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Histological effects of pesticides on the Spermatogenic tissue of testis in albino rats

Dr. Seema Sharma[1*], Dr. S Hamid[2], Dr. Sunanda Raina[3]
[1] MD Anatomy, JK Health Services, Jammu, Jammu and Kashmir, India
[2] Faculty, JK Medical College, Jammu, Jammu and Kashmir, India
[3] Professor and HOD Anatomy, GMC, Jammu, Jammu and Kashmir, India

Abstract
Introduction: Pesticides, today of scientific concern due to their high biological activity. Exposure of Pesticides not only reduced fertility but also embryo/fetal loss, birth defects, childhood cancer, and other postnatal or functional deficits. Carbaryl is one of the most important insecticides as it is widely produced and used which has prompted us to initiate this study.
Material and methodology: The present study was conducted on 40 male Wistar albino rats as experimental animals. The rats were procured from the Animals House. The rats were divided in four groups as normal control group I, group II, group III and group IV. All the rats were group housed and were fed with standard pellet diet and water for two weeks. After two weeks, the rats of group I were left as such and rats of groups II, III and IV were given 50 mg, 100 mg and 200 mg/kg body weight/day of carbaryl drug in 0.2 ml of groundnut oil orally, 6 days/week for 60 days, respectively. After 60 days, all the rats were anaesthetized by keeping them in an inverted glass jar containing large piece of cotton soaked in anaesthetic ether. The testis were dissected out from each rat and were cut into smaller pieces. These pieces were immediately fixed in 10% formalin. The blocks were prepared for section cutting with a microtome by paraffin wax embedding method. The sections of 7 µ thickness were mounted on glass slides and were stained by H&E and Masson's trichrome stain.
Observations: There were variation in the shape of seminiferous tubules, sloughing of the germinal cells from the basement membrane, depressed spermatogenesis and loss of sperms. Some tubules are showing accumulation of cellular masses in the lumen of seminiferous tubules of testis.
Conclusion: These findings are highly conclusive of reproductive toxicity produced by an insecticide, Carbaryl. Moreover, intensity of toxic effects both in peripheral and central parts increases with increase in dosage of the carbaryl drug.

Keywords: albino rats, testis, carbaryl, degeneration of spermatogenic tissue

Introduction
Pesticides is the general term for insecticides, acaricides, rodenticides, herbicides, fungicides etc. They are widely used in industry, agriculture and for public health purposes. Unfortunately, pesticides are toxic to a greater or lesser extent towards non-target organisms, including humans [1]. Pesticides, though present in the environment in small quantities as compared to other contaminants such as industrial wastes and fertilizers, account for public and scientific concern due to their high biological activity.
It has become more important to control insect pests of crops to reduce the losses and meet the requirements of increasing human population. Secondly, there is a need to check insect pest population for attaining economic threshold levels. Thus, insecticides play a pivotal role in saving the cropping enterprise from devastations of pests and in increasing food production.
The major factors which account for public and scientific concern of Pesticides is their biological activity.
Carbaryl is a broad-spectrum insecticide used to protect vegetables, cotton, fruits, cereals and other crops against a variety of insects and pests. Lot of work has been done on carbaryl indicating deleterious effects of chronic and subchronic administration of carbaryl on the male reproductive system. In the recent years, use of carbamate insecticides has gained importance due to ban of the insecticides belonging to Organochlorine groups that is D.D.T., Aldrin, Lindane and Endosulfan. These pesticides have a tendency to persist and have potential to bioaccumulate in the body

Material and methods
The present study is based on the findings carried out on 40 male Wistar albino rats as experimental animals.

Collection of material
Healthy male Wistar albino rats weighing between 50-80 grams were obtained from the Animals House. Forty rats were included in this study.

Grouping of animals
The rats were divided into the following four groups and identification number was given to the rats of each group.
Identification No.
Group I: Normal control - 10 rats N_1 (a to j)
Group II: Experimental group - 10 rats N_2 (a to j)
Group III: Experimental group - 10 rats N_3 (a to j)
Group IV: Experimental group - 10 rats N_4 (a to j)
All the rats were group housed in small iron cages in a room, where temperature was maintained at $23^0 \pm 1\ ^0$C. The rats

were fed with standard pellet diet and water for two weeks.

After two weeks the rats of normal control group I were left as such

Table 1: Administration of Carbaryl Drug to Experimental Groups

Experimental groups	Date of administration of 1st dose of carbaryl drug	Route of administration of carbaryl drug	Duration of carbaryl drug given	Dose of carbaryl drug administered to each group
Group II	28/12/2010	Oral	6 days/ week for 60 days	50 mg/kg body weight/ day in 0.2 ml of groundnut oil
Group III	28/12/2010	Oral	6 days/ week for 60 days	100 mg/kg body weight/ day in 0.2 ml of groundnut oil
Group IV	28/12/2010	Oral	6 days/ week for 60 days	200 mg/kg body weight/ day in 0.2 ml of groundnut oil

Dissection of experimental animals

After 60 days all the rats were sacrificed after anaesthetizing them in an inverted glass jar containing large piece of cotton soaked in anaesthetic ether and dissection of testis of albino rats was done on the same day

The scrotum was skinned and a midline incision was given with the help of scalpel and forceps. The testis were dissected out from the scrotum of each rat. The naked eye examination was done to see any external changes. The dissected out testis were cut into smaller pieces (5 mm) and were kept in tissue capsules along with a label indicating the serial groups I, II, III and IV. The dissected specimens were kept in 10% formalin solution for fixation. The fixation imparts firm consistency without excessive hardening and specimens were kept for optimum time 7 – 10 days for fixation. Paraffin wax embedding method was used for preparing the tissue for section cutting. Staining was done by:-

a) Harris Haematoxy line and Eosin Stain
b) Masson's Trichrome Stain.

Observations

Observations In Normal Control Group Microscopic Changes: Cut sections of testis in various planes show that the testis contain;

(A) Seminiferous tubules with many cells thickened walls.

Observations in carbaryl (50 Mg/Kg/Day) treated rats group II

The rats fed with 50 mg/kg/day for 60 days show following features in the testis:-

Microscopic changes:-

(A) Changes in seminiferous tubules

1. Some of the seminiferous tubules show mild variation in their size with focal distortion of normal shape which is mostly observed in peripheral seminiferous tubules.
2. A few sections of the tubules exhibit collection of cellular masses in their lumen.
3. There is detachment of the basement membrane from the seminiferous epithelium in few of the seminiferous tubules.
4. Light microscopy reveals mild to moderate degenerative changes in spermatogenic cells. In addition focal individual cell necrosis is also seen especially towards the lumen of tubules.
5. In few of tubules, spermatids and spermatozoa are identified.

Observations in carbaryl (100 Mg/Kg/Day) treated rats group III

The group of rats fed with 100 mg/kg/day for 60 days show following observations:-

Microscopic changes:-

(A) Changes in seminiferous tubules

1. Many of seminiferous tubules show distortion in the normal size. Variation in size is more marked than the previous group and is mostly observed in peripheral tubules.
2. There is collection of cellular masses in the lumen of several sections of the seminiferous tubules.
3. Several of the seminiferous tubules show the separation of the basement membrane from the spermatogenic series of cells, which is more marked than the findings seen in the rats given with low doses of carbaryl.
4. Several sections of seminiferous tubules show moderate to severe degenerative changes in spermatogenic cells with marked cell necrosis in seminiferous tubules towards the lumen.
5. Many of tubular sections show loss of spermatids and spermatozoa.

Observations in carbaryl (200 Mg/Kg/Day) treated rats group IV

The following findings are seen in the testis of rats fed with 200 mg/kg/day for 60 days:-

Microscopic changes:-

(A) Changes in the seminiferous tubules

1. As seen with light microscope, multiple sections of the seminiferous tubules show small atrophied seminiferous tubules with marked distortion in their shape and size. Variations seen in their shape are more pronounced than the previous groups with low doses of carbaryl. The seminiferous tubules show cell necrosis towards the lumen of tubules which is more marked than the previous groups. These changes are more prominent in the peripheral tubules of testis.
2. Several seminiferous tubules exhibit sloughing of the germinal cells from the basement membrane.

3. The spermatogenesis is much disturbed as compared with the previous groups with low doses of carbaryl.
4. Many of seminiferous tubules are showing loss of spermatids and spermatozoa.
5. Several of seminiferous tubules show accumulation of cellular masses in their lumen.
6. There is also evidence of detachment of germ cells from the basement membrane. This detachment of basement membrane is more marked as compared with groups with low doses of carbaryl.

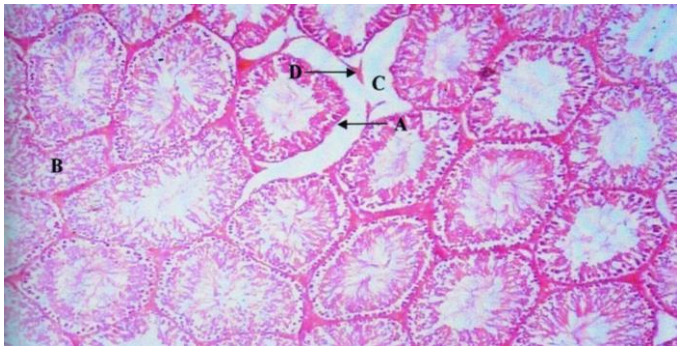

Fig 1: TS. of testes of Group II albino rats after giving carbaryl 50 mg/kg orally showing mild variation in shape of seminiferous tubules(A)Collection of cellular masses in the lumen of seminiferous tubules (B)Mild Interstitial edema (C) Degenerated Interstitial cells (D)

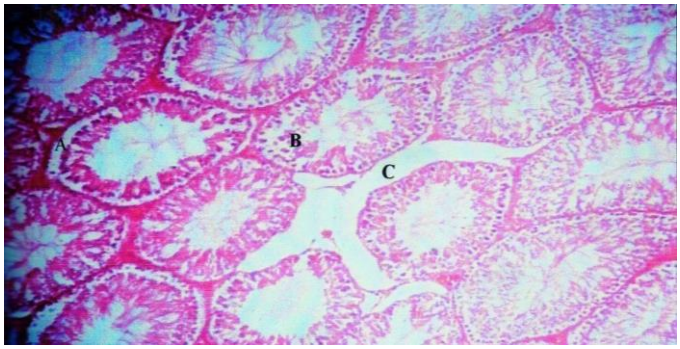

Fig 2: TS. of testes of Group II albino rats after giving carbaryl 50 mg/kg orally showing detached basement membrane from germ cells (A)Focal cell necrosis towards the lumen of seminiferous tubules (B) &Mild interstitial edema (C)

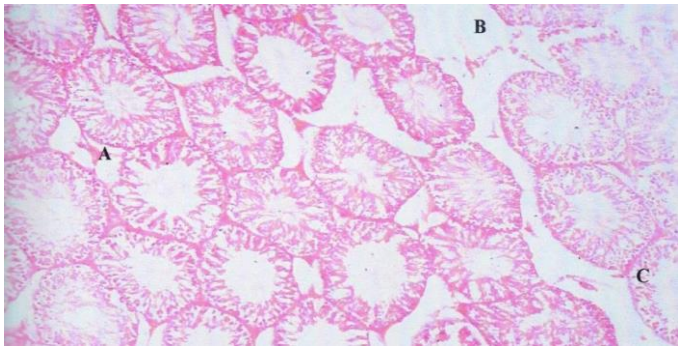

Fig 3: TS. of testes of Group II albino rats after giving carbaryl 50 mg/kg orally showing mild degenerative changes in the interstitial cells of leydig (A) Interstitial edema (B) And Cell Necrois (C)

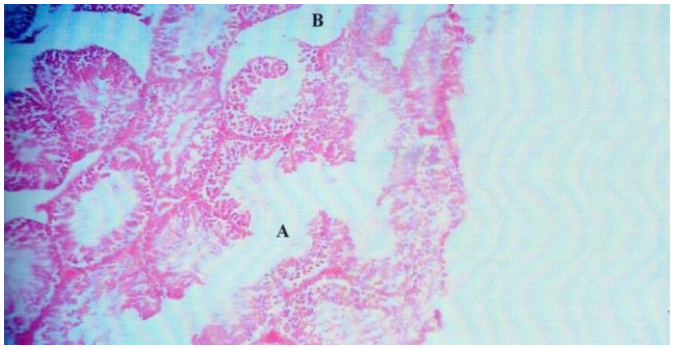

Fig 4: TS. of testes of Group II albino rats after giving carbaryl 50 mg/kg orally showing broken basement membrane (A) & Interstitial edema (B)

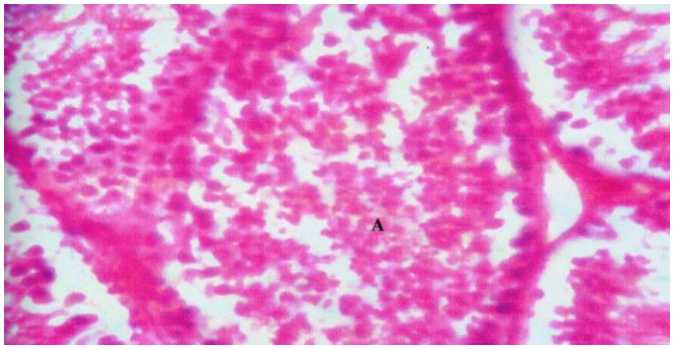

Fig 5: TS. of testes of Group III albino rats after giving carbaryl 100 mg/kg orally showing cellular collection in the lumen of testes (A)

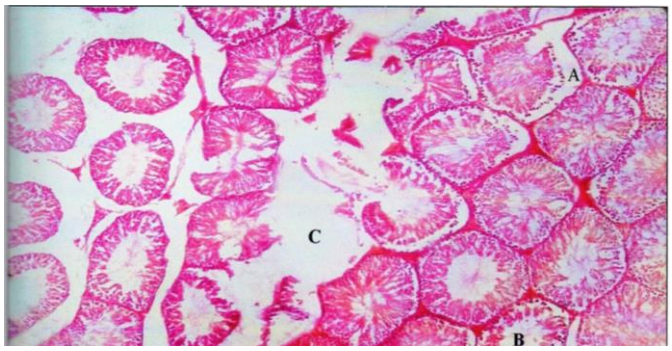

Fig 6: TS. of testes of Group III albino rats after giving carbaryl 100 mg/kg orally showing detachment of basement membrane from Germinal Cells (A),Marked Cell necrosis towards lumen of tubule (B) & marked Interstitial edema (C)

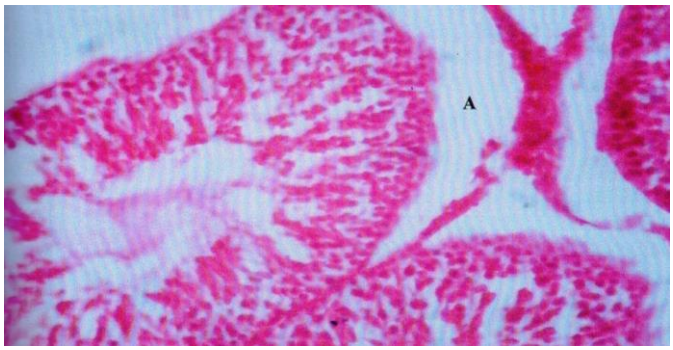

Fig7: TS. of testes of Group III albino rats after giving carbaryl 100 mg/kg orally showing detachment of basement membrane from Germinal Cells (A),

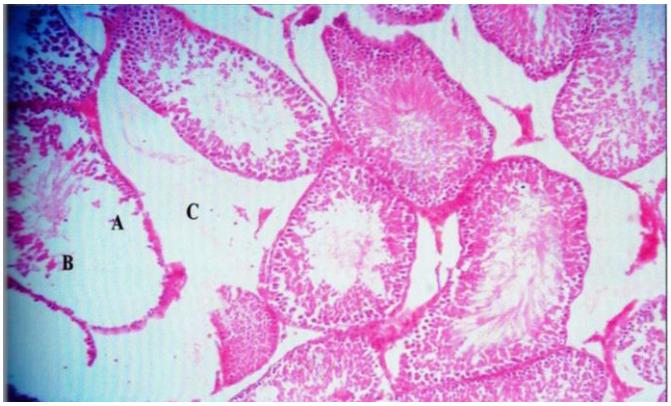

Fig 8: TS. of testes of Group III albino rats after giving carbaryl 100 mg/kg orally showing severe degenerative changes in spermatogenic cells (A) Loss of Spermatids and Spermatozoa (B) Interstitial edema (C)

Discussion

An extremely complex mechanism underlies the effects of various substances on reproductive components and functions. Various chemicals may interfere in different ways with components of reproductive system. They may affect directly by interference of the substance with reproductive components or indirectly by altering hormonal regulations.

The carbamate insecticides, one of which is Carbaryl, exert their insecticidal action by inhibiting cholinesterase enzymes. This inhibition is the primary mechanism by which these insecticides cause toxicity in mammals. The cholinesterase enzymes hydrolyze acetylcholine and other choline esters; consequently, their inhibition leads to the accumulation of endogenous acetylcholine and other choline esters most of the biologic effects of anticholinesterase agents, including carbaryl, are due to the inhibition of acetylcholinesterase which leads to the accumulation of endogenous acetylcholine, the principal choline ester that has demonstrated physiologic significance in humans. The aim of present experiment is to study normal histomorphological characteristics of the testis of albino rats and also to study dose-related effects of carbaryl on the testis of male albino rats with special reference to histomorphological changes.

The present study shows distorted shape of seminiferous tubules, disturbed spermatogenesis, accumulation of cellular mass in the lumen of seminiferous tubules, loss of sperms of varying degrees and detachment of germ cells from the basement membrane of seminiferous tubules of testis. Same findings were also reported by Rani *et al*. (2007) [2] in the testis of albino rats following administration of carbaryl in dose of 100 and 200 mg/kg body weight in 0.2 ml of groundnut oil orally/6 days week for 60 days. Similar findings were also reported by Rybakova (1966) [3], Vashakidze (1975) [4] and Pant *et al*. (1995; 1996), [5] who have reported spermatotoxic effect of carbaryl in adult and young male rats given with 50 and 100 mg/kg body weight. Male fed 5 days/week for 60 days, caused dose and age-dependent decline in epididymal sperm count and sperm motility and an increase in number of sperms with abnormal morphology. Young animals in comparison to adults exhibited pronounced spermatotoxic effects. Some of these findings are in accordance with the present study in which the dose-related decreased spermatogenesis and loss of sperms of varying degrees have been found.

The present study reveals marked histomorphological and degenerative changes of the lining cells of seminiferous tubules. These findings are in accordance with Rybakova (1966) [6], who observed deleterious effects of chronic and subchronic administration of carbaryl on male reproductive system of animals. These effects included damage to the germinal epithelium of seminiferous tubules and altered spermatogenesis.

Vashakidze (1965) [4] reported teratogenicity and decreased reproduction at subchronic intubated dosages of 100 mg/kg body weight/day and higher but not at 50 mg/kg/day. However, a single 50 mg/kg/day in intubated dose on gestation day nine or ten reported to cause teratogenicity. These findings correlate with the present study in which dose related effect is seen on spermatogenesis and loss of sperms of varying degrees in testis of male albino rats.

Kitagawa *et al*. (1977) [6] reported reduced number of spermatogonia and spermatozoa in rats given 3 mg/kg body weight of carbaryl orally for 1 year. These findings are also in accordance with the present study showing depressed spermatogenesis in rats fed with carbaryl orally.

The present study done on male albino rats by giving them carbaryl in dose of 50, 100 and 200 mg/kg body weight in 0.2 ml of groundnut oil orally/ 6 days week for 60 days showed distorted shape of seminiferous tubules and significant histomorphological changes in testis of albino rats. These findings are contrary to the findings by Dikshith *et al*. (1976) [7], who demonstrated that oral administration of carbaryl (200 mg/kg body weight for 3 days a week) for a period of 90 days did not produce any overt toxicological signs in male albino rats. There were no significant histological changes in testis, liver and brain. The activity of acetylcholine esterase in blood of carbaryl treated rats was however found to be decreased. Carbaryl did not affect the fertility of male albino rats at 200 mg/kg body weight upto 90 days.

Martin (1982) [8] and Osterioh *et al*. (1983) [9] showed that when carbaryl administered to mice up for 5 days at upto 800 mg/kg/day or by gavage at 150 mg/kg/every 2 days for upto 68 days, did not affect testis weight, histology, sperm count and frequency of sperm abnormalities. These findings are not in accordance with the present study which reveals the distorted shape of seminiferous tubules, disturbed spermatogenesis, loss of sperms in the testis of male albino rats. These findings are much significant with the high dose of carbaryl.

Carpenter *et al*. (1961) [10] reported that carbaryl in the diet of rats for 2 years at 400 ppm (about 20 mg/kg/day) slightly depressed organ weights in males but did not affect mortality, haematology or organ histopathology. No effect levels were at 9 mg/kg/day for males and 21 mg/kg/day for females. In shorter term studies at higher dosages, liver and kidney effects were noted, which were transient and may have been secondary to stress. Though our study does not affect mortality yet it shows significant histopathological changes on testis of albino rats.

Benson and Dorough (1984) [11] observed that when rats and

gerbils were given carbaryl orally for 70 days at dosages that were increased weekly, all deaths occurred with in 24 hours of the first administration of a given dosage. One of 12 rats died at dosage of 120 mg/kg/day, and cumulative mortality was 7/12 at a dosage of 180 mg/kg/day; no further deaths occurred at dosages of upto 200 mg/kg/day. In gerbils, mortality was 2/12 at the initial dosage of 60 mg/kg/day, and the last animal died at a dosage of 100 mg/kg/day. On the contrary there is no mortality of albino rats in the present study at doses of 50, 100 and 200 mg/kg body weight respectively.

The present study shows altered spermatogenesis which can lead to infertility by giving carbaryl to male albino rats. These findings do not correlate with the findings seen by Orlova and Zhalbe (1968) [12]

They could not find any change in fertility, gestation and viability of carbaryl (2 mg/kg) treated pups and rats. Similarly, studies of Benson and Dorough (1984) [11] showed that carbaryl (10 and 30 mg/kg body weight) induced no reproductive or teratogenic effects in mice. Weil *et al*. (1972) [13] observed no significant effects of carbaryl (10 mg/kg) on fertility, gestation, viability or lactation of rats. Studying non-human primates, Dougherly *et al*. (1971) [14] also could not find any teratogenic effects of carbaryl (2 and 20 mg/kg) in rhesus monkeys.

In the present study there is decreased sperm count. This is supported by decreased sperm number, postulated in the past 50 years in epidemiological studies (Bendvold *et al*., 1991; Carlsen *et al*., 1992) [15, 16].

Considering the effects of carbaryl on the testis in the present study and based on the findings of earlier studies, this compound may be designated as moderately toxic. This may affect spermatogenesis resulting in the production of decrease number of sperms.

Summary and conclusion

Carbaryl is being used extensively as a broad spectrum pesticide. It is known toxicant to the male reproductive system and, is therefore, under focus in the present study. The results of the present study have thrown some light on the toxic effects of carbaryl on testicular functions that are essential for reproductive success.

The present study was conducted on 40 male Wistar albino rats as experimental animals. The rats were procured from the Animals House of The rats were divided in four groups as normal control group I, group II, group III and group IV. All the rats were group housed and were fed with standard pellet diet and water for two weeks. After two weeks, the rats of group I were left as such and rats of groups II, III and IV were given 50 mg, 100 mg and 200 mg/kg body weight/day of carbaryl drug in 0.2 ml of groundnut oil orally, 6 days/week for 60 days, respectively. After 60 days, all the rats were anaesthetized by keeping them in an inverted glass jar containing large piece of cotton soaked in anaesthetic ether. The testis were dissected out from each rat and were cut into smaller pieces. These pieces were immediately fixed in 10% formalin. The blocks were prepared for section cutting with a microtome by paraffin wax embedding method. The sections of 7 thickness were mounted on glass slides and were stained by H&E and Masson's trichome stain.

The following findings are drawn from the study

1. There is variation in the shape of seminiferous tubules of testis.
2. There is sloughing of the germinal cells from the basement membrane.
3. There is depressed spermatogenesis and loss of sperms.
4. Some tubules are showing accumulation of cellular masses in the lumen of seminiferous tubules of testis.

However, since the study was conducted on experimental animals and results may not be exactly the same in humans, suffering from the carbaryl toxicity. But in no case it can be overlooked, while designing a therapy for pesticides, where it becomes necessary to take into consideration the effects of carbaryl on these tissues of vital importance.

It is concluded that the toxic effects are more pronounced in the peripheral parts of the sections of testis. Moreover, intensity of toxic effects both in peripheral and central parts increases with increase in dosage of the carbaryl drug.

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