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Review Article

Toxicological Effects of Carbendazim: A Review

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Abstract

Pesticides are usually inorganic substances used in agriculture to control various types of crops to kill insects and pests, weeds, rodents, fungi, and unwanted microorganisms. The biggest challenge today is that they are not limited to the agrochemical sector but are also used in households to kill mosquitoes, eliminate fungal and microbial activities that contaminate kitchens and food. One of these chemicals used as a fungicide to prevent fungal attack is carbendazim (CBZ) or methyl-1H-benzimidazol-2-yl-carbamate (MBC), one of the many chemicals used to protect crops from fungal attack. Several studies have been conducted to investigate the toxicological effects of CBZ on organismal health, environmental contamination, detection, and degradation. These studies have evaluated biochemical and histopathological changes in the liver, kidney, and testes, as well as concerns related to dermal, oral, and respiratory exposure to lethal and sublethal doses of CBZ. The current challenge is to develop less hazardous or organic alternatives to protect the environment and human health. This review highlights the hazards associated with CBZ and discusses potential alternative organic chemotherapeutics that are health friendly to the environment and organismic health.

Keywords: CBZ, health risks, environmental toxicity, degradation, organic chemotherapeutics.

Introduction

In general, pesticides are referred to as toxic chemical agents that act against insects, rodents, fungi, bacteria, slugs, and weeds. Pesticides are not new chemicals, but they have a long history of use in Rome and after World War II and the most used pesticides were 2,4-dichlorophenoxyacetic acid (2,4-D), betahexachlorocyclohexane (BHC), dichlorodiphenyltrichloroethane (DDT), alderin, dielderin, and endrin [1]. Undoubtedly, they are dangerous for health, but at the same time their importance in controlling the spread of diseases in humans and animals, reducing vectors for disease transmission internationally and killing them is another factor that cannot be ignored. Pesticides are increasingly being utilized, and there is an urgent need to work on pesticide chemistry in order to reduce the health hazards posed by pesticides and to introduce health-friendly, less hazardous chemicals that can be used in the agrochemical industry when needed [2].

Carbendazim, $C_9H_9N_3O_2$ or methyl-1H-benzimidazol-2-yl-carbamate (MBC) is a fungicide with wide range of applications [1]. As a fungicide, it is used to control fungal infections on fruits, vegetables, ornamentals, and cereals. In addition, CBZ is used in the laboratory as a reagent, as a preservative, in the manufacture of paints, in the paper industry, in leather processing, and in oil production. It is estimated that 80 to 85% of oral exposure

is absorbed and converted into various metabolites, such as 5-hydroxy-2-benzimidazole carbamate (5-HBC), 5-hydroxy-2-benzimidazole carbamate N-oxides (5, 6-HOBC oxides), etc. CBZ, which is commonly referred to as MBC, is also studied as a potent mutagen, an endocrine disruptor, and harmful to various body organs, and reports of its serious regulatory effects have been published [3].

Benzimidazole products such as benomyl and CBZ are widely used as fungicides in agriculture. At the same time, CBZ has been shown to possess some anticancer properties and to act as a suppressor of multidrug-resistant cell lines, mutagen, and an inhibitor of mammalian tumour proliferation. CBZ is in phase I clinical trials to test its properties as an anticancer agent. The acute and chronic effects cannot be ignored [4].

CBZ is notorious for its acute and chronic effects in agricultural practices in fields, while also affecting honeybee populations and being involved in pollen contamination. CBZ is also reported to cause reproductive disorders in daphnids and rats [5]. Chlorpyrifos (CPF) and CBZ are widely used in agricultural practices. CPF and CBZ had 96-h values LC_{50} of 247.72 g L(-1) and 200.82 g L(-1), respectively, for the marine invertebrate *Donax faba*. Lower accumulation of CBZ was compared to CPF. Tissues from gills, body and feet were used as target organs in biomarker and genotoxicity research [6]. CBZ concentration of (4.516, 0.4516 and 0.04516 ppm) in fields does not lead to negative results but may interfere with P450-mediated etoxification of honeybees and should not be sprayed during flowering season [7]. CBZ as a fungicide has a broad spectrum of acute and chronic toxic effects on living organisms, causing infertility in mammals, teratogenicity, embryotoxicity, apoptosis, hepatocellular dysfunction, testicular damage, endocrine system disorders, alterations in hematological parameters, abnormalities in cell division, and mutations in genetic molecules such as DNA and RNA [8]. A study by Jie Li., *et al.* [9] on *Caenorhabditis elegans* (*C. elegans*) showed that growth is affected by CBZ concentration up to 0.01 µg/L, which may interfere with the normal locomotion, antioxidant functions, reproductive toxicity, and shortening of lifespan. The aim of this review is to highlight the toxicities caused by CBZ. The current and latest findings explaining possible degradation methods, as well as suggestions of some medicinal plants to reduce toxicity.

Common toxicological effects of carbendazim

CBZ and reproductive toxicity

A sublethal dose of CBZ administered to male rats for eight weeks caused testicular damage, reproductive toxicity, and endocrine disruption. Rats exposed to CBZ showed impaired stages and poor spermatogenesis, reduction in testicular weight, poor sperm motility, and altered hormone levels with low sperm count [8,10]. Findings in male rat revealed that long-term exposure to CBZ causes testicular damage, resulting in vacuolization of the germinal epithelium, atrophy in the testicular tubules, marked indentations in the Sertoli cells, fibrosis in the interstitial cells, changes in the mitochondria and an altered mitochondrial structure with an expanded endoplasmic reticulum, leading to infertility in animals with poor testicular maturation when. Similar findings were reported in male goats [11]. Various studies confirm that a sublethal dose of CBZ and its derivatives also causes abnormal sperm head development, germ cell death, pathological changes in Leydig cells [12].

The toxicity of CBZ in the seminiferous tubules is caused by the oxidative stress induced by CBZ exposure. It is not limited to reproductive harm, but also alters foetal embryological health at a dose of 160 mg/kg over a period of 6 to 15 days in female rats, as it causes overt toxicity in the mother, resulting in foetal mental growth retardation, congenital defects, and embryonic lethality. It also promotes umbilical hernias, absent or short tails, and encephalocele. It can lead to skeletal system changes, poor development, delayed mental growth, kidney, and other body organ disorders [13]. CBZ and its metabolites, such as 2-aminobenzimidazole, are readily bio-transformable and have low toxicity in mammals with LD_{50} values of more than 2000 to 15000 mg/kg, depending on the animal and type of exposure. A CBZ dose of 300 mg/kg causes weight loss and poor nutrition in mothers. Certain biomolecules such as glucose, cholesterol, protein and creatinine, triglycerides, decrease in hormones such as progesterone and estradiol, decrease in live foetal birth rate, changes in visceral organs and skeletal deformities in the foetus are signs of CBZ toxicity [14].

Different concentrations of CBZ were administered sub-chronically to rats for eighty days before mating began. CBZ concentrations of 100 and 200 mg/kg were observed to lower

fertility, testicular weight, sperm motility, and sperm count. Likewise, it decreases the tendency of luteinizing hormone as compared to the control group and the 200 mg/kg treated group, with no effect on follicle stimulating hormone and testosterone. Histopathological findings showed atrophy of testicular tubules, stimulation of germ cells and decrease of germ cells. When testicular tissue was examined, flow cytometry showed that CBZ impaired spermatogenesis and inhibited mitotic division of the spermatogenic process [15].

CBZ and haematological alterations

The results showed that male rats exposed to 200 mg/kg CBZ for one month showed a decrease in the levels of haemoglobin, erythrocytes, haematocrit (Hct), plasma proteins and liver proteins, and on the other hand an increase in leukocyte count, triglycerides, liver glycogen, total lipids, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [8]. CBZ (50 mg/kg) was administered to male Wistar rats for nine days and the results showed significant changes in the levels of AST, ALT, ALP, blood urea nitrogen (BUN) and creatinine and the development of oxidative stress [16]. In another study, Jacobsen., *et al.* [17] documented hat CBZ administered at doses of 450 ppm and 16 mg/kg body weight daily for 28 days resulted in a decrease in red blood cells (RBC) and white blood cells (WBC). Male rats were administered CBZ intradermally, blood and liver samples were collected after 6 hours to study haematological parameters such as erythrocytes, leukocytes, neutrophils, haemoglobin, serum chemical parameters such as AST, ALT, creatinine and cholesterol and liver chemical parameters AST, ALT and malondialdehyde (MDA). Liver samples at same time were collected for histopathological studies. Findings showed that CBZ, even at modest doses, was toxic, had an adverse effect on the liver, and altered some hematological parameters in the rat [18].

CBZ and histopathological alterations in kidney, liver, and gonads

Histopathological studies in liver and kidney of rats treated with CBZ for 15 weeks showed histological changes in kidney and liver. Doses of 300 and 600 mg/kg per day caused changes in liver histology such as Kupffer cell proliferation, portal vein congestion, infiltration with mononuclear cells, sinusoid enlargement and hydrophobic degeneration. Regarding the kidney, fibrosis was observed at the highest dose by [19]. With other structural changes in renal tissue such as tubular degeneration, congestion and mononuclear cell infiltration. Liver damage was studied in rats at a dose of 50, 100, and 200 mg/kg for 14 days and the experimental results showed that CBZ induced changes in the histopathological structure of liver and kidney, biochemical changes in blood chemistry, oxidative stress, and impairment of liver and kidney function [15,20,21]. CBZ exposure damages the reproductive organs, affects the gonads' hormonal secretions, alter histological structures, and disrupts cellular processes, all of which result in oxidative stress in mammals [22-24].

Most studies on animal models exposed to CBZ confirm that there are changes in the liver (haemorrhage, dilation and congestion of blood vessels, enlargement of sinusoids, disorganization of hepatic cords and vacuole formation), in the kidney (damage to cortical and medullary tissues, changes in normal renal corpuscles, glomerulus, and Bowman's capsule), and in the testes (impairment of spermatogenesis, testicular weight, and testicular morphology) [8,25]. Among fungicides, CBZ is the most used fungicide in agriculture. It is not recommended in the United States of America (USA), most European countries, and Australia due to its acute and chronic effects and persistent nature [26].

In vitro and in vivo genotoxicity by CBZ

In vitro and *in vivo* studies also confirm the genotoxicity of CBZ. *In vitro* concentrations of CBZ and benomyl of 3.2-4.3 and 3.8-4.1 μM, respectively, are shown to stimulate chromosomal aneuploidy in cultured human lymphocytes by fluorescence *in situ* hybridization [27]. CBZ at 97% purity was administered to mice as a single oral dose of 500 or 1000 mg/kg body weight. The mice were killed after 24 and 48 hours of exposure. A statically significant increase in micronuclei was observed in the intestinal crypts. But in relation to bone marrow and polychromatic erythrocytes, non-significant results were obtained after exposure to such doses at irregular intervals [28]. The study by Winder., *et al.* [29] suggests that exposure to CBZ (100 μM) has no effect on tubulin structure and microtubular proteins (MAPs) isolated from rat brain and testes. However, it induces microtubule polymerization, which is compensated by MAPs, and it can also interfere with the normal binding of guanosine triphosphate (GTP) to tubulin. In comparison, colchicine (40 μM) and nocodazole (12.5 μM) induce

microtubule degradation and MAP -free tubulin isolated in parallel with glutamate.

During cell division, α- and β-tubulin are present *in vivo* as heterodimers and play their roles in cell division and chromosome segregation. CBZ interferes with microtubule assembly and polymerization in fungal and mammalian cells and leads to failure of normal cell division [30]. In various microorganisms, it affects normal cell structure, metabolic and physiological processes, cell division, and enzymatic activities. Zebrafish embryos exposed to various CBZ concentrations (LC_{50}) of 1.1, 1.19, 1.3, 1.41, 1.53, 1.66, and 1.68 mg/L showed sub-lethality, delayed development and dysfunction, pericardial edema, a decrease in heart rate, changes in biochemical measurements for various enzymes, changes in locomotor behaviour, and a decrease in body length after exposure of 96 hours [10].

Imazalil and cypermethrin both individually damaged DNA at alkali-labile sites, whereas CBZ alone had a tail moment (TM) comparable to that of the control but with a longer tail. The effects of imazalil and cypermethrin were enhanced in combination with carbendazim clastogen compared with the other treatments and the control. There were significant sex differences in the pattern of fragmentation between treatment groups. Females exhibited more long tail nuclei (LTN), suggesting that some female cells were more prone to complete nuclear decay. Low ambient concentrations of imazalil and cypermethrin in food, and especially their combinations with carbendazim, exhibit synergistic effects that can be very harmful to mammals with prolonged exposure [31].

The genotoxicity of CBZ was studied in a dose range of 125-2000 mg/kg and 250-2000 mg/kg in rats and it was confirmed to affect cytokinesis and karyokinesis, which is a clear indication of the extrusion of nuclei in bone marrow erythrocytes, an eneugenic effect, polychromatophilic with pyknotic nuclei, constriction of micronucleated polychromatic (PCE) and normochromatic erythrocytes (NCE) in mouse bone marrow [32].

Environmental toxicity of CBZ

Carbendazim is a persistent fungicide with a half-life of 12 months. As a result, samples of it can be discovered in plant products, soil, and water [33]. It induces changes in soil structure and affect microbial population in soil as well adversely affects animal health. Its use is not new, from decades CBZ is in use and there is no success achieved to remediate soil free from CBZ by different physiochemical processes and some degrading microbial strains [34,35]. CBZ not only pollute target plants but in parallel it affects the non-targeted plants and pollute soil for long term because its residues can be found in soil for 6 months to 12 years because it is readily to soil [5,36].

To check the long-term persistent nature of CBZ, an instrument lysimeter investigation using ^{14}C-labeled was used on the barley crop for four consecutive seasons. It was seen that the degradation of CBZ was more in the first phase and after four seasons, 33% of CBZ were found in soil bounded particles and in the roots of barley. The study showed that it causes environmental toxicity and retained in the soil if repeatedly applied then it could lead to more contaminated soil [37]. More CBZ sprayed as a fungicide to tobacco plants than the recommended quantity (5.2 mM) could be hazardous, dangerous, and lead to a decrease of nutrients, foliar pigments, and dry weight. It should be used within control limits to reduce risk and adverse environmental and physiological effects [38].

Sclerotinia sclerotiorum of rapes are fungus that are controlled by CBZ. The study was conducted to access field base results that how CBZ is transferred to apiculture. Pollens after 18 days, honey 24 and royal jelly after 22nd day of exposure was studies for CBZ residues by HPLC/ESI-MS/MS method. It was seen that CBZ diminish over spray time and not depends on gap of spray, however the residues of CBZ were 10 times more in pollens as compared to honey and royal jelly [37]. In China between 2014 to 2016 vegetable samples numbered 20496 in 24 vegetables samples commonly (cucumber, cereals, cowpeas, leeks and mostly lettuce plants) were analysed and 1674 were found to be polluted with CBZ but that was lower in concentration than Japan and US. 0.01 mg/kg in >18000 samples (91.9% of the samples analysed) [38]. In agriculture, CBZ (methyl 2-benzimidazolecarbamate) is frequently used to treat fungus-related disorders. Using marker techniques for intra-simple sequence repeats (ISSR) and inter-retrotransposon amplified polymorphisms (IRAP), ascertain the effects of CBZ (0, 0.1, 0.2, and 0.4 mM) on long terminal repeat (LTR) retrotransposon polymorphism, genomic template stability (GTS), and DNA damage. Findings demonstrated that retrotransposition polymorphism is present in all CBZ dosages. Additionally, it indicates that DNA damage increased in all CBZ

treatments, decreased the stability of the genomic template (GTS). These findings imply that the common agricultural chemical CBZ can affect the adverse growth and development of both target and non-target organisms, as well as pose a risk to creatures even at trace levels [39].

Promising organic chemotherapeutic agents against CBZ toxicity

According to the study, CBZ is a well-known pollutant that is toxic to the gonads and causes histopathological, hematological, and biochemical changes in rats when given in doses of 200 mg/kg. Olive leaf extracts are a promising chemotherapeutic agent to lessen the harm that CBZ induces as a pesticide [8]. Co-administration of licorice (*Glycyrrhiza glabra*) extract and CBZ enhanced histomorphological and histological changes in rats. In licorice supplementation significantly decreased MDA levels and increased superoxide dismutase (SOD) and catalase (CAT) activity. The above results suggest that the aqueous licorice extract may reduce the testicular toxicity of CBZ. This effect could be due to the antioxidant properties of one or more of its components [40]. The histomorphological and histological changes were observed in animals treated with CBZ. Remarkable improvement was seen in animals treated with fenugreek plus CBZ coadministration. Such as, fenugreek administration leads to a significant decrease in MDA levels and an increase in SOD and CAT activities. In conclusion, fenugreek extract can reduce the toxicity of CBZ on testes, and this effect may be related to the antioxidant properties of the plant [41]. *Gingo bilola* extract was given to rats against CBZ induced hepatotoxicity. Significant results were obtained. This extract improved liver health by decreasing AST, ALT level, improved cellular health and reduce oxidative stress markers [42].

A fruit name as *Guercus brantii* (internal layer called jaft), that is effective against oxidative damage, recover oxidative stress pathogenesis and pathological injuries, it can be used against inflammatory diseases, improving biochemical alterations in Wister mice males against CBZ pathological toxicity [16]. 6-Gingerol-rich fraction (6-GRF) obtained from ginger (*Zingiber officinale*)) administration gives chemoprotective results against induced CBZ toxicity and reduces oxidative stress as hepatoxicity. It improves liver and kidney health by improving oxidative health of cells and tissues [21].

CBZ/Mancozeb (MNZ) was administered to female mice to induce hepatorenal toxicity. *Nigella sativa* oil (NSO) was administered as a supplement, which remarkably improved the blood profile for macrocytic hypochromic anaemia, white blood cell count, normalisation of lymphocytes, eosinophils, and neutrophils that were perturbed by CBZ and MNZ. NSO also improves liver enzymes such as micronuclei abundance, lipid peroxidation, DNA damage and chromosome aberration abundance, and recovers antioxidant activity suppressed by CBZ and MNZ [43]. Quercetin was intragastrically injected into the testis against CBZ-induced toxicity. Quercetin proves effective in maintaining testosterone levels, production of proinflammatory cytokines, increasing cyclic guanine monophosphate (cGMP) levels, reducing oxidative stress, and better results in reproductive markers [44]. Mice were treated with seeds extract of *Nigella sativa* and *Foeniculum vulgar* against toxicity of CBZ. Both seeds have antioxidant properties, improve chemical composition of blood and haematological alteration [45]. Haematological-biochemical changes were induced by CBZ, and supplementation of vitamin C. proves to be ameliorative against such toxipathological changes induced by CBZ toxicity in male rats and female chickens [46,47]. In another study, banana peel was fed to rats. Supplementation of banana peel remarkedly improves performance of liver and kidney, lipid profile and histopathological alterations that were induced by CBZ toxicity [48].

Toxicity induced by CBZ in different ways		
Effects	**Findings**	**Reference**
Reproductive toxicity of CBZ	Endocrine disruptor, hormones imbalance, poor spermatogenesis, reduce testes weight, low sperm count and poor motility, atrophy in seminiferous tubules, vacuolization of germinal epithelium, marked indentation in sertoli cells, fibrosis in interstitial cells, changes in leyding cells, umbilical hernia, embryo lethality, skeletal and neural deformities in foetus.	[8-15].

Haematological alteration induces by CBZ	Toxicity induces decrease haemoglobin, erythrocytes, haematocrit, plasma protein and liver proteins. While increase in leucocyte count, triglycerides, liver glycogen, lipids, creatinine, AST, ALT, and BUN level.	[8,16-18].
Histopathological alterations in gonads and hepato-renal organs.	Increase in Kupffer's cells, congestion and dilation of hepatic portal vein and mononuclear cell infiltration, fibrosis, tubular degeneration, enlargement in sinusoids, liver haemorrhage, damage cortical and medullary tissues of kidney that leads to changes in glomerulus filtration rate. Reduction in testes weight, morphological changes with low sperm count and poor spermatogenesis.	[8,15,19-26].
In-vitro and *in-vivo* Geno- toxicity	Destroy normal cellular structure and metabolic reaction, polymerisation of mammalian and fungal alpha and beta microtubules assembly, uncontrolled gene activation, damage DNA in hepatocytes, retrotransspositional polymorphism, affects binding of guanosine triphosphates (GTP), aneuploidy in spermatogenesis, increase in micronuclei in intestinal and bone marrow cells, pericardial edemas in zebrafish embryos, epigenetic changes	[10,27-32].
Environmental toxicity	Induce changes in soil structure, affect microbial fauna, non-targeted plants, long resistivity, and half live almost one year, pollute vegetables and fruits, affects biological ecosystem and food chain.	[5,33-39].
Organic chemotherapeutic against CBZ	Different studies suggest that olive leaves extracts, licorice (*Glycyrrhiza glabra*), Fenugreek (*Trigonella foenum-graecum Linn.), Gingo bilola* extract, Ginger (*Zingiber officinale*), Jaft internal layer of *Quercus brantii, Nigella sativa oil, Quercetin,* Vitamin c., banana peel are best supplementations to reduce toxic effects of CBZ toxicity studied in different lab animals.	[8,16,21,40-48].

Table 1: Overall summary of CBZ toxicity.

Conclusion

Pesticides are used all over the world and their applications in agriculture are admirable. Without pesticides the desired quantity of food to compensate humans' hunger needs seems to be impossible. But in parallel they are polluting biosphere and creating life threatening issues for flora and fauna. CBZ is one of such toxicants that is employed to inhibit fungal attacks to save fruits and vegetables. It is creating many health complications and there is need to address its uses and need to alter its chemistry to make it less hazardous to life of non-targeted organisms. To address this issue, limits should be defined for its use and explore alternative, less toxic methods of fungal control. Photolytic and biodegradation methods are also solutions to reduce contamination caused by CBZ. Additionally, the use of organic chemotherapeutic agents should be encouraged as they may have fewer health risks than CBZ.

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