FINAL REPORT ON SHORT TERM RESEARCH PROJECT

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TITLED

To evaluate the toxic effects of commonly used insecticide Furadan on male rat reproductive organs

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Title of Research

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Objectives

To investigate

1. The morphological changes like increase or decrease in size, shape and weight of reproductive organs treated with furadan.
2. histological changes in germinal epithelium, sertoli cells of seminiferous tubules, Leydig cells of testis, epithelial cells of epididymis and ductus deferens, glandular cells and stroma of prostate gland and seminal vesicles..

The presence of pesticides in food items and their accumulation in tissues has direct toxic effects on humans and other non-target organisms. In situations where the safety measures are not applied properly these have direct effects on human health e.g. on the spraymen and other workers (WHO 1979). The organochlorine pesticides present in human and cow's milk are transferred to infants so the toxic effects are passed on to next generation. Continued use of pesticides have also resulted in more serious problems by contaminating water supplies (Carey, 1991) and degrading the cropland soil.

The current magnitude of translocation of food grown with pesticides is seen as a danger to mankind, even in areas where very little pesticides are used. It points to the reality that pesticide problem does not merely concerns the chemical industry and professional farmers, foresters and applicators, or one concerning only who wish to protect wildlife or those responsible for control of malaria and other vector born diseases, rather the pesticide problem concerns every person who wants food at a reasonable price and who wants his home free from vermin.

The ever increasing demand for more food production has been met by adoption of better crop protection measures. Since world war II synthetic chemicals / poisons have been extensively used for control of agriculture as well as household and medical insect pests. These poisons which otherwise are employed for human benefit can also be called as economic poisons. Pesticides used for control of different insect pests are the major component of such economic poisons. The circumstances to whom humans are exposed are as follows:

1. Suicide ———— High doses ———— Terminal fatal/ Occasionally survive
   Low doses ———— Survive
2. Genocide ———— Terminal fatal
3. Intentional ———— Experimental ———— Volunteers
   Poisoning ———— Therapeutical ———— Patients
4. Unintentional —— Accidental ———— Fatal, Sometimes survive
   Poisoning ———— Occupational ———— Survive with side effects
5. Environmental ———— Survive with side effects

There is a lot of concern about the health hazards and the chronic effects of pesticides. It is impossible to avoid some sort of direct or indirect exposure to these chemicals in present era. Contaminated diet is the main problem now in the world. Due to globalization, the pesticides used in one part of the world and then contaminated grain are shipped and exported to the other part of the globe. In the same way, within a country the translocation of food items is on a massive scale. The pesticides used for crops enter into them and when used by livestock build up milk and meat. The milk and meat when taken by the humans can

transfer theses pesticides into human body. Contaminated raw vegetables and fruits can also be a source of pesticides residues found in human tissues.

PESTICIDES

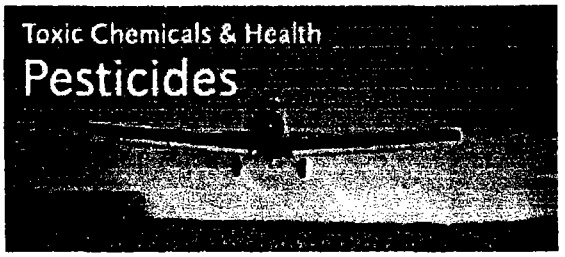

Fig. 1 Pesticide spray on crops

Fig. 2 Pesticide spray resulting into toxic effects

The word "Pesticide" is a general term that describes chemicals used to combat a variety of pests harmful to people, domestic and wild animals, crops and forests. It includes insecticides, fungicides, herbicides, rodenticides, bactericides, miticides, nematocides and molluscicides, which can enter the body through the mouth, lungs, skin contact and wounds.

The list of chemicals used for these purposes is very long and is expanded continuously. Therefore, it is not possible to list them in the Handbook. Suffice it

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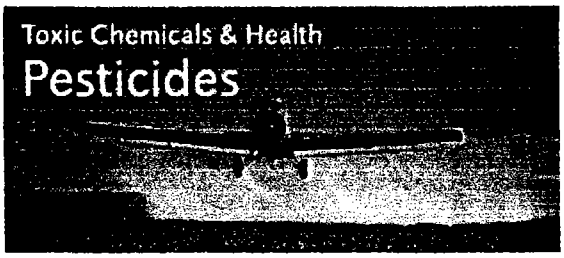

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to say that a journalist should not assume that any pesticide is completely harmless to humans whether absorbed or ingested directly or eaten on crops or in wildlife.

There is heavy use of pesticides throughout Asia, and millions of dollars are being spent yearly to subsidize their sale to untrained farmers, according to a World Resources Institute reports that the subsidies by countries such as China, Indonesia and Pakistan encourage farmers to use more chemicals than they would if they had to pay the full cost of the chemicals. It also discourages farmers from controlling pests by methods that do not rely as heavily on chemicals.(Fig.1, Fig.2)

Experts estimate that, in developing countries, 10,000 people die and 40,000 suffer acutely from pesticide poisonings. A larger number of people are reported to be chronically exposed to pesticide residues on food and in water. In China and India, where persistent pesticides (long-lived ones that remain in the environment for a number of years) are used, residues are prevalent in mothers' milk.

Regulation of pesticides is ineffective in many countries and farmers using the pesticides often do not understand the instructions. In 1987, farmers in the Cameron Highlands of Malaysia were found to be spraying too much fungicide on their leafy vegetables so that the vegetables would not have spots or holes in the leaves. According to the Chairman of the Malaysian Agriculture Chemical Association, the overdose of fungicide could not be avoided because the farmers were illiterate and did not follow the directions on the label.

The widespread use of paraquat as a weed killer in Malaysia and 130 other tropical countries has also created problems. Figures from the Health Ministry show that 1,200 Malaysians died of paraquat poisoning between about 1980 and 1987. Many more were thought to have died but their deaths had not been reported, according to environmentalists.

The use of these and hundreds of other chemicals continues to be questioned by health and environ mental specialists who are concerned about the chemicals' effects on the environment and human health. Not only do these chemicals affect people, but they also kill animals and birds. Eventually many pesticides become in effective after the insects they control become immune to them.

The controversy over pesticides is a tangled one, where the benefit of increased agricultural yield must be weighed against the costs of acute and chronic exposure to sometimes deadly chemicals. Unfortunately, it takes many years before scientific evidence can be amassed to prove or disprove any danger.

Pesticides in Ecosystem

Food contamination by pesticides

Ingestion of pesticides through contaminated food supplies is a cause of concern for almost everyone. This was most serious at the time when persistent insecticides,(organochlorines) were commonly used on crops. There are many examples of pesticide poisoning through food (Hayes, 1982).

Classification of insecticides

Insecticides are classified into four major groups

1. Persistent organochlorines.
   Examples: DDT, BHC, Dieldrin
2. Organophosphorus insecticide
   Examples: Malathion, methyl-parathion, diazinon, endosulfan, dimethoate, chlorpyrifos, monocrotophos
   These are less persistent and their primary mode of action is on the nervous system mainly inhibiting the acetyl cholinesterase enzyme.
3. Carbamate insecticides (based on carbamic acid)
   Examples: Pyrethroids, derived from the chrysenthemum. Pyrethroids could be natural or synthetic. They are least persistent.
4. Cypermethrin insecticides
   Examples: Deltamethrin and fenvelerate
   They have quick knock-down effects and are most commonly used against flying insects (e.g., as aerosols for the control of household insects like flies and mosquitoes.)

Pesticide Use in Malaysia and its Health Implications

Agrochemicals are widely used in Malaysia especially in the plantations. Mainly dominated by herbicides, these are most commonly used in the approximately 4 million hectares of plantation crops—palm oil, rubber and cocoa. In1993,the agrochemical market in Malaysia was worth RM 262million,with herbicides accounting for76.3 percent of the share.In1997,the figure rose to the level of RM 326million,with herbicides still accounting for three-quarters of the share at 75.1 per cent. The herbicide market itself was estimated at RM200 million in 1993and at RM 245millionin1997

16.0% Insecticides
3.5% Rodenticides
5.4% Fungicides
75.1% Herbicides
1997 Total: RM326 Million

Herbicides 76.3%
Insecticides 14.9%
Fungicides 5.0%
Rodenticides 3.8%
1993 Total: RM262 Million
Source: Malaysian Agricultural Directory & Index, 1999/2000

About 50% of the estimated 10,600 vegetable farmers in peninsular Malaysia have already begun using naturally occurring pest-control agents to protect their crops Called bio-pesticides, these agents are harvested from plants, insects and micro-organisms (such as viruses, fungi and bacteria), and are used to kill pests. Unlike chemical pesticides, they are safe for human and animal consumption and environmentally friendly, said Science, Technology and Environment Minister Datuk Seri Law Hieng Ding ... Law said the Government hoped that Malaysia would become a globally recognised hub for bio-pesticide research and production."Malaysia's biodiversity puts it in a position to contribute to the discovery of new bio-pesticides, and we hope to phase out all chemical pest control agents by the year 2005," he said..he added that following Malaysia's signing of the UN's Persistent Organic Pollutants treaty with 87 other countries in Stockholm, Sweden last year, the Government has started phasing out the use of 12 dangerous pesticides known as " dirty dozen".

Women and pesticides

This gives you an overview of what PAN-AP has done in the past and is now doing, and I will now talk a bit about our Women and Pesticides project. I will focus actually on our activities in two countries - the first is from Malaysia, called Victims without Voice, and looks at women pesticide workers in plantations in Malaysia; the second which I will discuss briefly is called Invisible Farmers, and involves our work in Pakistan.

Presently in Malaysia, about 40% of the women who are involved in work outside the home or in paid employment are women in agriculture. There are about 30,000 women who spray pesticide in the plantations, and there are also about 50,000 women who are involved in general work in the plantations. Women are the lowest paid workers in the agricultural sector, and always end up doing the work nobody else wants to do.

In 1991, an organisation called Tenaganita, which is a women's organisation in Kuala Lumpur, and PAN undertook a study to look at the extent of problems in the plantations. In this study we did interviews with 50 farm workers in 6 estates in Kuala Lumpur, which is in the southern part of the peninsula, and we looked at the role and status of women, the effect of pesticide on their lives, and we also looked at the laws of pesticides in Malaysia and how well they were implemented.

To describe the situation, I thought I would let you hear a bit of the voices of the women who don't have a voice. For instance, Veena has been spraying pesticides for the past 20 years: "I spray Gramazone (paraquat) all the time. It is so strong that the odour makes me sick most of the time. In the beginning, I used to cry (tearing in my eyes from the strong fumes). Now my only problem is nose bleeds and chest pains. I also have bad stomach pains. Most days when we come back from work we are soaked with the chemical top to bottom - we are used to it." (Arumugam 1992). And these women actually go in every day and spray pesticides, 5 or 6 days a week, 52 weeks a year.

One of Veena's co-workers is Meena, 48. She lives in an oil palm plantation, and she started work 25 years ago. Every day she sprays paraquat. She feels run down, and has never had a regular menstrual cycle. "Sometime back, I developed a rash on my legs. I went to the hospital and the Hospital Assistant told me it was a heat rash. He gave me some cream and assured me it would go away in a few days. The rash persisted and I went back. This time he shouted at me, gave me more cream and that was the end. The rash did not go away. I still have the rash. But now I have learned to live with it." (Arumugam 1992).

Therefore even if there are symptoms of pesticide poisoning, often they are not treated as such. The chemical these women were using was paraquat. To put their stories in context, I would refer to Table 1, which is a list of the most popular pesticides in Malaysia that are being used on different crops - oil palm, rubber, cocoa, tobacco. If we go through them, it will be seen that many of them are very highly toxic, and many of them are part of the dirty dozen.

In terms of pesticides which have caused poisoning, paraquat is the number one pesticide poisoning agent, and actually it tops the list by a lot. This is followed by malathion and endosulphan. These are the official statistics of poisoning - however, we know that the real numbers are much higher than these, mostly because a lot of the long term health problems are never recorded as poisonings and only the acute ones are recorded. What did we find out when we went through and talked to the plantations workers?

First of all, most of them, some 90%, knew that pesticides were dangerous. And most of the ones that used it knew that you could get poisoned if you actually ate or drank it, but few of them realized you could become poisoned just by contact through the skin; more of them knew that you could get poisoned by breathing the pesticide.

Fully 90% had no idea how to tell if they were being poisoned - they had no idea of the early symptoms of pesticide poisoning. If they were aware that they were poisoned, most of them would go to the local hospital to try to get treated - however, as we heard previously, if you do go to the hospital you may not be recognized as having suffered poisoning from pesticide.

So we looked at the conditions on the plantations, and examined to what extent they actually operated within the laws of Malaysia regarding pesticide usage, and if fact of you look at the availability of sanitation, of water to wash with, most of the plantations do not meet the requirements of having piped, clean water; most depend on the monsoon rain for water, and many of them actually have no water available for washing. So that is a clear breach of the Malaysian law, in terms of access to clean water.

We also examined labeling. Although the labels are in Bahasa Malayu, the Malay language, most of the estate workers did not know how to read or write in Bahasa Malayu - most of them are Camal, they speak Camal, some of them can read Camal. And we now have workers from Bangladesh on the plantations, and of course they cannot read Bahasa Malayu. Most of those interviewed knew that the name of the pesticide would be on the label, but very few were aware that the label should contain other information relating to the toxicity of the pesticide and even fewer could understand what the label was saying.

After they spray the pesticide, most people do not really do anything in terms of cleaning themselves properly until well after they reach home in the evening, therefore all this time they are exposed to the pesticide through their clothing and so forth. Therefore the whole point of the sanitation and safe use regulations - that you're supposed to wash immediately after use - are not followed; also many people did not follow necessary sanitation procedures; as you see, very few people even just washed their hands with soap and water before eating, and all of them eat with their hands.

Then we looked at the availability of protective clothing, and in fact the only thing that is available in 50% of the plantations is protective boots, whereas all people should have access to protective clothing; however even though you may have access to protective clothing you may not use it because it is not suitable. So in general most of the pesticide sprayers do not wear protective clothing, and very few of them are trained to even mix pesticides properly - of 50 people who were interviewed only 2 were trained for mixing pesticides and only 5 were trained in spraying pesticides.

All of these facts indicate that in general the people who are out there spraying pesticides are not the people who are trained in how to use the pesticides. All of these examples clearly show that the laws are not being implemented in Malaysia in terms of the health and safety of pesticides.

In conclusion, the women plantation workers are among the most disadvantaged workers in Malaysia. They are further disadvantaged by the male dominated culture. Rural illiteracy and lack of awareness make them even more vulnerable, and overall the conditions on the plantations do not allow for conditions of safety of pesticides.

Our conclusion from working with the women working with pesticides on the plantations is that the state of the women plantation workers can only change when all the factors that lead to their exploitation are addressed - we must look at literacy, gender relations, poverty and all of the other social factors that are involved with keeping the women t the lowest rung in society.

This project is called Invisible Farmers, and it is a study of the role of women in farming and the impact of pesticides.

This quote is a summary of what the farmers have found in terms of the usage of pesticides in Pakistan. In Pakistan, compared to Malaysia, agriculture is a most important part of the economy and accounts for 75% of foreign exchange. Most of the export is agricultural export. The main crops are wheat, cotton and fodder, or feed for the animals. 38% of the land is smallholdings, less than 12.5 hectares.

National agricultural policy at this time is to improve the performance of the farming sector through industrialization and commercialization, so they are in fact trying to make the agricultural economy much more modern, much more integrated in world trade. Part of the agricultural policy recommends reduction in the cost of pesticides so that then they'll buy cheaper ones to use, and also encourage more local production of pesticides so that more of it is available, and hopefully to create more competition to lower the price of pesticide. At the same time it does say we'll try to use Integrated Pest Management (IPM) practices, and look at the identification of indigenous natural pesticides. However, the first two depend on companies, and companies will go out and sell pesticides; the last one depends on government which has no money, so the first two will win out.

Findings in Pakistan's project

The study was conducted in Pakistan in 1993-4. In each of 7 villages about 30 small farmers were interviewed for at total of 210 farmers, including both men and women farmers in this case, and we found out that most farmers were not aware that pesticides cause problems. One farmer said, pesticides don't kill insects - how can they kill humans?

In general, they will use whatever pesticide is given to them either by the landowner or a trader. They will not necessarily use a specific pesticide for specific pests. They also paid probably one third more than the market price for the pesticide, usually because they get the pesticide on loan, a loan in kind from the trader, so they generally use whatever pesticide is given by the seller, and of course the dealer of pesticides will choose to sell the pesticide for which he gets more money.

There is widespread advertising on radio and television promoting the use of pesticides. In general the only protective clothing people use is a piece of cloth around the face, and then any washing that is done is done is in the irrigation

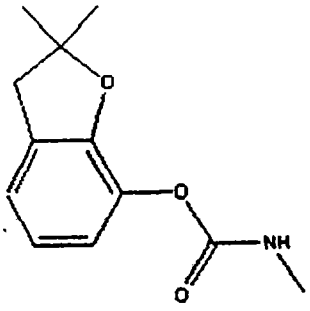
canal on the farm itself, so the pesticide gets washed into the irrigation water. In Pakistan about 85% of the pesticide used is on cotton. The two most popular are endosulphan and midathol. There is a big problem of pest resistance due to overuse and also use of adulterated or impure pesticides. Also, in general there is a very low level of literacy in Pakistan so most farmers cannot read or understand the labels. Now we'll look a bit at the situation of women farmers in Pakistan, and although the last census shows a decrease of women employed in age between 1980 and 1990, the census does not take into account family members - wives and daughters - working on the farm.

Women raise animals, contribute to most farm activities, look after the household, and are also involved in non-farm activities in getting income for the farm and also are primary care givers in rearing the family. However, women are totally dependent on their main relatives for status and protection, and in general are illiterate, cannot read or write. Effectively, the woman in the private sphere of the home does not have any access to any training opportunities, any chance to learn how to use pesticides properly or to learn how to farm. Although in Pakistan women are not involved in spraying pesticides, we found that women had exposure to pesticides. In the first study, 74% of the women had significant blood inhibition, which means that there was an impact on the nervous system due to exposure to pesticides; and there were many other symptoms also, many of them closely linked to pesticides.

So, in spite of not being in the field, the woman actually are exposed to pesticides. This exposure occurs through a number of vectors: in the mixing of pesticides, washing of tanks, disposal of empty containers, washing clothes, storage of pesticides, cleaning out the plant, weeding and picking of cotton, cotton residues, and so forth. So these other various activities are all significant exposures to pesticides, although many people question how can the women be affected by pesticides because they are not spraying them. So we came to similar conclusions in Pakistan as we did in Malaysia. Pesticide use in Pakistan is highly unregulated, and literacy and training are essential components of safe pesticide use. Pesticide use is probably not the appropriate form of pest control, especially among smallholders. Even when women are not directly involved in spraying they can still be overexposed. Women must be fully recognized as making an important contribution to the agricultural economy - as it stands now they are completely ignored. Finally, problems related to pesticide use need to be addressed within the social contexts of poverty, illiteracy and the marginalisation of women in society.

FURADAN (Carbofuran)

Chemical structure of Carbofuran



Chemical Formula: C12H15NO3

Boiling Point	200□ C
Solubility in Seawater	not found anywhere!!
Vapour Pressure	2E-05 mm Hg @ 33□ C
K_{ow}	Log Kow =2.32
Soil sorption coefficient	Mean Koc of 29.4
Melting Point	153-154 C

Synthesis of Carbofuran

Carbofuran is synthesized by the following process:

"A cold solution of 16.4 parts 7-hydroxy-2,2-dimethyl-2,3-dihydrobenzofuran in 14 parts of ether was treated with 5.8 parts methyl isocyanate and 0.1 part triethylamine. The mixture was stirred at room temperature, and a white crystalline product precipitated. Separation of the solid yielded 17.5 parts of product, MP 151 to 152 C. (Siting 1977)

Carbofuran is a white crystalline solid with a slightly phenolic odor. This broad spectrum insecticide is sprayed directly onto soil and plants just after emergence to control beetles, nematodes and rootworm. The greatest use of carbofuran is on alfalfa and rice, with turf and grapes making up most of the remainder. Earlier uses were primarily on corn crops. Carbofuran is allowed for use on only a few U.S. crops, and will soon be banned from use on corn and sorghum in California.

The list of trade names given below may help you find out whether you are using this chemical at home or work.

Trade and Other Names: Trade names include Furadan, Bay 70143, Carbodan, Carbosip, Chinofur, Curaterr, D 1221, ENT 27164, Furacarb, Kenafuran, Pillarfuron, Rampart, Nex, and Yaltox.

Niagara
Furadan
Brifur
Crisfuran
Chinufur
Curaterr
Yaltox
Pillarfuran
Kenofuran
Carbofuran

In 1974, Congress passed the Safe Drinking Water Act. This law requires EPA to determine safe levels of chemicals in drinking water which do or may cause health problems. These non-enforceable levels, based solely on possible health risks and exposure, are called Maximum Contaminant Level Goals.

Following a Special Review, the EPA initiated a ban on all granular formulations of carbofuran which became effective on September 1, 1994. Before 1991, 80% of the total usage of carbofuran was in granular formulations. The ban was established to protect birds and is not related to human health concerns. Bird kills have occurred when birds ingested carbofuran granules, which resemble grain seeds and when predatory or scavenging birds have ingested small birds or

mammals which had eaten carbofuran pellets. There is no ban on liquid formulations of carbofuran. Liquid formulations of carbofuran are classified as Restricted Use Pesticides (RUP) because of their acute oral and inhalation toxicity to humans. Granular formulations are also classified as RUP's, but for a different reason; their toxicity to birds. Liquid formulations bear the Signal Word WARNING. Granular formulations bear the Signal Word DANGER. Formulations of carbofuran are in toxicity class I - highly toxic or toxicity class II - moderately toxic.

Chemical Class

carbamate

Introduction

Carbofuran is a broad spectrum carbamate pesticide that kills insects, mites, and nematodes on contact or after ingestion. It is used against soil and foliar pests of field, fruit, vegetable, and forest crops. Carbofuran is available in liquid and granular formulations, but as stated avove the granule form is banned in the U.S.

Formulation

Carbofuran is available in liquid and granular formulations, but as stated above, the granular form is banned in the U.S.

Toxicological Effects

- **Acute toxicity:** Carbofuran is highly toxic by inhalation and ingestion and moderately toxic by dermal absorption. As with other carbamate compounds, carbofuran's cholinesterase-inhibiting effect is short-term and reversible. Symptoms of carbofuran poisoning include: nausea, vomiting, abdominal cramps, sweating, diarrhea, excessive salivation, weakness, imbalance, blurring of vision, breathing difficulty, increased blood pressure, and incontinence. Death may result at high doses from respiratory system failure associated with carbofuran exposure. Complete recovery from an acute poisoning by carbofuran, with no long-term health effects, is possible if exposure ceases and the victim has time to regain their normal level of cholinesterase and to recover from symptoms]. The oral LD50 is 5 to 13 mg/kg in rats, 2 mg/kg in mice, and 19 mg/kg in dogs. The dermal LD50 is >1000 mg/kg in rabbits. The LC50 (4-hour) for inhalation of carbofuran is 0.043 to 0.053 mg/L in guinea pigs.
- **Chronic toxicity:** Rats given very high doses (5 mg/kg/day) for two years showed decreases in weight. Similar tests with mice gave the same

results. Prolonged or repeated exposure to carbofuran may cause the same effects as an acute exposure.

- **Reproductive effects:** Consuming high doses over long periods of time caused damage to testes in dogs, but carbofuran did not have any reproductive effects on rats or mice. Available studies indicate carbofuran is unlikely to cause reproductive effects in humans at expected exposure levels.
- **Teratogenic effects:** Studies indicate carbofuran is not teratogenic. No significant teratogenic effects have been found in offspring of rats given carbofuran (3 mg/kg/day) on days 5 to 19 of gestation. No effects were found in offspring of mice given as much as 1 mg/kg/day throughout gestation. In rabbits, up to 1 mg/kg/day on days 6 to 18 of gestation was not teratogenic].
- **Mutagenic effects:** Weak or no mutagenic effects have been reported in animals and bacteria. Carbofuran is most likely nonmutagenic .
- **Carcinogenic effects:** Data from animal studies indicate that carbofuran does not pose a risk of cancer to humans].
- **Organ toxicity:** Carbofuran causes cholinesterase inhibition in both humans and animals, affecting nervous system function.
- **Fate in humans and animals:** Carbofuran is poorly absorbed through the skin [32]. It is metabolized in the liver and eventually excreted in the urine. The half-life in the body is from 6 to 12 hours. Less than 1% of a dose will be excreted in a mother's milk. It does not accumulate in tissue.

Ecological Effects

- **Effects on birds:** Carbofuran is highly toxic to birds. One granule is sufficient to kill a small bird. Bird kills have occurred when birds ingested carbofuran granules, which resemble grain seeds in size and shape, or when predatory or scavenging birds have ingested small birds or mammals that have eaten carbofuran pellets.Red-shouldered hawks have been poisoned after eating prey from carbofuran-treated fields.The LD50 is 0.238 mg/kg in fulvous ducks, 0.48 to 0.51 mg/kg in mallard ducks, 12 mg/kg in bobwhite quail, and 4.15 mg/kg in pheasant.The LD50 is 25 to 39 mg/kg in chickens consuming carbofuran as a powder. The LC50 (96-hour) in Japanese quail is 746 ppm.
- **Effects on aquatic organisms:** Carbofuran is highly toxic to many fish. The LD50 (96-hour) is 0.38 mg/L in rainbow trout and 0.24 mg/L in bluegill sunfish. The compound has a low potential to accumulate in aquatic organisms. The bioconcentration factor ranges from 10 in snails to over 100 in fish.
- **Effects on other organisms:** carbofuran is toxic to bees except in the granular formulation.

Environmental Fate

- **Breakdown in soil and groundwater:** Carbofuran is soluble in water and is moderately persistent in soil. Its half-life is 30 to 120 days. In soil, carbofuran is degraded by chemical hydrolysis and microbial processes. Hydrolysis occurs more rapidly in alkaline soils. Carbofuran breaks down in sunlight. Carbofuran has a high potential for groundwater contamination. Carbofuran is mobile to very mobile in sandy loam, silty clay, and silty loam soils; moderately mobile in silty clay loam soils; and only slightly mobile in muck soils. Small amounts of carbofuran have been detected (1 to 5 ppb) in water table aquifers beneath sandy soils in New York and Wisconsin.
- **Breakdown in water:** In water, carbofuran is subject to degradation by chemical hydrolysis under alkaline conditions. Photodegradation and aquatic microbes may also contribute to degradation. The hydrolysis half-lives of carbofuran in water at 25 C are 690, 8.2, and 1.0 weeks at pH values of 6.0, 7.0, and 8.0, respectively. Carbofuran does not volatilize from water, nor does it adsorb to sediment or suspended particles.
- **Breakdown in vegetation:** The half-life of carbofuran on crops is about 4 days when applied to roots, and longer than 4 days if applied to the leaves.

Physical Properties

- **Appearance:** Carbofuran is an odorless, white crystalline solid. Heat breakdown can release toxic fumes. Fires, and the runoff from fire control, may produce irritating or poisonous gases. Closed spaces (storage, etc.) should be aired before entering.
- **Chemical Name:** 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate
- **CAS Number:** 1563-66-2
- **Molecular Weight:** 221.25
- **Water Solubility:** 320 mg/L @ 25 C
- **Solubility in Other Solvents:** acetone v.s.; acetonitrile v.s.; benzene v.s.; cyclohexone v.s
- **Melting Point:** 153-154 C
- **Vapor Pressure:** 2.7 mPa @ 33 C
- **Partition Coefficient:** 1.2304-1.4150
- **Adsorption Coefficient:** 22

Environmental Effects

Fate

- Persistence: carbofuran is moderately persistent in soils, with a half-life from 30 to 120 days, depending on conditions. In soil, carbofuran is degraded by chemical hydrolysis and biodegradation. Carbofuran is soluble in water and is highly mobile in soils. Carbofuran has a high potential for groundwater contamination, and has been detected in aquifers and surface waters.
- Bioaccumulation: carbofuran does not accumulate in animal tissue.

Ecotoxicity

- Carbofuran is highly toxic to fish. The LC50 for bluegill sunfish is 0.24mg/L.
- Carbofuran is highly toxic to birds. Carbofuran has been documented in hundreds of avian mortality events sometimes involving large numbers of birds in each incident. Birds are susceptible to carbofuran from direct spraying, ingestion of granules or contaminated drinking water and from the consumption of contaminated prey. One granule of carbofuran is enough to kill a small songbird.

LD50 fulvous whistling ducks	0.238 mg/kg
LD50 mallard	0.51 mg/kg
LD50 Northern bobwhite	12.0 mg/kg
LD50 pheasant	4.15 mg/
LD50 house sparrow	1.3 mg/kg

Incidents

- Tishomingo National Wildlife Refuge, Oklahoma, 1976. Approximately 500 Canada geese died after feeding in a field treated with 0.5 lbs / acre liquid carbofuran. This level of use is only one-sixth of the application rate of some crops (2.96 lbs / acre is used on grapes, 1997 average usage figures, USDA.)
- New Jersey, 1990. After carbofuran application to a fruit orchard, approximately 100 carcasses were discovered, including blue jay, American robin, and dark-eyed junco. Laboratory analyses confirmed carbofuran as the cause of death.

- Saskatchewan, Canada, 1986. Forty-five California gulls were found dead after a landowner applied liquid carbofuran to a grain field. Gulls had crops full of grasshoppers; analysis of the grasshoppers showed 4.2-7.2 ppm carbofuran.

- Linden, California, 1990. Liquid carbofuran was applied by irrigation, with exposure via puddle water. Carcasses of 30 mourning doves, 100 American robins, 200 European starlings, red-winged blackbirds and grackles, and 700-800 goldfinches, sparrows and house finches were recovered.
- Colusa, California, 1989. 1,985 dead ducks, approximately 97% northern pintails, and 3% green-winged teal were found in an area where carbofuran had been used. Carbofuran residues were found in duck and mud samples. The dead birds were not found in an agricultural field, but in an area that is routinely flown-over by airplanes moving between two local airstrips and rice fields.
- Stevens County, Oklahoma, 1985. Carcasses of 150-160 American wigeon and ten Canada geese were found in an alfalfa field treated with a flowable formulation of carbofuran.

Yountville, California, 1990. Carcasses of one acorn woodpecker, one bushtit, one white-breasted nuthatch, one western bluebird, one American robin, one cedar waxwing, four hermit thrushes, seven yellow-rumped warblers, one chipping sparrow, one white-crowned sparrow, eleven dark-eyed juncos, nine house sparrows, three house finches, and six lesser goldfinches were reported following drip irrigation of a vineyard with a 6 lb / acre usage rate of flowable carbofuran.

Anatomy of the male reproductive organs

Testis

The primary function of the male reproductive system is reproduction, which includes the **production** of spermatozoa, the **transportation** of spermatozoa from the testes out of the male body, the **secretion** by glands, and the **placement** of spermatozoa in the female reproductive tract. Spermatozoa are **produced** in the testes then **transported** from the testes by a series of ducts which become gradually larger and connect with the urethra of the penis. Various accessory glands in the male system **secrete** materials which together with the spermatozoa constitutes the semen. A secondary function of the male reproductive system is the production of the male hormones which are responsible for the secondary sex characteristics of the male animal.

The male reproductive system is composed of several distinct organs. These include the testes, epididymis, deferent ducts, accessory glands, and the penis. The testis (plural, testes) is both an exocrine organ (compound, coiled, tubular gland) producing cells, i.e., spermatozoa, and an endocrine organ, secreting hormones, i.e., testosterone. Accessory glands (not all are present in all species) include the ampullary glands, vesicular glands, prostate gland, bulbourethral gland and urethral glands.

The testes are paired organs, and each one is enclosed in a fibrous white capsule of dense connective tissue **(tunica albuginea)** containing blood vessels (the stratum vasculare). A layer of peritoneum is tightly adhered to the tunica albuginea of each testis. The stallion has obvious smooth muscle fibers in the capsule. The connective tissue of the capsule continues into the testis on the posterior aspect as the mediastinum testis.

The dense connective tissue of the tunica albuginea is continuous with the loose areolar connective tissue of the **septuli testis** (septa) which extend through the parenchyma of the testis and divide it into lobules. Each **lobule** is composed of several seminiferous tubules (tubuli contorti) and the surrounding connective tissue. Spermatogenesis (formation of spermatozoa) occurs in the epithelial lining of the **seminiferous tubules**. The **interstitium** is composed of loose connective tissue containing fibroblasts and Leydig cells **(interstitial cells).** Spermatozoa produced in the seminiferous epithelium move through the lumen of the tubules to the **tubuli recti** (straight tubes) which extend to a network of spaces in the mediastinum, the **rete testis** (except in the stallion). **Efferent ductules** (ductuli efferentes) carry the spermatozoa from the rete testis, then converge to form the **ductus epididymis,** a convoluted duct. The ductus epididymis straightens and becomes the **ductus deferens.** In domestic mammals, testes are not in a major body cavity, but are enclosed in the scrotum. Each testis is suspended at the end of a tissue called the spermatic cord which contains the **ductus deferens,** the blood vessels, and the nerves supplying the testis.

Each testis is composed of an exocrine part (seminiferous tubules) and an endocrine part (interstitial or Leydig cells). The testis is divided into lobules by septa consisting of loose areolar connective tissue. Several seminiferous tubules are found in each lobule, and interstitial cells are found in the connective tissue septa surrounding the seminiferous tubules. The seminiferous tubules are the exocrine portion of the testis producing and "excreting" spermatozoa. These tubules are lined by a stratified epithelium that consists of the developing spermatozoa and supporting cells (Sertoli cells).

Seminiferous tubules

The stratified epithelium of the seminiferous tubules is composed of different stages of developing sperm cells. Spermatogonia are stem cells located near the basement membrane of the tubule which proliferate by mitosis. Some of the progeny cells differentiate into sperm and move away from the basement membrane toward the lumen of the tubule. These differentiating cells first undergo meiosis then undergo a morphological change to become spermatozoa. Some of the progeny cells undergo mito***Meiosis:*** Cells in prophase of the first meiotic division are **primary spermatocytes**. They are characterized by highly condensed chromosomes giving the nucleus a coarse chromatin pattern and an intermediate position in the seminiferous epithelium. This is a long stage, so

many primary spermatocytes can be seen. Primary spermatocytes go through the first meiotic division and become **secondary spermatocytes.** The cells quickly proceed through this stage and complete the second meiotic division. Because this stage is short there are few secondary spermatocytes to be seen in sections. You are not responsible for identifying secondary spermatocytes in lab. Meiosis is the process by which the diploid number of chromosomes present in spermatogonia (the stem cells) is reduced to the haploid number present in mature spermatozoasis again to produce more progeny cells providing a continuous source of stem cells for the production of spermatozoa.

Spermatogenesis

Spermatogenesis: the process by which stem cells develop into mature spermatozoa. There are three phases: (1) Spermatocytogenesis (Mitosis), (2) Meiosis, and (3) Spermiogenesis.

Spermatocytogenesis:(also called Mitosis): Stem cells (**Type A spermatogonia**; singular = spermatogonium) divide mitotically to replace themselves and to produce cells that begin differentiation (**Type B spermatogonia**). Spermatogonia have spherical or oval nuclei, and rest on the basement membrane. (You are not responsible for distinguishing between Type A and Type B spermatogonia in lab.)

The products of the second meiotic division are called **spermatids**. They are spherical cells with interphase nuclei, positioned high in the epithelium. Since spermatids go through a metamorphosis into spermatozoa, they occur in early through late stages. You are not responsible for distinguishing the different stages of spermatids, but you are required to identify a spermatid.

All of these progeny cells remain attached to each other by cytoplasmic bridges. The bridges remain until sperm are fully differentiated.

Genital ducts

After production in the testes, spermatozoa pass through a series of ducts in their journey out of the male system.

Epididymis

The **ductus epididymis** is lined with a pseudostratified stereociliated columnar epithelium. Stereocilia are actually nonmotile, long microvilli which serve to increase the absorptive and/or secretory surface of the epithelium.

Prostate gland

Grossly the prostate gland can be divided into two parts: the body and the disseminate part. Low cuboidal to low columnar epithelium provides the lining for this compound, tubuloalveolar gland which consists primarily of serous secretory end pieces. The secretion of this gland is more serous in dogs and more mucous in bulls. It serves to promote the movement of spermatozoa and to form a vaginal plug. Additionally, in bulls, the secretion contains high amounts of fructose and citric acid. Concretions may be present in the secretory end pieces as well as parts of the duct system.

Accessory glands

The products of these glands serve to nourish and activate the spermatozoa, to clear the urethral tract prior to ejaculation, serve as the vehicle of transport of the spermatozoa in the female tract, and to plug the female tract after placement of spermatozoa to help ensure fertilization. Although the glands are usually described as being branched tubular or branched tubuloalveolar, they vary in their organization and in their distribution in different species.

Literature Review

The widespread use of pesticides in public health and agriculture has caused severe environmental pollution and health hazards including cases of severe, sub- chronic and chronic human poisoning.(Ellenhorn et al, 1997, Abdollahi, 1995, Abdollahi et al 1996, 1997, 1999, Jalali 2000, Pajoumand 2002)
Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Banergee et al, 1999, Etemadi-Aleagha et al, 1999)

Carbofuran is an insecticide and a nematicide, first brought on to the market in 1965. Two forms of the pesticide, granular and flowable (liquid) have been registered for use in the United States. The manufacturer of carbofuran agreed to a phase-out of most of the granular products based solely on the dangers it presented to birds, in 1994. Some granular formulations are still in use today, however flowable product accounts for most carbofuran use in the United States. In 1991, a Virginia state monitoring project documented wildlife mortalities in 10 of 11 farm sites where carbofuran was in use. Following this monitoring effort, the state of Virginia banned all granular formulation of carbofuran for sale or use in the Commonwealth. Canada has also banned the use of granular carbofuran in 1998.

Hundreds of bald eagle deaths have been linked to carbofuran. Eastern blue birds, Northern pintails, American robins, owls, swallows, grackles, killdeer and kestrels: more than one hundred bird species have been documented as having died from carbofuran poisoning. The number of birds involved in any single incident ranges up to 2,450. Carbofuran has also killed mammals and fish. US Fish and Wildlife biologists have stated, *"there are no known circumstances under which carbofuran can be used without killing birds."* In 1989, US EPA estimated that 1 to 2 million birds were killed each year by carbofuran alone.

The U.S. Fish and Wildlife Service maintains that the use of carbofuran poses unreasonable hazards to birds. In 1992, it requested that the EPA cancel all registrations for carbofuran. Many prominent environmental organizations oppose the continued use of carbofuran, but its use is still sanctioned by the EPA. North American migratory birds are at risk of exposure from carbofuran while on their wintering grounds in Latin America. International attention and cooperation is needed to address pesticide use in all of the Americas to adequately protect migratory birds.

N-methyl carbofuran (Furadan) is a carbamate which is widely used insecticide on crops in Malaysia. Many incidents of acute carbamate poisoning has been reported from contamination of crops. Chronic toxicity results in behavioral and neurochemical changes (Boyd, 1990).All carbamate inhibits 17- beta estradiol and progesterone activity in human braest and endometrial cancer cells in

vitro(Klotz; 1997). Gonadal toxicity has been reported in male after chronic exposure to various carbamates (Kackar, 1997). Primary effects of low doses of benomyl on rats testes resulted in morphological changes in sperms while high doses cause occlusion of efferent ducts (Hess, 2000)). Same type of toxicity has been reported by benzimidazol carbamates (Hess, 1990). Harmonal changes resulting by chronic exposure to carbamate methomyl include decrease in testosteron level and significant increase in FSH, LH and prolactin (Malgomb, 2001). Toxic effects of carbofuran on semen characteristics has also been reported (Yousaf, 1995)). As the agricultural workers are contaminated by low doses of insecticide over long periods, little is known about long term effects of repeated small doses (sub-chronic and chronic toxicity)of most commonly used insecticide "Furadan"in Malaysia. These experiments were conducted on male albino rats. Treated group received Furadan (2 mg kg in saline) daily for two months while control group received saline. Animals of both groups were sacrificed on day 60 and those of third group were kept without treatment for another one month till sacrificed on day 90.

The influence of carbofuran metabolism on acetylcholinesterase inhibition has been defined after low dose (50 µg/kg, iv and oral) carbofuran exposures to male Sprague–Dawley Rats. Red blood cell acetylcholinesterase (RBC AchE) inhibition (83% at 2 min, 37% at 15 min for iv and oral, respectively, with recovery by 3 hr), was correlated with carbofuran plasma concentrations ($r = 0.97$). Eight-hour sample collection indicated that ultimate carbofuran fate (41–47% CO_2, 14–15% urine, <1% feces, and 30–31% carcass) was independent of exposure route. Carbofuran absorption (peak plasma levels < 7 min), distribution, and elimination ($t½ = 29 \pm 5$ min) occurred rapidly. 3-Hydroxycarbofuran, a significant oxidative metabolite of carbofuran with anticholinesterase activity, was rapidly formed and subject to enterohepatic circulation (plasma $t½ = 64 \pm 5$ min). Results indicated that rapid RBC AchE recovery closely paralleled carbofuran metabolism and the primary *in vivo* disposition of 3-hydroxycarbofuran was metabolic conjugation. (Furgoson 1984).

The acceptable daily intake (ADI) of carbofuran has been derived by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as follows:

$$\text{ADI} = \frac{\text{1.0 mg/kg bw per day}}{100} = \text{0.01 mg/kg bw per day}$$

where:

1.0 mg/kg bw per day is considered to be the NOAEL for cholinesterase inhibition and growth suppression in a two-year study in rats

100 is the uncertainty factor.

Based on the above ADI, the maximum acceptable concentration (MAC) for carbofuran in drinking water is derived as follows:

$$\textbf{MAC} = \frac{\textbf{0.01 mg/kg bw per day X 70 kg bw X 0.20}}{\textbf{1.5 L/d}} = \textbf{0.09 mg/L}$$

where:

0.01 mg/kg bw per day is the ADI established by the FAO/WHO
70 kg bw is the average body weight of an adult
0.20 is the proportion of daily intake of carbofuran allocated to drinking water
1.5 L/d is the average daily consumption of drinking water by an adult.

MATERIAL AND METHOD

Twenty four adult male albino rats of Sprague Dawley strain weighing 150 – 200 grams of same breed were taken from veterinary institute, University Sains Malaysia. The animals were divided into three groups as A, B and C each comprising of eight animals. Each A, B and C group was further subdivided into A1, A2, B1, B2 and C1, C2 groups comprising of four animals in each group. Group A1 and A2 were given saline orally for a period of two months as control group. The rats were housed under standard laboratory conditions and free access to food and water ad libitum. Group B1 and B2 animals were given 2mg / kg body weight of Furadan as a pure compound in the form of powder obtained from FMC Limited (PVT) as a gift dissolved in saline. The equivalence dose of furadan for rats is about six times of that of human being.

Each animal was given 1 ml / kg body weight daily for the same period as the control group. Group C1 and C2 were treated like group B for a period of two months and then left for another one month without treatment. The animals were then sacrificed by euthanasia with sodium phenobarbitol 100mg/ kg body weight (appendix I). Testes, prostate, epididymis and vas deferens were removed. Testes, prostate glands and seminal vesicles of each animal were weighed by electrical balance and examined for any morphological change. Each tissue was fixed in 10% formaline and was processed to get paraffin sections for histological study to identify the changes in parenchyma and stroma by the insecticide using Haematoxylin and Eosin stain (Drury 1967) with image analyzer. The results were analysed.

RESULTS

Gross examination

There was steady rise in the weights of both control and experimental animals at the end of experiment.It was most probably due to free access to laboratory food throughout the experiment (table.1).

No significant gross morphological changes were observed in testes, epididimys, ductus deferens, seminal vesicles and prostate gland of experimental animals at the end of 60 days treatment or 90 days without treatment (table.2).

Table. 1 Weights of the animals at 15 days interval in grams.

Group	Wt 0 1st day	Wt1 15th day	Wt 2 30th day	Wt 3 45th day	Wt 4 60th day	Wt 5 75th day	Wt 6 90th day
A1	259.5 ± 14.2	273.2 ± 12.0	314.7 ± 7.2	323.2 ± 9.2	244.5 ± 5.9	248.5 ± 4.5	352.2 ± 4.6
A2	261.2 ± 7.7	272.5 ± 7.3	288.0 ± 9.1	312.5 ± 4.5	322.7 ± 4.9		
B1	301.2 ± 4.7	296.2 ± 6.6	310.5 ± 7.5	319.0 ± 7.8	316.7 ± 12.1		
B2	293.7 ± 6.2	282.7 ± 2.3	301.7 ± 8.4	333.0 ± 12.8	345.0 ± 10.8		
C1	297.5 ± 12.3	288.7 ± 9.9	301.7 ± 6.9	323.7 ± 3.7	331.0 ± 2.4	336.7 ± 2.9	349.0 ± 3.5
C2	288.7 ± 6.6	292.2 ± 6.1	300.2 ± 5.5	321.7 ± 5.8	334.0 ± 5.2	345.0 ± 5.2	349.0 ± 3.8

Table.2 Weights of the reproductive organs of control and experimental male rats in grams at the end of experiment.

Group	Rt. testis	Lt. testis	Prostate	Seminal vesicle
A1	1.32 ± 0.13	1.40 ± 0.12	0.60 ± 0.07	0.82 ± 0.08
A2	1.40 ± 0.09	1.50 ± 0.07	0.65 ± 0.09	0.77 ± 0.06
B1	1.5 ± 0.17	1.42 ± 0.11	0.62 ± 0.08	0.80 ± 0.07
B2	1.4 ± 0.11	1.45 ± 0.09	0.67 ± 0.06	0.80 ± 0.06
C1	1.3 ± 0.09	1.3 ± 0.12	0.80 ± 0.04	0.80 ± 0.06
C2	1.3 ± 0.07	1.32 ± 0.07	0.77 ± 0.06	0.65 ± 0.06

Histological findings

Testes

Group A (control)

Testes of normal control rats stained with haematoxylin – eosin stain appeared to be formed of sections of seminiferous tubules surrounded by connective tissue coat, the tunica albugenia (Fig.3). Each seminiferous tubule was surrounded by adventitial cells i.e fibroblasts and myoid cells and an inner basement membrane. In between seminiferous tubles, the interstitial connective tissue showed fibroblasts, blood vessels and interstitial Leydig cells (Figs. 4,7,11).The seminiferous tubules appeared almost rounded, uniform in size and shape.(Fig. 4)Each tubule was lined with regularly arranged rows of spermatogenic cells in different stages of maturation (Figs. 5,6,7,9,10). The lumen of each seminiferous tubule was filled with mature spermatozoa (Fig. 5)

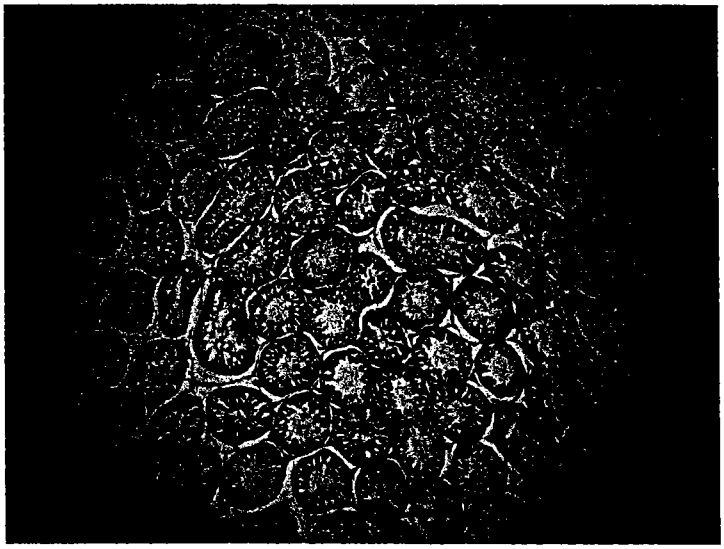


Fig.3 Photomicrograph of rat testis showing seminiferous tubules, interstitial tissue with Leydig cell. H & E 20X

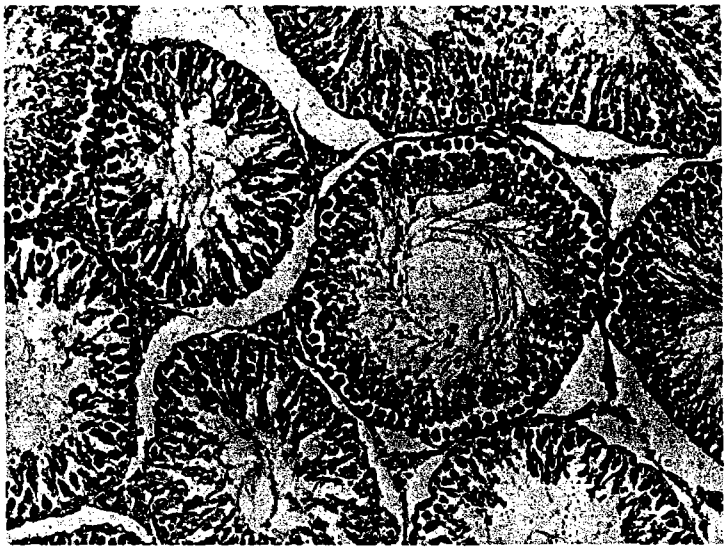


Fig. 4 Photomicrograph of rat testis showing rounded seminiferous tubules, interstitial tissue with Leydig cells. H&E 100x

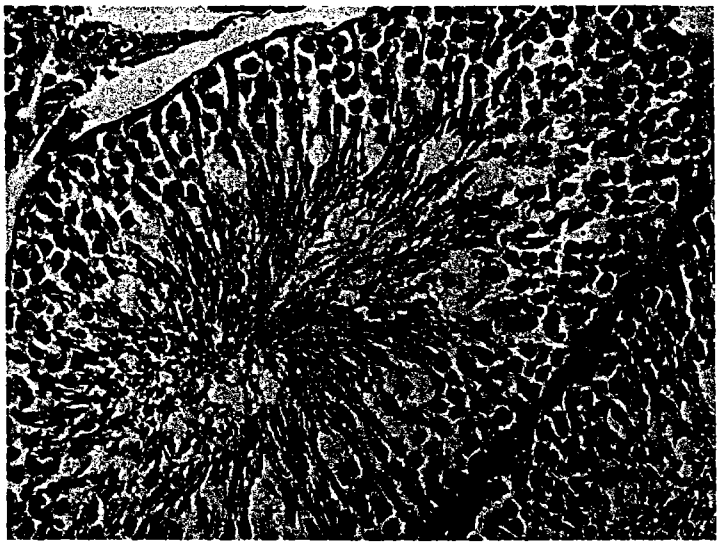


Fig. 5 Cross-sectioned seminiferous tubule showing basement membrane, myoid cells and different stages of spermatogonia. H&E 200x

Fig. 6 Cross-sectioned seminiferous tubules showing, spermatogonia, primary spermatocytes and cells of Leydig. H&E 200x

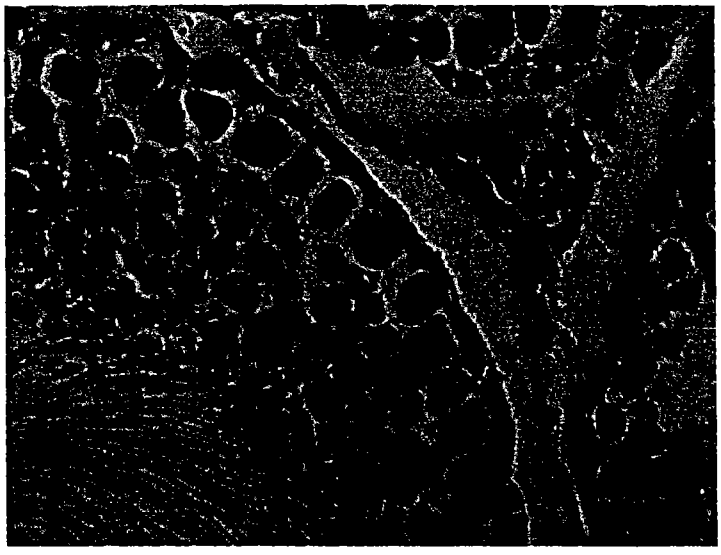


Fig. 7 Micrograph of rat testis showing seminiferous epithelium at different maturation stages. H&E 400x

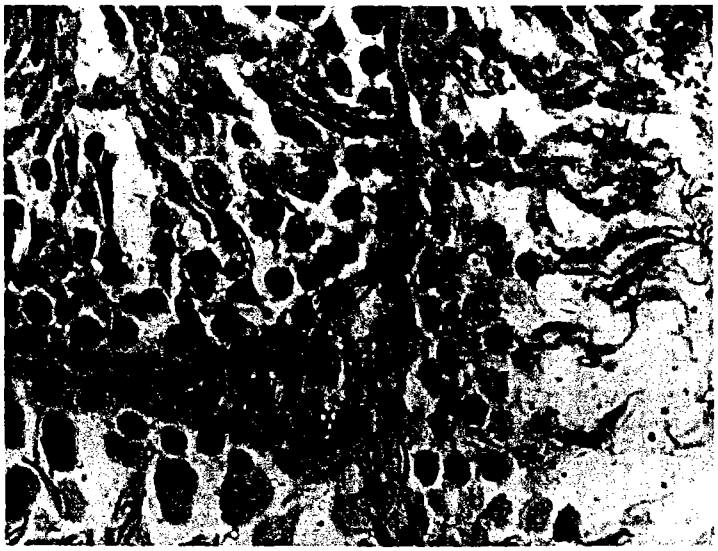


Fig. 8 Photomicrograph of seminiferous tubules showing interstitial tissue and cells of Leydig. H&E 200x

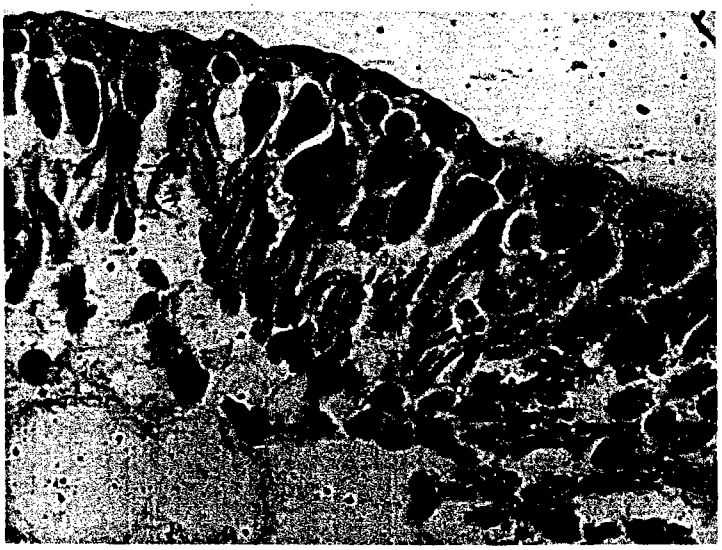


Fig. 9 Micrograph of a seminiferous tubule of rat testis showing Sertoli cells, spermatogonia and spermatozoa. H&E 400x

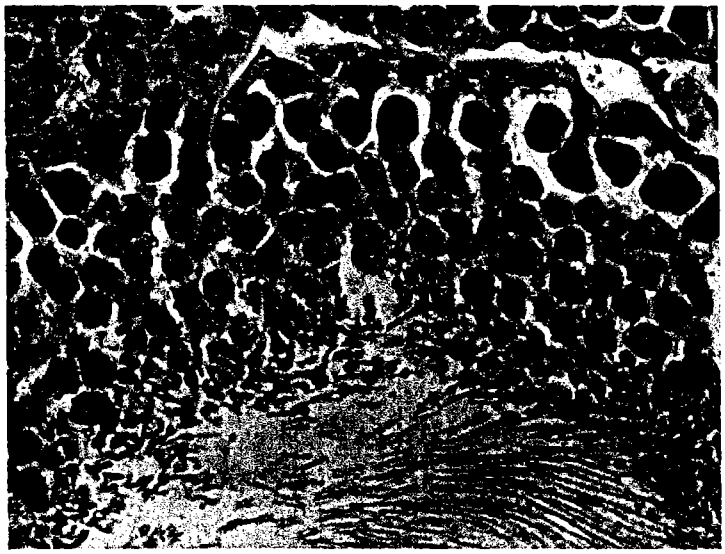


Fig. 10 Photomicrograph of cross section of a seminiferous tubule showing spermatogenic cells at different stages of maturation. H&E 400x

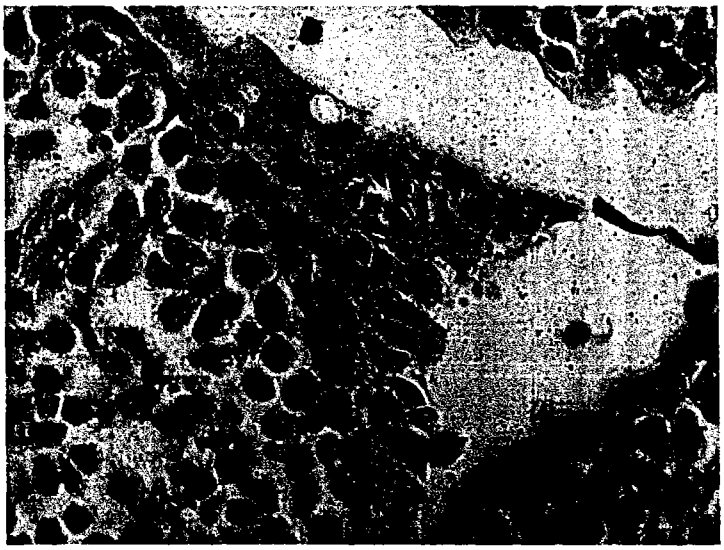


Fig. 11 Photomicrograph of a seminiferous tubule showing Leydig cells. H&E 400x

Group B (experimental)

The seminiferous tubules showed variable grades of degenerative changes in spermatogonia ranging from cloudy swelling to absolute cellular destruction. Most of the seminiferous tubules were of variable shape and size with swelling in their interstitial tissues (Fig. 12). They were disorganized and haphazardly placed in the stromal tissue of the testes (Fig. 13). Most of them were filled with sloughed cells in their lumina. Tunica albugenia was thinned out (Fig.12) There was moderate vascular engorgement of tunica vasculosa (Fig. 14). Proportional hypoplasia with marked thinning of spermatogenic cell rows was very prominent feature observed (Fig. 15). Epithelial architecture of each seminiferous tubule was disorganized and disoriented (Fig. 16).The spermatogonia revealed evident cytoplasmic vacuolation (Fig.17). Primary spermatocytes were scattered in tubal epithelium showing swelling, ill defined and irregular nuclear membrane with destroyed cell membrane. Some of the nuclei were pyknotic, shrunken and small sized (Fig. 18).

Chromatin pattern was seems to be normal. Secondary spermatocytes and spermatids showed extreme degree of destruction. Most of the cells were swollen with ill-defined membranes and dispersion of chromatin and nucleoli (Fig. 19).On the luminal surface of seminiferous tubules, degenerated spermatids with few spermatozoal heads with abnormal morphology were noticed (Fig. 20). The lumen of tubules was filled with cellular debris and flagella of the dead spermatozoa (Fig. 21). Sertoli cells showed normal nuclei with slight swelling of cytoplasm (Fig. 22).

Inflamatory cells were present in moderate amount in the interstitial tissue in between seminiferous tubules. The interstitial cells of Leydig showed variable degrees of degeneration. The cellular outline was looking irregular with patchy vacuolation in the cytoplasm. Some of the cells showed dark staining two or more nuclei (Figs.20,22,23,24,25).

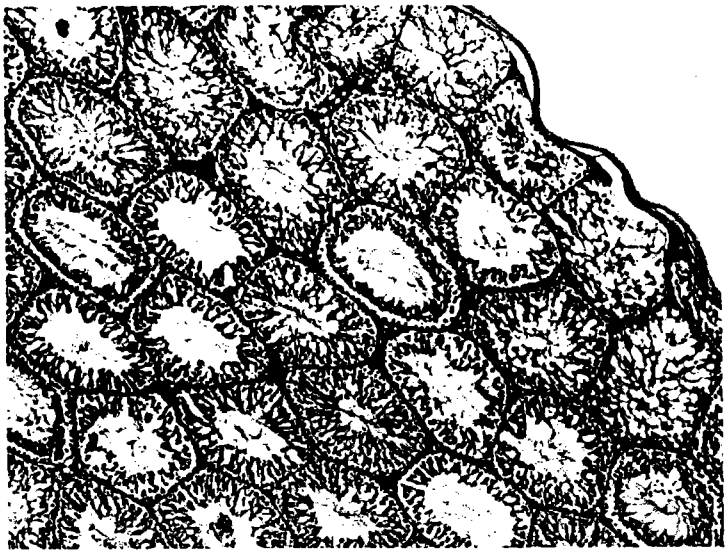


Fig.12 Photomicrograph cross- section of rat testis treated with furadan for 60 days showing thinning of tunica albuginea and change in shapes of seminiferous tubules. H&E 40x

Fig. 13 Photomicrograph of treated animal testis showing degenerated seminiferous with interstitial oedema. H&E 40x

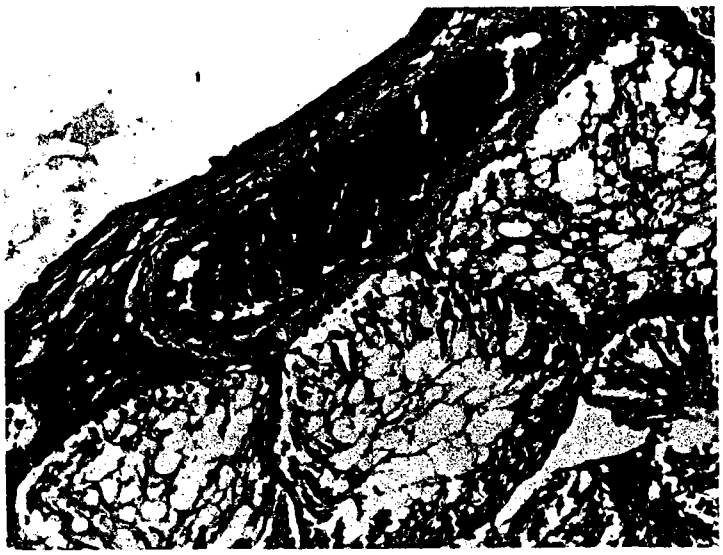


Fig.14 Photomicrograph of treated rat testis showing engorged blood vessel of tunica vasculosa and degenerated tubules. H&E 100x

Fig.15 Photomicrograph of testis showing peritubular oedema with marked thinning of spermatogenic rows. H&E 100x

Fig. 16 Photomicrograph of treated rat testis showing disorganized epithelial architecture of seminiferous tubules. H&E 100x

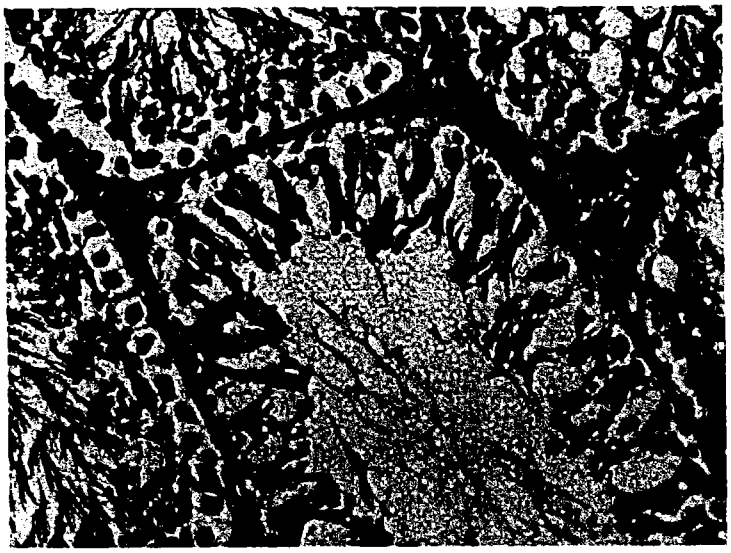


Fig. 17 Photomicrograph of treated testis showing cytoplasmic vacuolation of spermatogonia. H&E 200x

Fig. 18 Photomicrograph of cross-section of a seminiferous tubule of a treated rat testis showing spermatogenic cells with pyknotic nuclei. H&E 400x

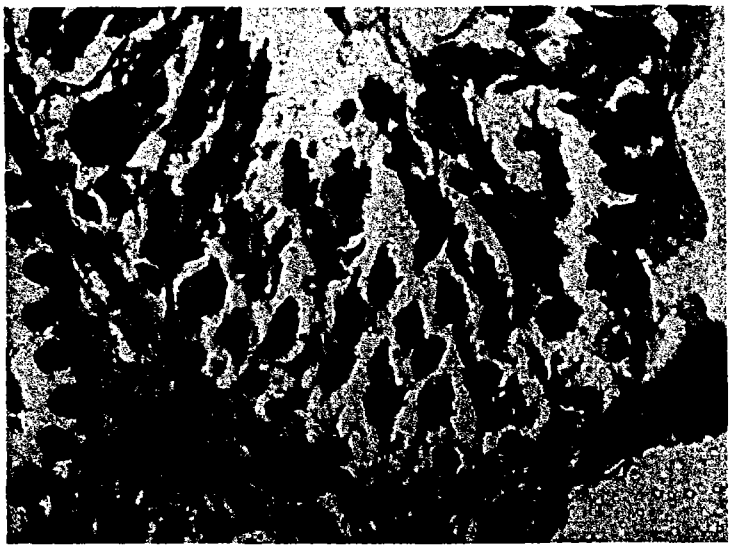


Fig. 19 Photomicrograph of section of a seminiferous tubule showing disruption of spermatogenic epithelium with ill defined membranes. H&E 400x

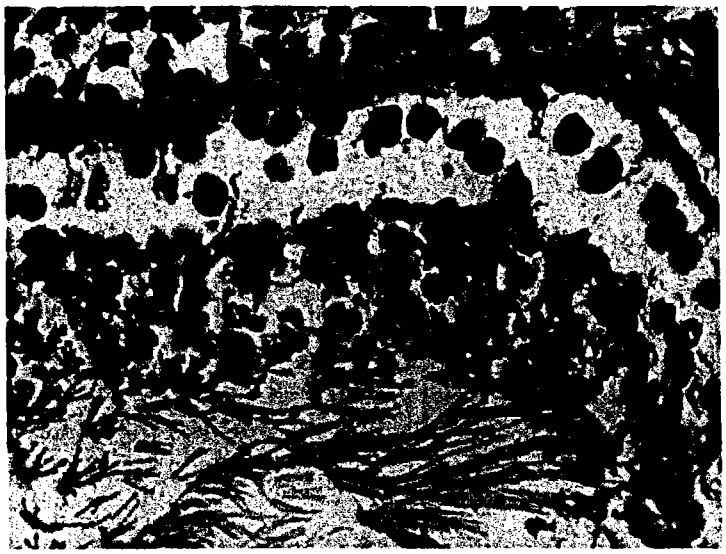


Fig. 20 Photomicrograph of seminiferous tubule showing degenerated spermatids with spermatozoal heads. H&E 400x

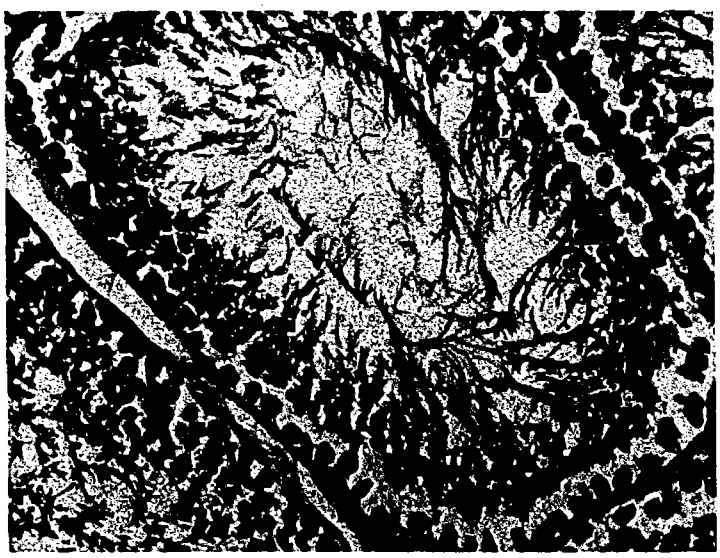


Fig.21 Photomicrograph of a treated seminiferous tubule showing dead spermatozoa and their flagella in the lumen. H&E 40x

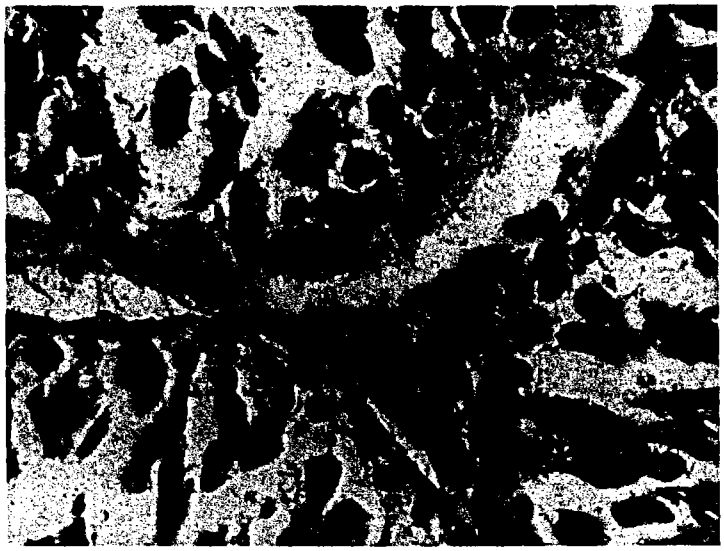


Fig. 22 Micrograph of testis showing Sertoli cells and cells of Leydig. H&E 400x

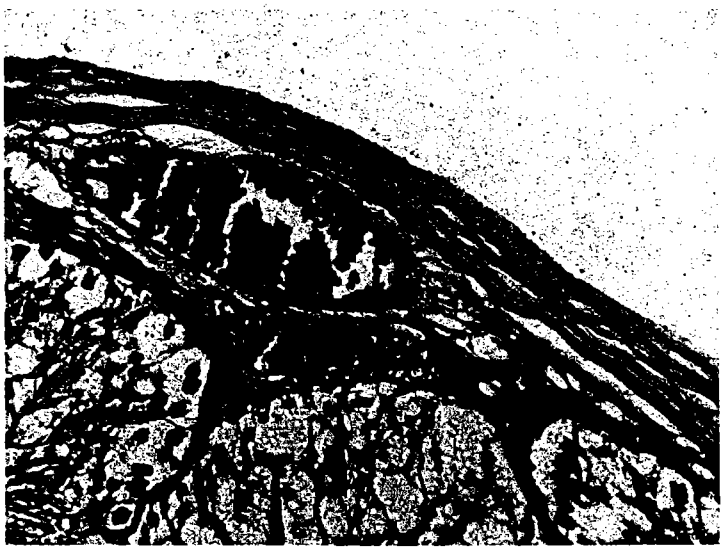


Fig. 23 Photomicrograph of treated testis showing vascular congestion and inflammatory cells in interstitial tissue. H&E 200x

Fig. 24 Micrograph of treated testis showing degenerated cells of Leydig. H&E 400x

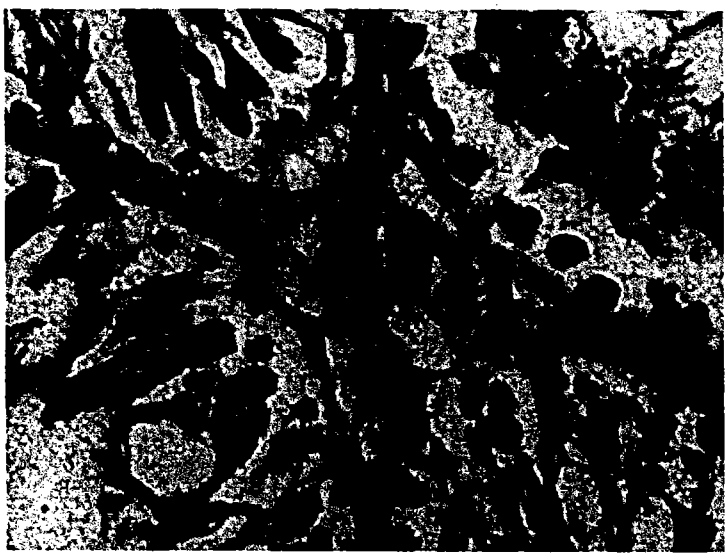


Fig. 25 Photomicrograph of treated rat testis showing patchy vacuolation in the Leydig cells cytoplasm and vascular congestion of interstitial tissue. H&E 400x

Group C (experimental)

Tunica albugenia was comparatively thick with fibroblasts, collagen and elastic fibers (Fig. 26).Vascular congestion was there in tunica vasculosa (Fig.27). Stromal swelling was reduced and semineferous tubules were attaining their normal rounded shape and configuration (Fig.26).Each seminiferous tubule was having thin basement membrane with very few fibrblasts and myoid cells.The epithelium of the seminiferous tubules consists of degenerated, highly undifferentiated spermatogonial cells with pyknotic nuclei (Fig.28,29).Degenerated sperm heads were sufficient in amount.The cytoplasm appeared totally disintegrated. The lumen of the seminiferous tubules were filled with degenerated membranes and flagella of the sperms in whorls form forming myelin like figures.(Fig.28) Sertoli cells were also degenerated with dark staining pyknotic nuclei (Fig.30) In the interstitial tissue, cells of Leydig were reduced and degenerated.(Fig .29)

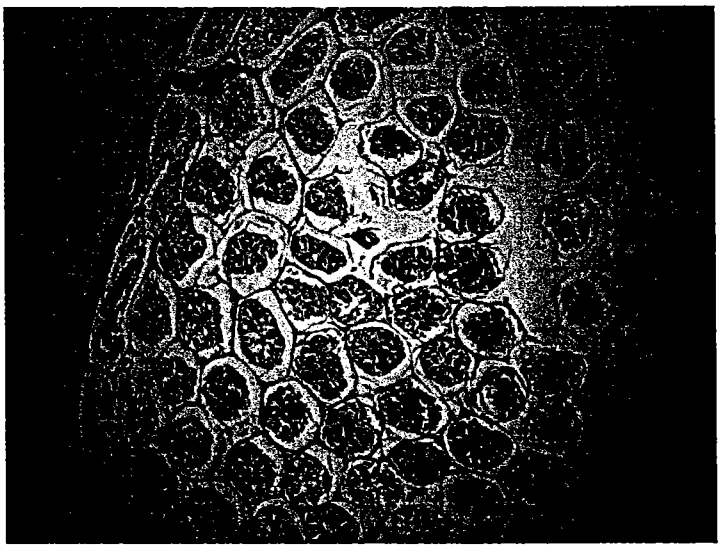


Fig. 26 Photomicrograph of treated rat testis of group C showing comparatively thick tunica albuginea with vascular congestion in interstitial tissue and mild peritubular swelling. H&E 40x

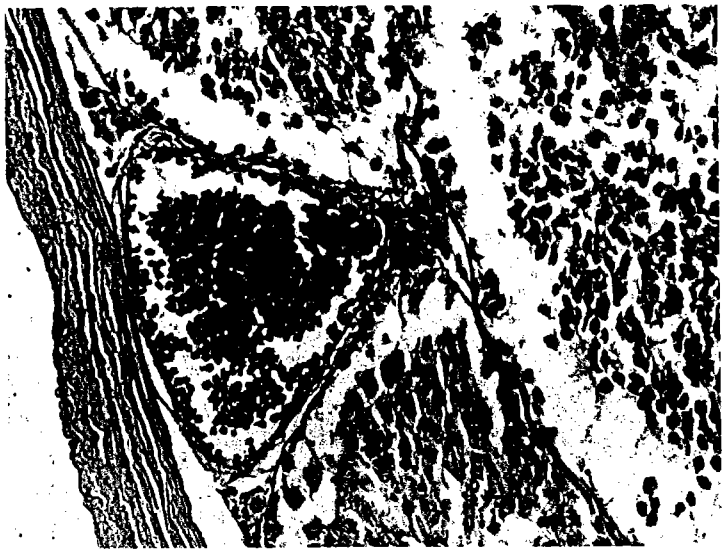


Fig. 27 Micrograph of testis of group C animal showing tunica albuginea and mild vascular congestion in tunica vasculosa. H&E 200x

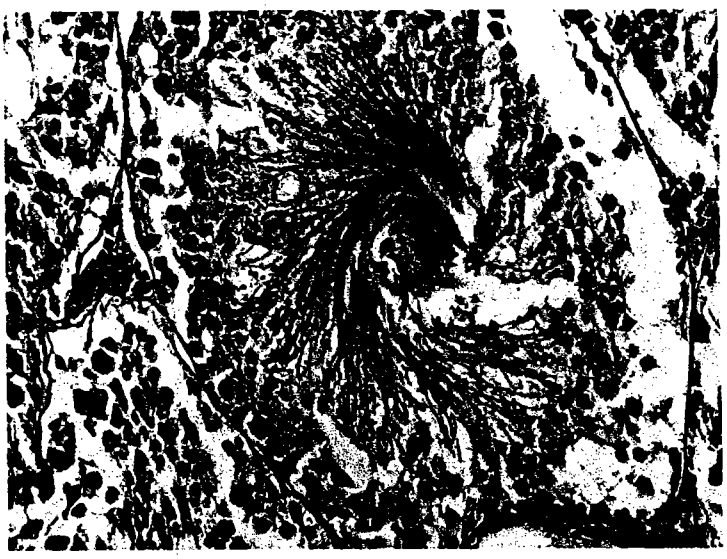


Fig. 28 Photomicrograph of sectioned seminiferous tubule showing degenerated epithelial cells, spermatozoa with their flagella in a whorl form. H&E 200x

Fig. 29 Photomicrograph of seminiferous epithelium showing regenerating spermatogonial cell, myoid cells and Sertoli cells. H&E 400x

Fig. 30 Photomicrograph of interstitial tissue of treated rat testis showing degenerated Leydig cells and vascular congestion. H&E 400x

EPIDIDIMYS

Group A (control)

Sections of highly coiled tube with surrounding connective tissue and blood vessels (Fig. 31)The tube was lined with pseudostratified columnar epithelium composed of rounded basal cells and columnar cells. The basal lamina is supported by smooth muscle cells and loose connective tissue rich in blood capillaries. The surface of epithelial cells is covered by stereocilia (Fig.32, 33) The lumen of the epididymis is filled by spermatozoa (Fig. 34).

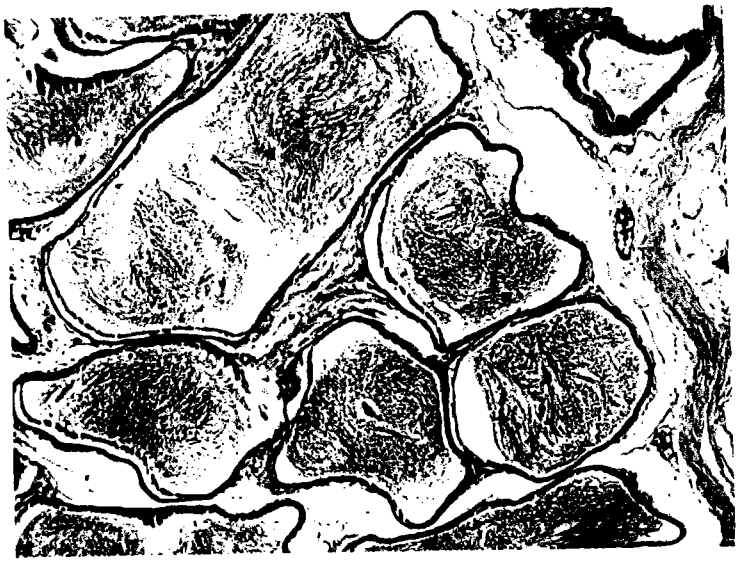


Fig. 31 Photomicrograph of group A (control) rat epididimys showing highly coiled tube with surrounding connective tissue and blood vessels. H&E 40x

Fig. 32 Microphotograph of sectioned epididimys of rat showing tubular epithelium resting on basement membrane with spermatozoa filling its lumen. H&E 100x

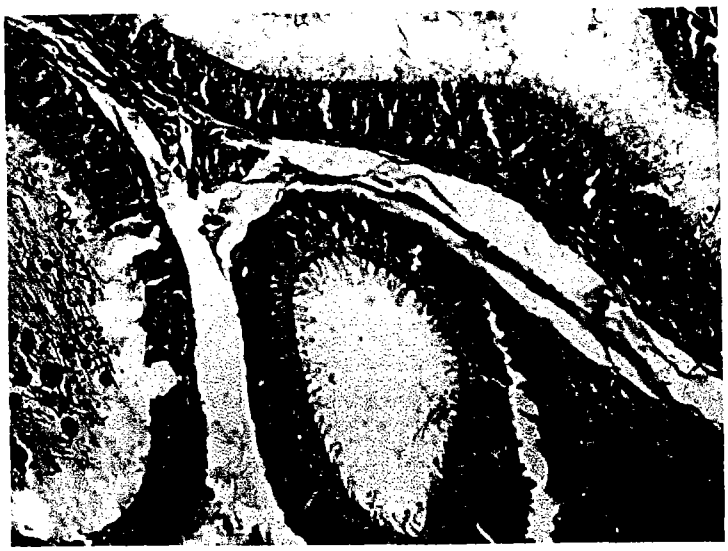


Fig. 33 Photomicrograph of cross-section of epididimys showing basal cells and pseudostratified columnar cells with stereocilia. H&E 200x

Fig. 34 Photomicrograph of rat epididymis showing epithelial cells and stereocilia and microvilli. H&E 400x

Group B (experimental)

Degeneration of epididymal epithelium was observed in most of the sections.(Fig.35) Nuclei of the cells were pyknotic with irregular shape. The lumen showed very much reduction in spermatozoa.(Fig. 36)

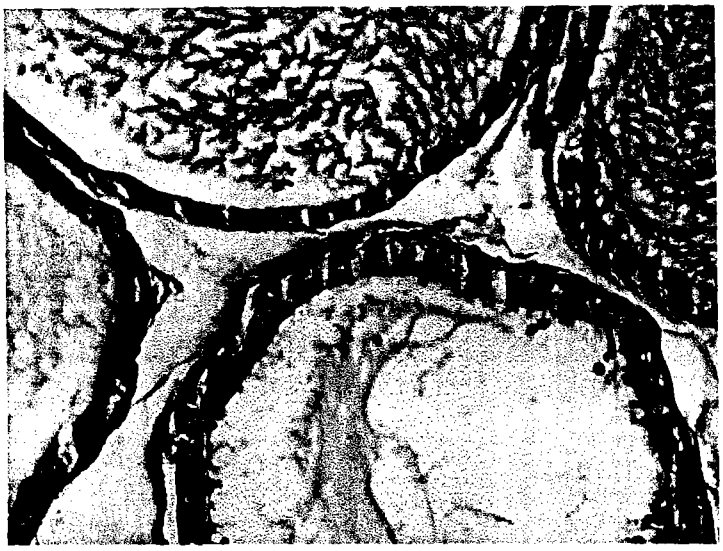


Fig. 35 Photomicrograph of group B treated rat epididimys showing degeneration of epithelium. H&E 200x

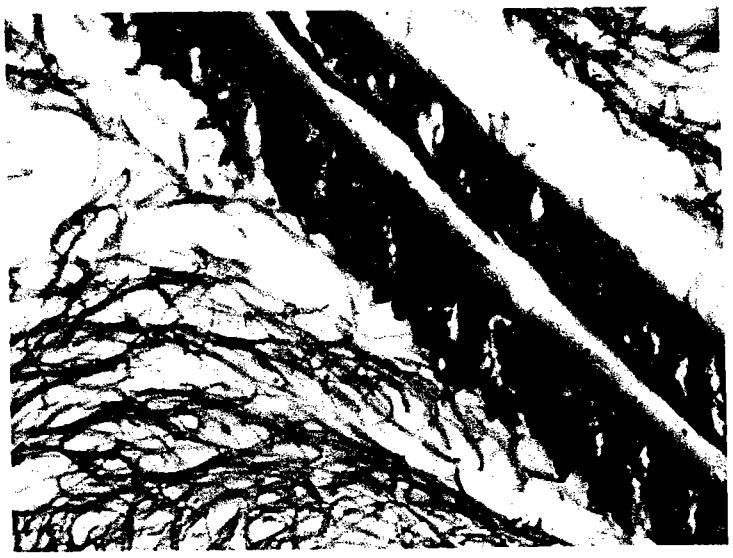


Fig. 36 Micrograph of treated epididimys showing degenerated basal and columnar cells with loss of stereocilia. H&E 400x

Group C (experimental)

The connective tissue around the epididymis was infiltrated with inflammatory cells and dilated blood vessels (Fig.37). The lumen was still having very few spermatozoa and cellular debris (Fig. 38)Basal cells and muscle cells were present in sufficient number Columnar cells were occupying various positions in the epithelium. Their apical surfaces were having stereocilia while lumen was having scanty spermatozoa and desquamated cells.

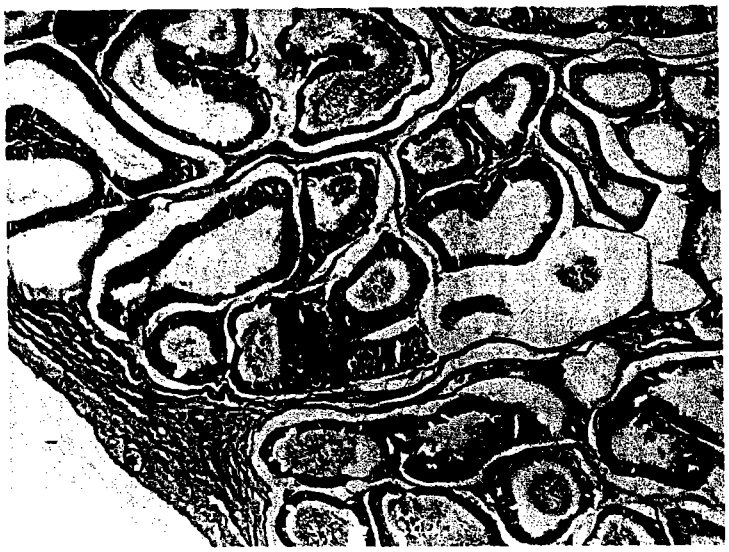


Fig. 37 Photomicrograph of group C rat epididymis showing inflammatory cells and blood vessels in surrounding connective tissue. H&E 40x

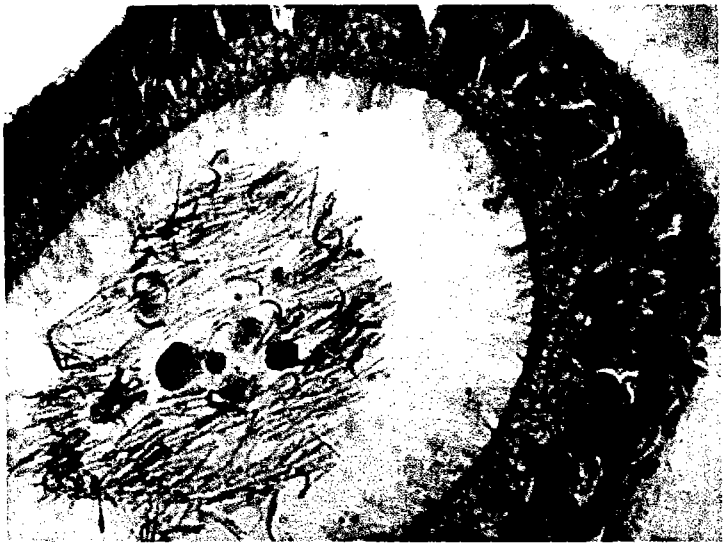


Fig. 38 Photomicrograph of cross-sectioned epididymis showing basal and columnar cells with stereocilia and few spermatozoa with desquamated cells in the lumen. H&E 400x

Ductus (vas) deferens

Group A (control)

Transverse section shows a tubular structure with thick, muscular wall, star shaped lumen and a mucosa with longitudinal folds. (Fig.39) Lumen is lined by pseudostratified columnar epithelium with stereocilia.(Fig.40) The lamina propria is rich in elastic fibers,(Fig.41) and the thick muscular layer consists of inner longitudinal and middle circular and outer longitudinal muscle fiber layers.(Fig.42)

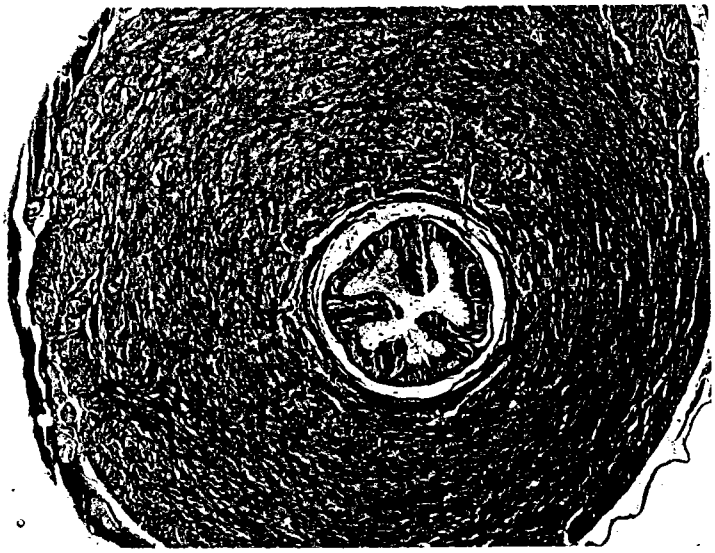


Fig. 39 Photomicrograph of ductus deferens of group A (control) rat showing outer connective tissue layer, thick smooth muscle wall, star shaped lumen and mucosa with longitudinal folds. H&E 40x

Fig. 40 Photomicrograph of vas deferens showing psudostratified columnar cells with stereocilia and spermatozoa in lumen. H&E 200x

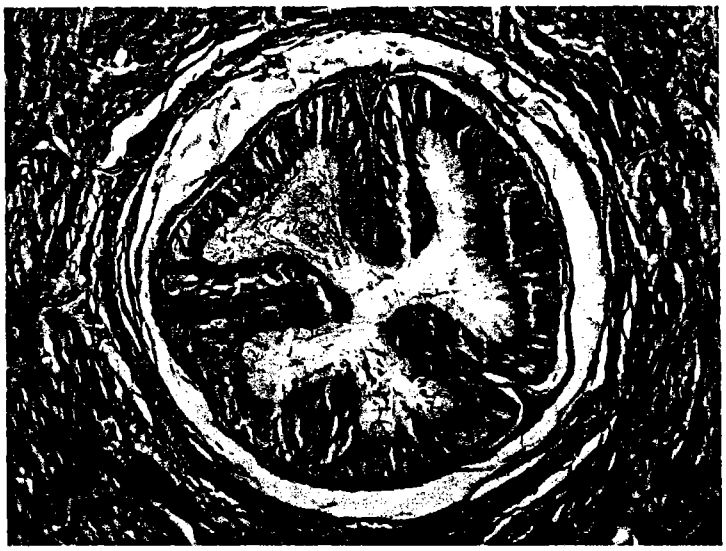


Fig. 41 Photomicrograph of cross section of rat vas deferens showing lamina propria with elastic fibers and connective tissue cells. H&E 100x

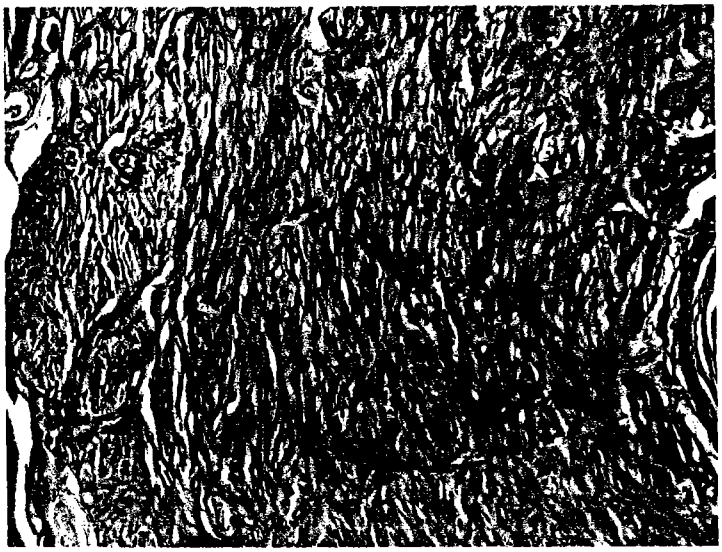


Fig. 42 Photomicrograph of cross section of rat vas deferens showing inner and outer longitudinal and middle circular smooth muscle fibers. H&E 100x

Group B (experimental)

Muscular layer is hyperplastic and congested with inflammatory cell and lymphocytes.(Fig.43, 44) The oedematous tunica adventitia showed engorged blood vessels and inflammatory cell.(Fig.45) .The lumen was almost empty with degenerated mucosal cells merging into lamina propria. Highly disorganized epithelium with darkly stained multiform nuclei.(Fig.46)

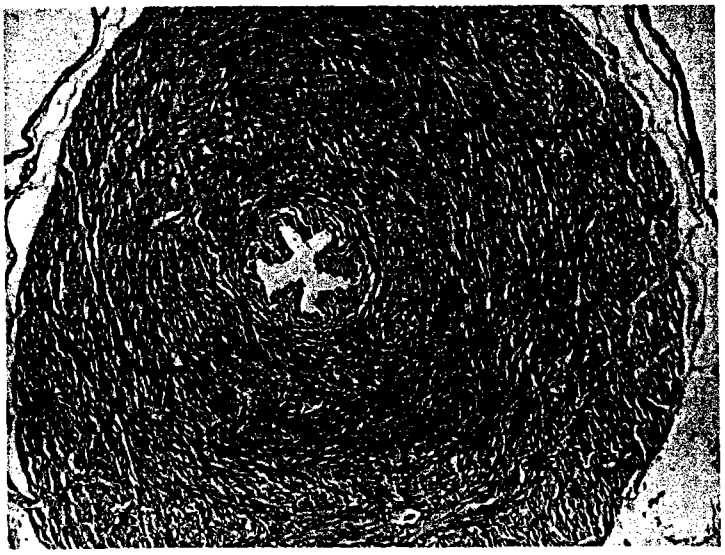


Fig. 43 Photomicrograph of cross section of rat vas deferens of group B showing hyperplasia of muscular layers. H&E 40x

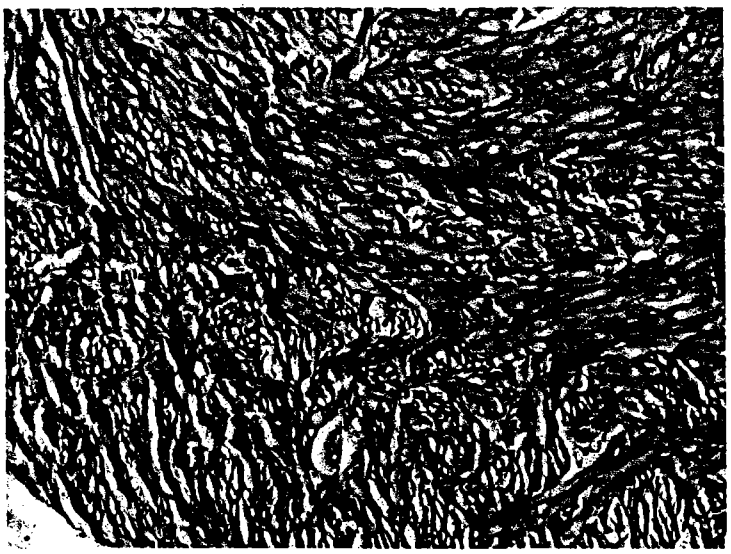


Fig. 44 Photomicrograph of muscular layer of vas deferens congested with inflammatory cells and lymphocytes. H&E 100x

Fig. 45 Microphotograph of vas deferens showing oedematous tunica adventitia with vascular congestion and lymphocytic infiltration. H&E 100x

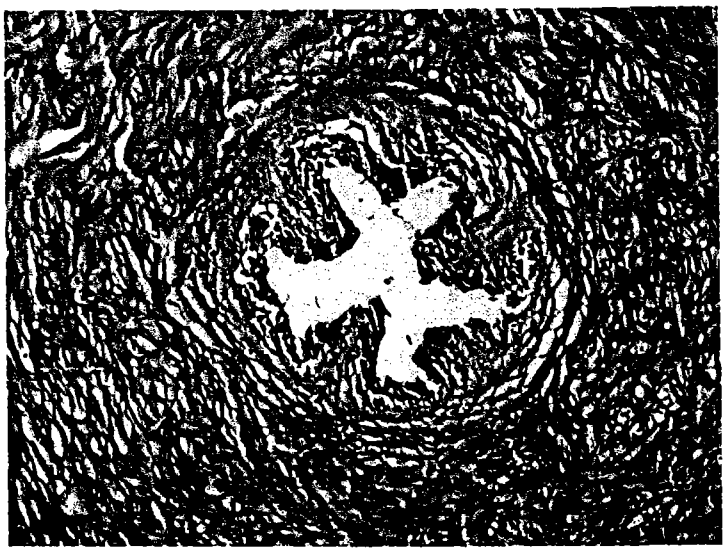


Fig. 46 Microphotograph of vas deferens of treated rat showing empty lumen, highly disintegrated epithelium. H&E 200x

Group C (experimental)

Swelling was reduced but still present in muscular layer and lamina propria (Fig. 47) mucosa was reverting back its normal cell types. Pseudostratified columnar epithelium was regenerating in the mucosa. Stereocilia were also developing on the luminal surface of the columnar cells. Lumen was almost filled by seminal fluid (Fig.48, 49).

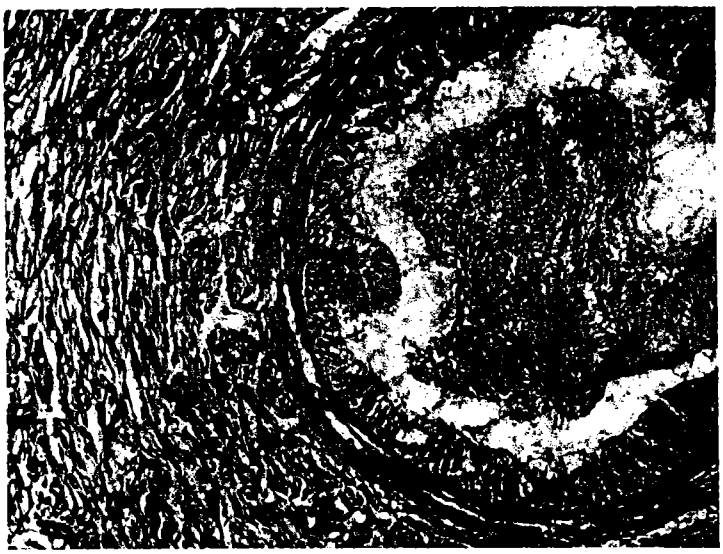


Fig. 47 Photomicrograph of group C treated rat vas deferens showing slight swelling of muscular layer and lamina propria. H&E 100x

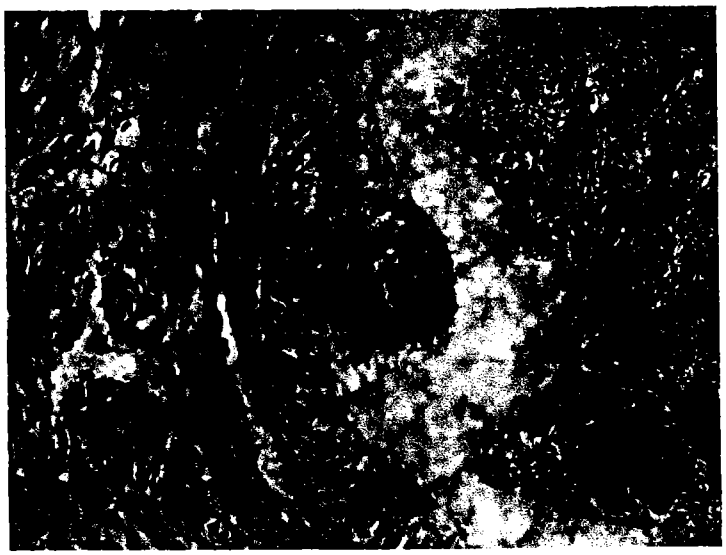


Fig. 48 Photomicrograph of cross sectioned vas deferens of group C rat showing pseudostratified columnar epithelium of mucosa. Lumen is filled with seminal fluid. H&E 200x

Fig. 49 Microphotograph of section of treated rat vas deferens mucosa showing columnar cells with stereocilia. H&E 400x

Seminal vesicle

Group A (control)

It is highly tortuous tubular gland showing different orientations when sectioned (Fig.50) It has folded mucosa lined with cuboidal or pseudostratified columnar epithelium rich in secretory granules. Lamina propria is rich in elastic fibers and surrounded by a thin layer of smooth muscles (Fig. 51)

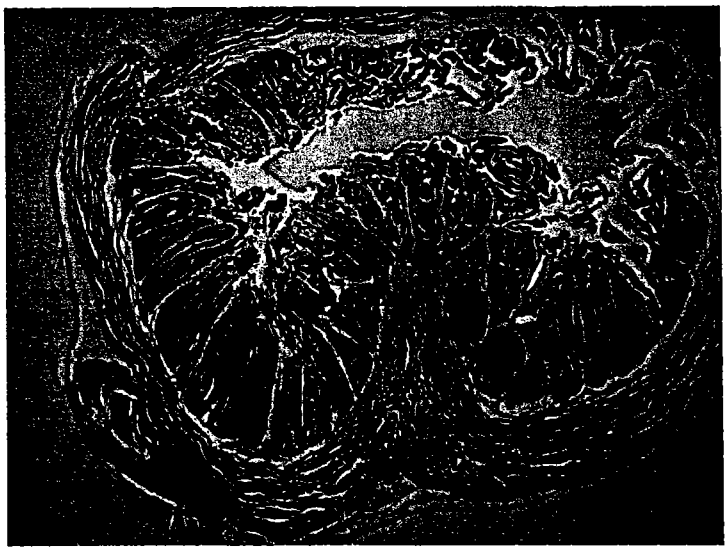


Fig. 50 Microphotograph of seminal vesicle of group A (control) rat showing tubular glands surrounded by smooth muscle layer. H&E 20x

Fig. 51 Microphotograph of seminal vesicle showing folded mucosa lined by secretory epithelium. H&E 200x

Group B (experimental)

Mucosal folds are prominent.The connective tissue in lamina propria is swollen.Glandular epithelium looks degenerated (Fig.52)Mucosal epithelium is disintegrated. Connective tissue around the mucosa is infiltrated with inflammatory cells (Fig. 53) Glandular (columnar) epithelium is degenerated. Vacuolation in the cytoplasm is evident and nuclear membrane is distorted (Fig. 54)

Fig. 52 Microphotograph of group B (experimental) rat seminal vesicle showing thin muscle layers and degenerated glandular epithelium. H&E 40x

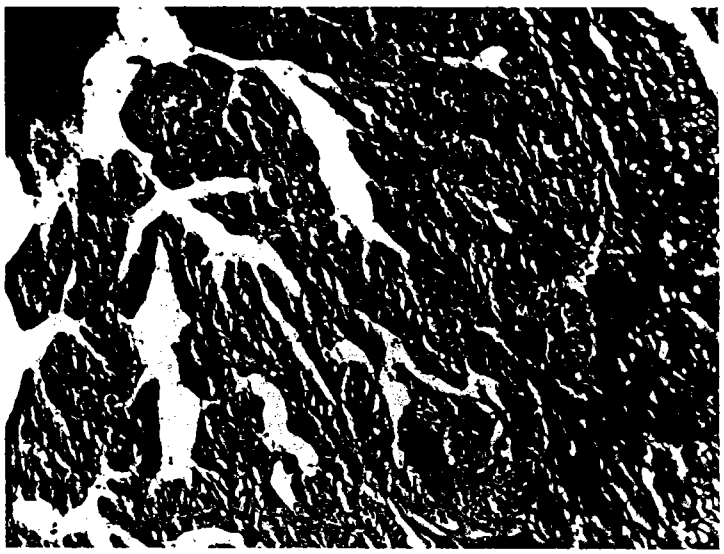


Fig. 53 Microphotograph of treated seminal vesicle showing degenerated glandular epithelium, lymphocytic infiltration in muscle layer. H&E 100x

Fig. 54 Microphotograph of treated seminal vesicle with degenerating secretory columnar cells. H&E 400x

Group C (experimental)

Connective tissue around the gland is moderately thick and congested with blood vessels and connective tissue cells (Fig. 55).The glandular epithelium is present on mucosal folds. Lamina propria is thick (Fig. 56). Epithelium is degenerated. There is swelling and cellular congestion in the connective tissue (Fig. 57, Fig. 58)

Fig. 55 Microphotograph of section of group C rat seminal vesicle showing almost normal glandular epithelium. H&E 100x

Fig. 56 Microphotograph of seminal vesicle showing smooth muscle layer, lamina propria and glandular epithelium. H&E 200x

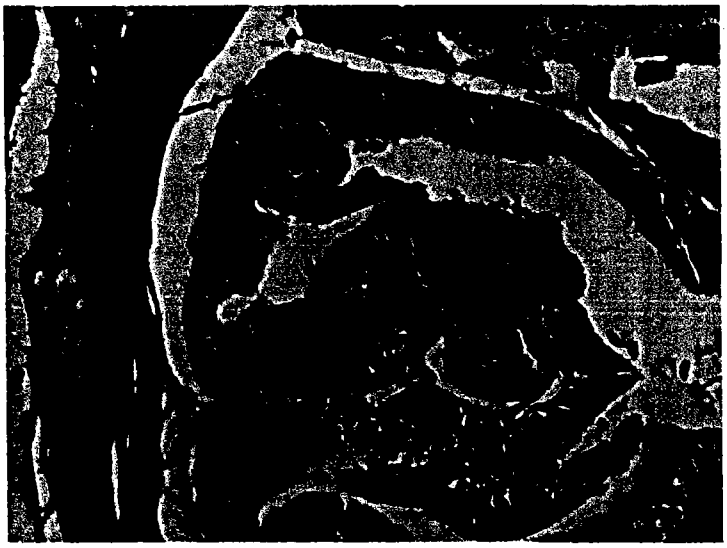


Fig. 57 Cross section of rat seminal vesicle showing secretory epithelium. H&E 200x

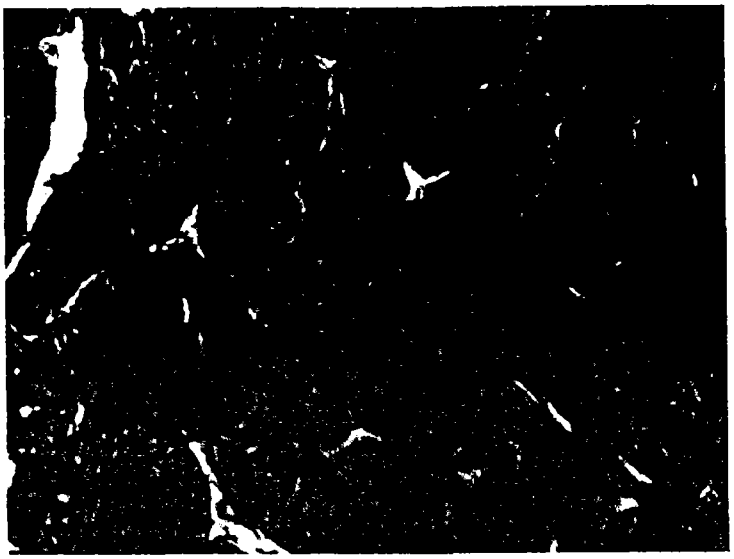


Fig. 58 Microphotograph of treated seminal vesicle showing active secretory columnar epithelium. H&E 400x

Prostate Gland

Group A (Control)

A collection of tubuloalveolar glands. The parenchyma of the glands is formed by cuboidal or pseudostratified columnar epithelium (Fig. 60). The stroma is formed by thick fibro-muscular tissue. It is surrounded by a fibroelastic capsule rich in smooth muscle fibers.The septas from its capsule penetrate the gland to divide it into lobes (Fig.59).

Fig. 59 Microphotograph of group A (control) rat prostate gland showing thick fibromuscular stroma and glandular epithelium. H&E 40x

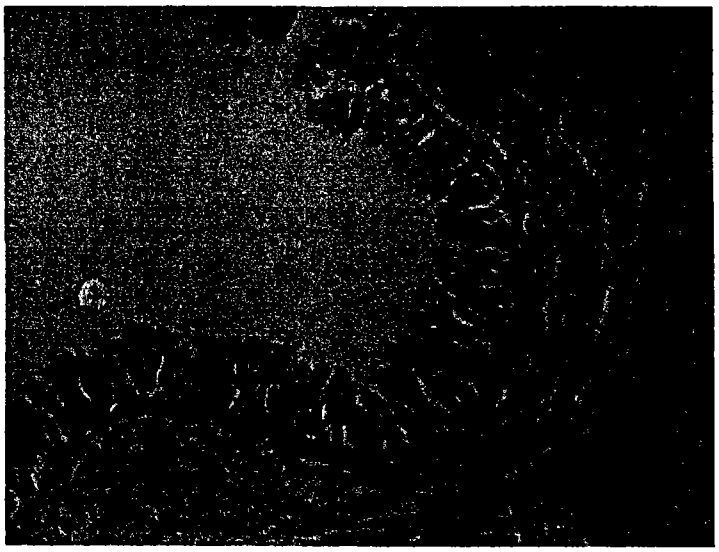


Fig. 60 Microphotograph of rat prostate gland showing cells of tubulo-alveolar glands and connective tissue around them. H&E 200x

Group B (experimental)

Fibromuscular stroma is infiltrated with lymphocytes and other inflammatory cells. The parenchyma is showing degenerated glandular cells. The lumen of the tubuloalveolar glands is empty (Fig. 61)

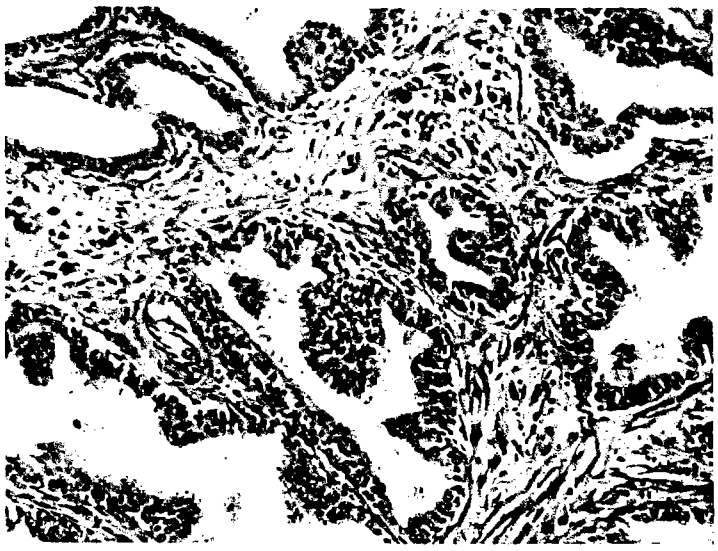


Fig. 61 Microphotograph of group B (experimental) rat prostate gland showing mild degeneration of glandular epithelium and cellular infiltration of stromal fibromuscular tissue. H&E 100x

Group C (experimental)

Highly congested fibromuscular stroma filled with inflammatory cell. Degenerated and distorted glandular epithelium is prominent (Fig.62, Fig. 63).Swelling of the cytoplasm and ruptured nuclear membrane shows the degeneration of the glandular epithelial cells. (Fig. 64)

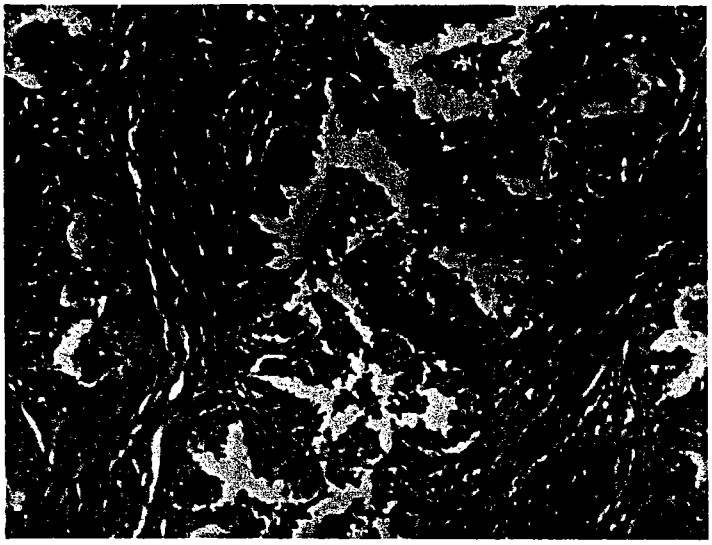


Fig. 62 Microphotograph of group C rat prostate gland cross section showing fibromuscular stroma and slight degeneration of glandular epithelial cells. H&E 100x

Fig. 63 Microphotograph of glandular epithelium and stroma of rat prostate gland showing moderate degeneration of glandular epithelium. H&E 200x

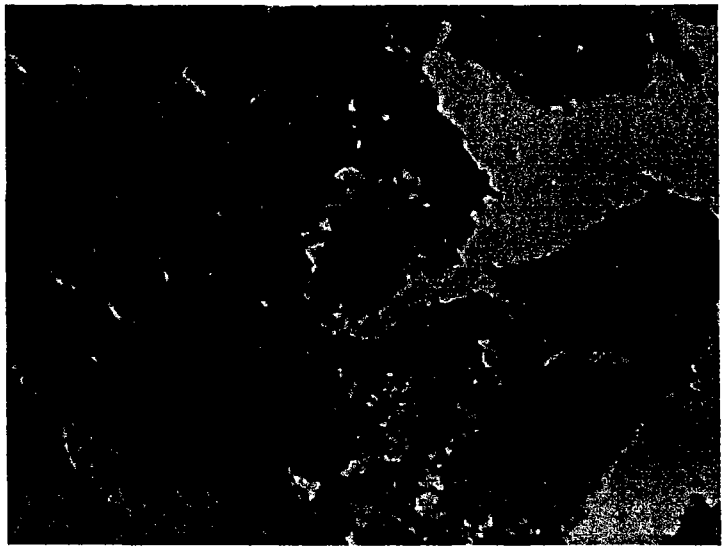


Fig. 64 Microphotograph of treated rat prostate gland showing degenerated secretory columnar cells, fibroblasts and smooth muscle cells. H&E 400x

DISCUSSION

Carbofuran has the potential to cause damage to the reproductive system and to health by prolonged exposure. The substance has been shown to produce developmental toxicity in several studies at doses, which cause minimal maternal toxicity. The male reproductive organs are severely damaged at doses around 0.2 mg/kg bw in both rats and dog. The described effects are degeneration of seminiferous tubules, loss of spermatogenesis, degenerative changes in Sertoli cells, and depletion of a variety of cell types.

The dramatic increase in the use of carbamate compounds substitutes for chlorinated hydrocarbon insecticides was has resulted in a new dimension of occupational hazard for agriculture industry.(Baron, 1991)

The importance of the androgens for normal spermatogenesis is well documented. Spermatogenesis depends on testosterone production by Leydig cells in response to stimulation by FSH and LH. FSH increases Sertloi cell synthesis of an androgen binding protein needed to maintain high concentrations of testosterone. LH stimulates testosterone production by the interstitial cells of the testis.(Kackar et al, 1997)

In the present study it was shown that the chronic administration of furadan led to significant histological changes in male reproductive organs.
Similar results were obtained in rat testis only for methomyl a carbamate, 17 mg/Kg body wt in saline daily for two months.(Afaf 2001)
In this study, other organs of male reproductive system were also examined were toxic effects of furadan, So rat testis, epididimys, ductus deferens, seminal vesicle and prostate glands were observed. All these organs revealed variable degrees of degenerative changes least in the prostate gland.

It has been proved by many studies that hormonal changes produced by carbofuran compounds are in favour of direct toxic effect of the insecticide or possibly through an alteration in the neuroendocrine environment.(Goldman et al, 1989)

According to comments on the classification of carbofuran based on its toxic effects by KEM, a Swedish Chemical Inspectorate published on 2005-05-30, these effects occurring at very low doses in two species justify a **classification** as Repr. Cat. 2;R60. On basis of the following results it can be classified as Repr. Cat.3; R63 and R64 warranted. Additionally a classification for damage to health by prolonged exposure, R48/22, should be considered due to inhibition of brain, RBC and plasma acetyl cholinesterase. This together with clinical symptoms indicates effects on the peripheral nervous system.

According to the European Union criteria, developmental toxicity occurring in association with maternal toxicity cannot be dismissed although concern may be

reduced. Thus, an appropriate classification of carbofuran into category 3 for developmental toxicity is warranted. In studies with rats effects such as reduced faetal body weight, decreased pup survival and mean number pups born and born alive were observed together with additional signs of increased incidence of malformations (situs inversus, sternebrae absence, major vessels variations).

In a 2- generation study on rats with diet, 20,50,100 ppm, the offspring survival index was significantly lower in the high dose group pups. Mean body weight for the mid and high dose pups (F1 and F2) were also significantly lower. The effect was greater for the F2 pups. The level of concern is elevated due to the fact that the findings are progressively higher in subsequent generation.(Schardein, 1990)

Another study on in utero and lactational exposure of carbofuran to rats, there was no mortality or toxic symptoms observed during the treatment periods. Both *In utero* exposure and lactation exposure resulted in testicular- and spermatotoxicity at dose levels of 0.4 mg/kg bw/day.(Pant et al, 1997)

Development rat study + postnatal study, dietary, 20, 60, 160 ppm , gestation day 6-19. Statistically significant decreases in mean pup body weights were noted in the top dose (10.96 mg/kg bw/day). In the mid and top dose a number of malformations were observed (situs inversus, scoliosis, protruding tong, limb anomalies, caudal vertebrae anomalies and absent of anal opening) (Rodwell, 1981, FMC).

Developmental neurotoxicity study, dietary, 20, 75, 300 ppm, GD6 through LD 10, 24 mated females Spraque Dawley CD rats/dose received in diet carbofuran on gestation day 6 through lactation day 10. Maternal survival was not affected and no adverse effect of treatment was evident from physical observation data. During the lactation period and post weaning, pup weight was statistically significant lower than the control. Also, pup survival was decreased in a dose related manner. Pup viability index lactation day 0-4 was decreased from 98.5 to 33.8 in the high dose group. Litter survival index was 9/23 in the high dose group compared to 23/23 in the controls. The decrease in pup weight is culminated day 11 during lactation. Developmental landmark data showed a delay in sexual development at the mid and top dose. Pups from mid and top dose also exhibited delays in angle development in swimming development tests.(Ponnock, 1994, FMC)

According to Directive 67/548 the risk phrase serious damage to health is to be considered when major functional changes in the central or peripheral nervous system is evident. In studies with rat, dog and rabbit, a critical effect of carbofuran is acetyl cholinesterase inhibition. The inhibition of plasma and erythrocyte AChE, supported by clinical signs, demonstrates a toxic effect on the peripheral nervous system. In addition, dose-related inhibition of brain AChE was observed in studies with rat and rabbit.

In the 90-day rat study, 20,129,720 ppm, brain and plasma cholinesterase activity was significantly inhibited in all dose groups. The decrease in brain AChE activity provides a direct evidence of potential adverse effects. In the lowest dose group (20ppm, corresponding to 1 mg/kg bw/day) there was a significant decrease of 13% (male). At 120ppm (corresponding to 6.2-6.8 mg/kg bw/day) the decrease in brain tissue AChE activity for males was 32% and for females 22%. (Abe, 1986, Dianica)

Carbofuran was administered in the diet to groups of 4 male and 4 female beagle dogs in concentrations equivalent to 0, 0.43, 3.1, and 10.6 mg/kg bw/day. Dose related inhibition of plasma (13, 27 and 70%) and erythrocyte (11, 29 and 68%) cholinesterase activity was observed in all treated groups. Increased salivation was determined in the lowest tested group and in the highest group clinical signs consisted of muscular spasms, ataxia, decreased motility and so on. (Bloch et al, 1987)

In a 21 day, rabbit study, 25, 100 and 400 mg/kg bw/day, 5 New Zealand white rabbits/sex/dose were exposed to carbofuran, topically for 6 hours/day daily for 21 days. A dose-related reduction in brain cholinesterase levels were observed in males reaching statistically significant difference at 100 (25%) and 400 (52%) mg/kg bw/day. Even in females a dose-related reduction was observed but this was not statistically significant. However a 10% inhibition was noted in the mid dose and in the top dose the inhibition was 30% compared to the control group. (Elliot et al, 1986, Dianica)

Testicular degenerative changes of the seminiferous tubules have been reported in experimental animals with various insecticides (Ezeasor, 1990, Chapin et al, 1994)

The histological results are in agreement with the observations of Hess , 2000 and Afaf 2001 who found that benomyl and methomyl respectively induces sloughing of germ cells at moderate to low doses.

In the present study, cytoplasmic vacuolation and degeneration were evident in Sertoli cells and germ cells which is an indication of cell necrosis. Such cellular vacuolation was most probably a cellular defence mechanism against injurious substances.(Mitchel, 1997)

The mitochondria of most germ cells, Sertoli cells and Leydig cells were swollen with loss of their cristae and granularity of their matrix. The mitochondria are labile structures that can be readily altered by the action of various agents, thus being one of the most sensitive indicators that could reflect injury to the cell. (Moussa, 1981)

Organophosphate insecticides cause disturbance of other metabolic processes apart from inhibiting acetylcholine esterase activity.Insecticides are capable of

binding to the lipid component of mitochondrial membrane resulting into change in mitochondrial function. (Sitkewicz 1975)

Dilatation of the smooth endoplasmic reticulum observed in Sertoli, spermatogenic cells and Leydig cells might be the consequence of intracellular redistribution of water and ions. The diminished mitochondrial function in the degenerating cells resulted in reduction in ATP and ATP-ase causing failure of the active transport system. So accumulation of sodium will retain waterintracellularly.It lead into the swelling of the cell (Dunnick et al, 1984)

Some investigators have attributed the testicular changes to the hypoxia that follows to chronic exposure to organophosphorous compounds (Barger et al, 1973). The effect on germ cells might be due to the decrease in testosterone level resulting due to injury of the cells of Leydig (Mitchell (1997)

Acid phospatase enzyme plays an important role in the process of cell metabolism, autolysis, differentiation and many related processes.(Sugar 1997). Dilatation of blood capillaries in between seminiferous tubules is the result of acid phosphatase enzyme activity. The increase in acid phospahatase enzyme activity could be explained on the bases of enhancement of cell membrane permeability with disturbance in the transphosphorylation process as a result of cellular degeneration. (Linder et al, (1988)

In the present study this may be the cause of cellular degeneration whereas dilatation and increased vasculature is most probably a compensatory mechanism to the hypoxia induced by furadan intoxication. Chronic insecticide intoxication may also lead into cellular disintegration. The cellular membrane degeneration of Leydig cell seen in present study is in accordance to the study of Sitkiewicz. (1975) in which it was proved that increase in alpha estrase activity observed in interstitial Leydig cells could be attributed to a disturbance in lipid metabolism induced by cellular membrane degeneration due to insecticide intoxication.

As it is clear from the results of this study that sixty days after the discontinuation of furadan (group C) to the rats, the histopathological changes reverted back slightly in the reproductive organs but persistence of these histological changes in moderate amount indicate long-lasting reproductive damage.

Male Druckrey rats were dosed with technical carbofuran (97.2% purity) by gavage (peanut oil vehicle) at 0, 0.1, 0.2, 0.4, or 0.8 mg/kg/day (10/group), 5 days/wk for 60 days. At sacrifice, reproductive structures were weighed, testes were taken for histopathology and testicular enzyme assays, and epididymal sperm were evaluated for motility, count, and abnormalities. Seven of the 10 high dose rats died survivors showed lethargy and imbalance. Other groups did not show clinical signs. Progressive body weight decrements occurred at 0.2 mg/kg/day and above. Weights of epididymides, seminal vesicles, ventral

prostate, and coagulating glands were significantly reduced at 0.2 mg/kg/day and above. Sperm motility and sperm counts were reduced at these dose levels. Increased numbers of sperm necks and tails were bent or curved, or tails were otherwise misshapen at the same dose levels. Testicular enzyme levels were altered at 0.2 mg/kg/day and above: reduced glucose-6-phosphate dehydrogenase and sorbitol dehydrogenase were considered to reflect disturbed germ cell maturation, whereas elevated (-glutamyl transpeptidase and lactate dehydrogenase levels were taken to indicate alterations in Sertoli cells and germinal epithelium, respectively. Histopathology of testes at 0.2 mg/kg/day and above included moderate edema and congestion among seminiferous tubules. There was moderate vacuolization of Sertoli cells and germinal cells at and above 0.2 mg/kg/day, with no change in Leydig cell appearance. Progressively higher dose levels led to tubular atrophy, disturbed spermatogenesis, and in some cases, atrophy of affected cell types (Pant et al, 1995).

Druckrey female rats of proven fertility were dosed with technical carbofuran (97.2% purity) by gavage (peanut oil vehicle) as follows: 6/group were dosed with either 0 or 0.4 mg/kg/day carbofuran daily throughout pregnancy, or 4/group were dosed with 0, 0.2 or 0.4 mg/kg/day carbofuran daily during lactation days 0 to 21. In all instances, pups were weaned at day 21, and 5/litter were examined at day 90 p.c. for epididymal sperm appearance and motility, activities of key testicular enzymes, sperm motility, sperm count, and sperm abnormalities. At 0.4 mg/kg/day in gestation and lactation treatment groups, there were reductions in sorbitol dehydrogenase, and increases in lactate dehydrogenase and glutamyl transpeptidase. Also, at 0.4 mg/kg/day after gestation or lactation treatment, epididymal sperm evaluations showed decreased sperm motility, decreased sperm count, and increased sperm abnormalities. Histopathology was most strongly evident after in utero exposure: individual seminiferous tubules lacked spermatogenic activity and Sertoli cells were frequently degenerated. There were no effects at 0.2 mg/kg/day. The consistency and magnitude of the changes at 0.4 mg/kg/day indicate "possible adverse effects." (Pant et al, 1997)

According to summary of "Potential toxicity of carbofuran to the testis: critical review of data" prepared for FMC Corporation as project number: FM17704 published on 9th June 2005 The initial European Union review of carbofuran for inclusion in Annex 1 of Directive 91/414/EEC suggested that the ADI should be based on the lowest NOAEL of 0.1 and 0.25 mg/kg bw/day seen in the one-year dog studies. The review states "... at the LOAEL, clinical signs (miosis and soft stool) and inhibition of AChE were reported at 1 mg/kg bw/d in one study and testicular degeneration was seen at 0.5 mg/kg bw/d in the second study. The choice of 0.1 mg/kg bw/d is further supported by NOAEL of 0.1 mg/kg bw/d in a published study from Pant et al (1995) in which, rats exposed for 60 weeks (sic) by gavage, showed testicular damage and toxic effects on sperm at 0.2 mg/kg bw/d." In addition, some regulators have suggested that carbofuran be classified as a reproductive toxicant.

The NOAEL in the chronic dog study by Taylor (1983) is 20 ppm in diet or 0.7 mg/kg bw/d. The effects on the testes observed in this study occurred at the high dose level (500 ppm in diet) that caused severe toxicity, which caused a delay in sexual maturity. The incidence of testicular effects at the lower dose levels were within the range that would be expected based on historical data from the literature. The study by Pant *et al.* (1995) – of 60 days only - has an irretrievably flawed scientific basis. This study and others by the same group of workers have not been reviewed critically by the RMS. These studies use test carbofuran of a different source (not FMC) than those for which approval is sought, and the results cannot be supported by GLP studies that are of substantially greater statistical, scientific and regulatory value. Other toxicology studies including the 13-week dog study by Bloch, the one-year dog study by Spicer (1990), a 90-day study in rats by Abe (1986), and the multi-generation studies in rodents by Goldenthal (1979) and Schardein (1990) all demonstrated an absence of testicular effects.

In the one of three GLP-compliant regulatory dog studies, carbofuran was associated with testicular lesions; these findings are concomitant with and attributable to prolonged severe general toxicity at the top dose which delayed the onset of sexual maturity, a delay which is misinterpreted as a specific testicular effect. There is no other evidence in GLP compliant guideline studies, of an effect of carbofuran on the testis or on spermatogenesis. The proposal for classification of carbofuran on the basis of testicular effects is inappropriate for the same reasons.

The conclusion from this review is that there is no specific effect on the testis from treatment with carbofuran and that carbofuran should be therefore regulated based on the NOAEL of 0.22 mg/kg bw/d based on inhibition of red blood cell cholinesterase and systemic toxicity in the 4-week dog study.

In contrast to this report, present study showed marked histological changes in testes, epididymis. seminal vesicles, prostate gland and vas deferens at the end of 60 days treatment.These changes persisted to large extent even at the end of another 30 days in (group C) animals kept without treatment with furadan.

CONCLUSION

Present study shows that the long term use of insecticide N-Methyl carbofuran (furadan) at the dose of 2 mg/Kg body weight orally is responsible for irreversible damage to male reproductive organs if precautionary measures are not taken while using them.Although furadan has high acute oral toxicity with an oral LD 50 in rats of 8 – 14 mg/ kg body weight. These structural changes persisted in the organs even after stoppage of furadan for 60 days. Structural damage to mammalian testes, epididimys, vas deferens, seminal vesicles and prostate gland may lead into infertility and carcinogenicity. So the utilization of pesticides must be predicted in selecting the quantities and mode of usage which will minimize the possibility of exposure of non target organisms to injurious quantities of these chemicals. This can be achieved through public health education to make people aware of the hazardous effects of these compounds. It is therefore recommended that great precautions must be taken to minimize the harmful side effects of such chemicals to the environment especially to men, animals and agriculture products aiming to avoid environmental pollution. The precautionary measures like wearing of impermeable gloves and masks to reduce the risk of inhalation of spray liquid.

This study will add to the knowledge on the subject which can be utilized by agricultural, environmental medicine, community medicine and health department personals to educate the end users of this insecticide about its toxic effects
In the past studies it was regarded that furadan is relatively harmless for human beings but the results of present study on experimental animals indicate that these toxicological features should not be ignored.

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Associate Professor Dr. Nasir Aziz

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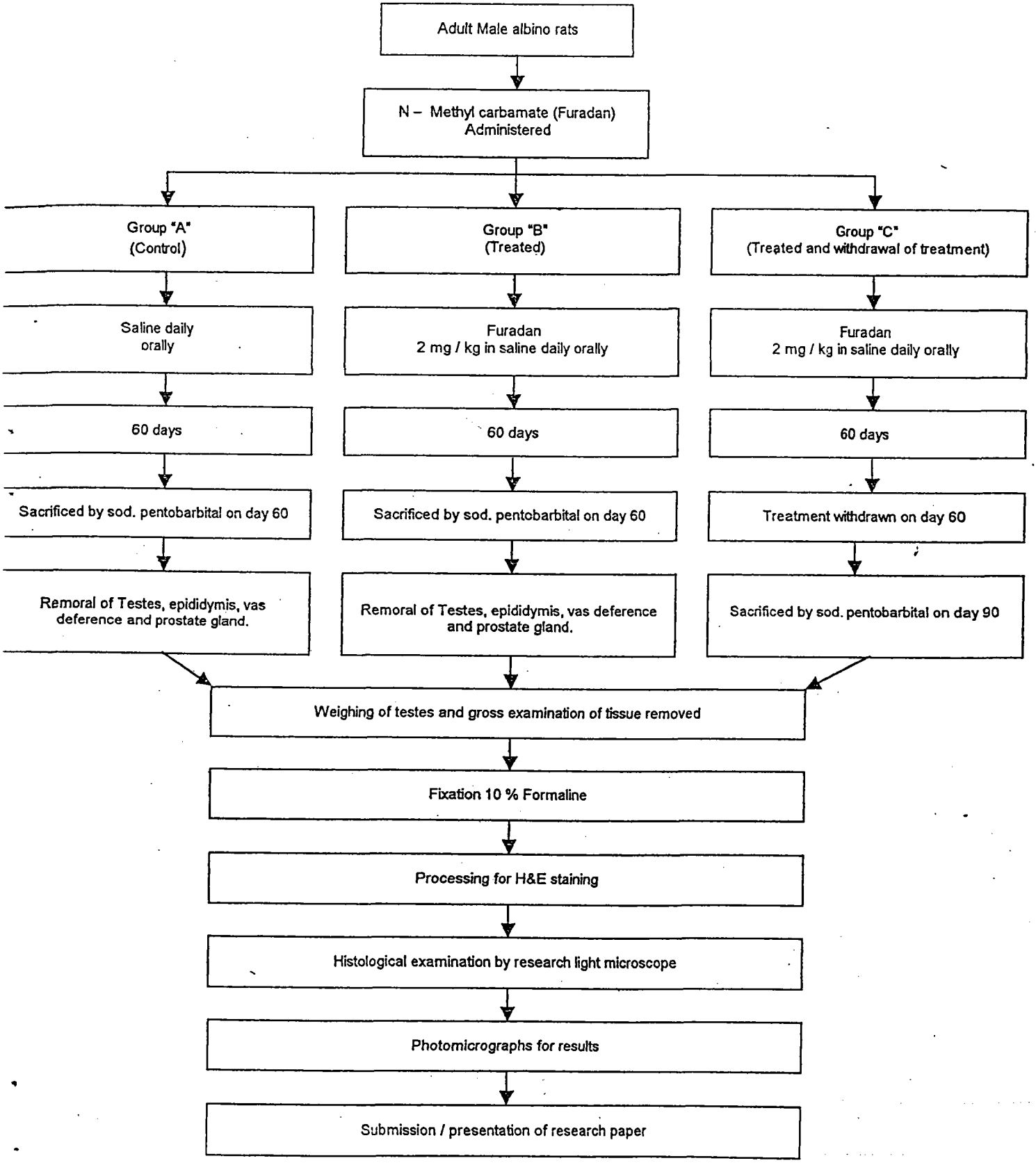
Appendix I

Flow Chart For Experimental Design



RCMO
Research Creativity & Management Office
PEJABAT PENGURUSAN & KREATIVITI PENYELIDIKAN

Tarikh : 12 Jun 2003 304/PPSP/6131256

Prof. Madya Nasir Aziz
Jabatan Anatomi
Pusat Pengajian Sains Perubatan
Kampus Kesihatan USM
16150 Kubang Kerian
KELANTAN DARUL NAIM.

Tuan,

Permohonan Geran Jangka Pendek

Sukacita dimaklumkan bahawa Jawatankuasa Sains Bio-Perubatan & Kesihatan di Mesyuarat ke 16 pada 9 April 2003 telah meluluskan permohonan penyelidikan tuan di atas tajuk ***"To Evaluate the toxic effects of Commonly used insecticide Furadan on male Rats Reproductive Organs"*** daripada geran **USM Jangka Pendek.** Sebanyak **RM 9,170.00** diluluskan dengan perincian seperti berikut:-

Vot 21000 *(Perbelanjaan Perjalanan & Sarahidup)*	RM	870.00
Vot 23000 *(Perhubungan & Utiliti)* -	RM	300.00
Vot 26000 *(Bekalan Bahan Mentah dan Bahan-Bahan Untuk Penyelenggaraan dan Pembaikan)*	RM	500.00
Vot 27000 *(Bekalan dan Bahan-Bahan Lain)*-	RM	3,500.00
Vot 29000 *(Perkhidmatan Ikhtisas & Perkhidmatan Lain yang dibeli dan Hospitaliti)* -	RM	2,500.00
Vot 35000 *(Harta Modal)* -	RM	1,500.00
* (diluluskan untuk Pembelian Rats Cages)		
JUMLAH BESAR	**RM**	**9,170.00**

Seterusnya, sekiranya peruntukan diluluskan di bawah Vot 11000, dibawah geran penyelidikan Jangka Pendek, hanya Pembantu Pelajar ataupun Pembantu Projek boleh dilantik. Kadar elaun yang boleh dibayar ialah RM15.00 sehari ataupun pada maksimumnya RM450.00 sebulan.

Dari segi perjalanan pula, kadar tuntutan yang dibenarkan ialah berasaskan tuntutan Pegawai A Tingkatan Biasa dengan tidak mengambil kira jawatan hakiki tuan/puan.

Lain-Lain Penyelidik : ***Prof. Madya Othman Mansor***
Dr. Mohammad Nazrul Islam
[Jabatan Anatomi]
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