

Figure 4. Action of pesticides on harmones.

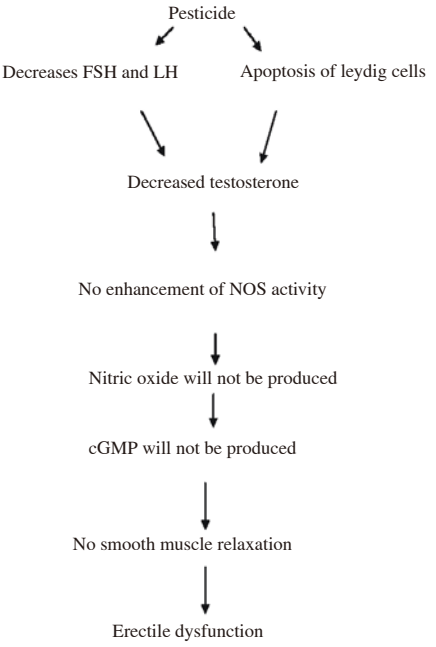

Figure 5. Mechanism of action of pesticides.

6.8. Effects on tunica albuginea

The tunica albuginea is the tough fibrous envelope of connective tissue that surrounds the corpora cavernosa of ths penis. It consists of approximately 5% elastin, an extensible tissue that is primarily made up of the amino acids glycine, valine, alanine, and proline.

The majority of the remaining tissue is collagen, which is made up of lysine, proline, glycine, alanine, and other amino acids[117]. The tunica albuginea is directly involved in maintaining an erection. On administration of imidachloroprid to the rats, the investigations revealed increased thickness of tunica albuginea[118]. Alterations in the microarchitecture of the tunica albuginea, including a decrease in the elastic fibers, may contribute to impotence in men[119]. Microstructural disorders of tunica albuginea have been reported in patients affected by impotence. In impotent patients, a reduction in the elastic fibers in the TA appears to produce disorders in the arrangement of the collagenic fibers. These alterations in the architecture of the TA in impotent patients can give rise top erection disorders[120]. During erection intracorporal pressure of patients with venogenic erectile dysfunction was significantly lower. Tunica albuginea collagen fibers exhibited degenerative and atrophic changes which presumably lead to tunica albuginea subluxation and floppiness. These tunica albuginea changes seem to explain cause of lowered intracorporal pressure which apparently results from loss of tunica albuginea veno-occlusive mechanism[121]. Atrazine also effects tunica albuginea. Atrazine increases thickness of tunica albuginea[70]. Tunica albuginea of patients showed degenerative and atrophic changes of collagen fibers; elastic fibers were scarce or absent [121].

6.9. Pesticides and neurotransmitters

Neurotransmitters are endogenous chemicals that transmit signals across a synapse from one neuron (brain cell) to another 'target' neuron. Decrease or increase in level of neurotransmitter effect the normal functioning of body. Different neurotransmitters like GABA when increased in body regulate FSH and LH in negative manner by suppressing release of Gonadotrophin releasing hormone. Hence suppress normal reproductive functions[53–57]. Acephate and metamidophos stimulate the secretion of hypothalamic corticotropin-releasing hormone, which in turn stimulate adrenocorticotropic hormone (ACTH) and so cortisol[49]. Receptor of cortisol are located on GnRH neurons, RFRP-3(mammalian ortholog of GnIH) and on gonadotrops. High cortisol level inhibits the secretion of GnRH, LH and testosterone while positively regulates RFRP-3 secretion, thus suppressing reproductive system[122]. Decrease in testosterone may be the reason of erectile dysfunction as testosterone is activator of nitric oxide synthase enzyme involved in production of nitric oxide [99].

The manuscript is suggestive of a significant place of erectile dysfunction as a causal factor for infertility. In this communication it is inferred that pesticides are having multiple mechanisms of action through which these chemicals cause erectile dysfunction. It is also reflected that out of mentioned widely used pesticides, acetamiprid is having most devastating effect causing erectile dysfunction as it acts through multiple inhibitory pathways.

Conflict of interest statement

We declare that we have no conflict of interest.

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