



Research Article

SUB CHRONIC AND SUB ACUTE TOXICITY STUDIES OF CARBOFURAN IN WISTAR RAT: APPLICATION TO NEUROBEHAVIOURAL EVALUATION

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ABSTRACT

Carbofuran is a carbamate pesticide used in fields for promoting crop yield as it cuts down the expectancy of pests. It has been contaminating water in the nearby water sources which ultimately drained into water supply of towns and villages. It is a pollutant and is hazardous if consumed directly. We have conducted sub-acute and sub chronic toxicity studies on wistar rat for studying neurobehavioral effects using carbofuran as the key toxicant. The sub-acute studies were carried out daily dosing and experimenting toxicity studies on 7, 14, 21, 28 & 42 days of study and the sub chronic studies were carried out on giving repeated doses for 90 days and studying the toxic effects. The dose was administered in subsequent groups as 1 mg/kg b.wt, 2.5 mg/kg b.wt, 5 mg/kg b.wt dissolved in olive oil respectively. A control group of animal receiving saline was also established to compare the results and evaluate a clear outcome of the effects and toxicities. The neurobehavioural tests were performed using grip strength test, rota rod test, pain threshold, water consumption evaluation and weight evaluation were monitored during the course of experiment. So as to understand the behavior patterns exhibited by the Wistar rats during the course of experiment in comparison to the control group and the treated groups. The extent of damage was being evaluated by studying various doses of the pesticide and three groups were divided which were administered three different doses of Carbofuran drug at different concentrations.

KEYWORDS: Pesticides, Food Toxicity, Neurological Behavior, Pain Threshold, Toxicity

INTRODUCTION

It has been reported that Carbofuran intake causes a lot of toxicities and WHO has reported that the main route take by the chemical to enter our bodies in through the drinking water.

This pesticide has been entering the human body through our lifestyle of consuming the raw vegetable or half cooked meals. The chemical stays with the food we eat and enters the body causing major toxicities therefore producing serious abnormalities¹.

The pesticide Carbofuran is very popularly used in crop fields since 1997 as recorded by WHO¹. It is considered as a hazardous substance when administered orally and is therefore classified as a hazardous chemical by WHO in 2001². The pesticide Carbofuran falls in the category of anticholinesterase Carbamate which is also very popularly used as insecticide and nematicide. It is used enormously and is therefore contaminating water, vegetables, fruits which are targeting the health of all living organisms³. Amongst the major pesticide used, carbamates are commonly used in developed and developing nations for commercial farming as well as for kitchen gardens⁴.

In this research we have focused on the effects of Carbofuran on neuro-behavior of the wistar rat. The sub-acute and chronic studies were performed using the grip Test method, Rota Rod test, and pain threshold and water consumption analysis.

Toxicity

Marine Toxicity

Fishes: It has been reported that the behavioral changes has been observed in fishes when they were exposed to furan contaminated water. They have shown erratic movements, abnormal swimming in slant angles, jerking through occasional convulsions and profuse mucus productions. The Hct content of furan treated fishes was found to be reduced throughout the complete study duration. The fishes were exposed to furan contaminated water and showed major decrease in the RBC count ($P < 0.05$) as well as GOT ($P < 0.05$) in gill activity, functioning of liver and kidney⁵.

The enzymes GPT and GOT are regarded as useful biomarkers to determine the impairment and rupture of cell⁶.

The poisoning caused by pesticide as pollutants through the run-off water from the agricultural grounds is causing toxic effects as well as neurological effects.

In rodents (wistar rats)

The Carbofuran drug was able to generate oxidative stress in the heart of rat when they were treated for 30 days at an interval of 24 hours. This further caused an elevation in the GSH, Total thiols and MDA in the heart tissues. This caused a decrease in the activity of catalase, SOD and GST which further added to the level of oxidative stress. The increase in levels of LDH in rat blood serum was

reported which further marked a leakage in enzymes due to the damage in cardiac cells⁷.

The Hyperlipidemia effect has also been reported in rats when they were exposed to doses of Carbofuran and Carbtap⁸.

OBJECTIVE OF STUDY

The major objective of the study is to find the effect of Carbofuran on brain by studying various behavioral patterns.

This study was helpful to find out the potential damage caused to animals while consuming Carbofuran. We are accessing the neurological damage by accessing parameters for neurological evaluation.

We have also listed out the behavioral patterns of the different groups of rat being fed with regular doses of Carbofuran (variable doses are monitored in different groups) pesticide along with food.

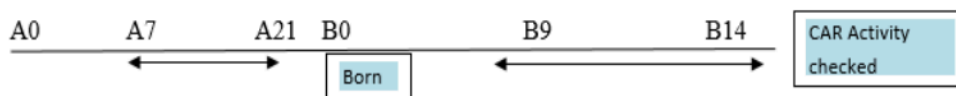
MATERIALS AND METHODS

Work plan

The research work has been designed to study the acute, sub-acute and sub chronic toxicity of the pesticide Carbofuran. The work involved the use of Wistar Rats which were administered doses of Carbofuran at different dose levels in different groups. The toxicities were reported by studying various parameters ruled by four different groups of rats given Carbofuran doses of 0 mg/kg, 1 mg/kg.b.wt, 2.5 mg/kg.b.wt, 5 mg/kg.b.wt respectively.

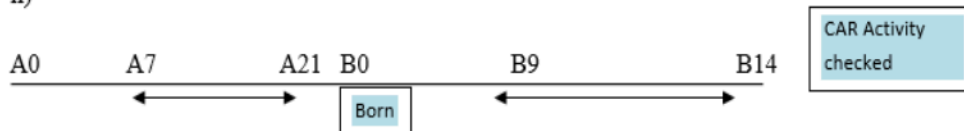
EXPERIMENTAL DESIGN:

i)



Vehicle normal saline, subcutaneously

ii)



0.5ml Carbofuran (1mg/ml), Subcutaneously

Chemicals

We have purchased Carbofuran from Sigma-Aldrich. It was dissolved in olive oil (Figaro) and administered to the rats in different doses in 0.5 ml oil volume. The control group of animals were administered 0.5 ml of saline water during the complete dosing schedule.

Animals

All the animal experiments were conducted as per the instructions of the Committee for the Purpose of Control and Supervision of Experiments on Animals and approved by the Institutional Animal Ethical Committee.

Young adult male (Average weight 200 g) Wistar rats 7–8 weeks old were taken from Animal House in Pranveer Singh Institute of Technology, Approved by Animal Ethical Committee for the Institution, India (CPCSEA/09/1273) and were housed in well ventilated cages under controlled conditions of light and humidity and provided with normal soya bean pellets as food and water ad libitum.

Sub-acute toxicity

Sixteen rats were divided in to 4 groups having 4 animals in each (8 males and 8 females placed in different cages). In the experiment all doses of Carbofuran were administered orally dissolved in olive oil to groups of 16 wistar rats of 180–200 g. The dosage levels were 1 mg/kg.b.wt, 2.5 mg/kg.b.wt and 5 mg/kg.b.wt Carbofuran in olive oil. The control group animals were given saline dose orally for the

complete duration in morning. All number of deaths were recorded. Experiments for sub-acute studies were set up for 7, 14, 21, 28 & 42 days consequently for group II, III and IV.

Proper monitoring of the groups was done by studying the clinical and behavioral symptoms like Motor activity, Tremors, Convulsions, Arching and Rolling.

We further studied the symptoms like Lacrimation, Muscle spasm and the onset of toxicity for the Carbofuran dose was noted.

Our study design also included the proper record of the food consumption for the first and the third day throughout the entire study. The regular intake of water was also being calculated throughout the study for each group. Considering the weight and water consumption the body weight of each animal was also kept tracked and recorded on every day.

Sub chronic toxicity

Groups of 16 rats of 180-200 g were administered with 1 mg/kg.b.wt, 2.5 mg/kg.b.wt and 5 mg/kg.b.wt. in olive oil upto 90 days. The control group received saline daily.

The drug Carbofuran was given through an oral gavage procedure was given for a duration of 90 days. The study involved the proper monitoring and recording of body weight, water and food consumption for each group every third day during the complete research work. The bottles feeding water had markings which were properly noted down for all consecutive days and the pellets dispensed was counted and written in the record data. The body weight was recorded daily and a record of mortality of animals was kept. Neurobehavioral monitoring was performed on every 14 day to keep a record of the neurological behavior. Sixteen Wistar rats were divided into 4 groups having 4 animals in each (8 males and 8 females)

METHODOLOGY

Sub chronic toxicity: Grip Strength Test in Rats

This method was performed for studying the pattern of Neurobehavioral studies for sub chronic toxicity in rats.

The Carbofuran administered animals were showing behavioral changes when they were analyzed by the grip strength test method. The procedure was followed by analyzing the Wistar rat using Rota Rod and Pain Threshold test methods.

These tests pointed out the affected muscular strength due to regular dosage in rodents. The fatigue time was calculated as the time when both the fore limbs and hind limbs lost grip and strength and the animal left the grip on mesh.

This same procedure was repeated for five consecutive days and the fall time was noted for five consecutive days. The average of the fall time was calculated for all groups.

Sub chronic toxicity: Pain Threshold Test in Rats

This test was performed by pinching the root of the tail of Wistar rat by an Artery Clip. This induced pain in the animal and the response time of the animal was noted down for all groups respectively. The animal started biting near the tail location which showed the animal's response to the artery clip. The duration of locking the artery clip and the biting or licking response of the animal was recorded by stop watch as the response time. The same procedure was repeated for five consecutive days and the average response was written down as the final reading.

Sub chronic toxicity: Motor coordination by Rota rod test

The skeletal muscle relaxation was tested for the all the groups on the Rota rod treadmill. All animals from all groups were made to run on the Rota rod tread mill to estimate the time of fall. The start of the movement of the rotating rod was marked as the onset time and the fall of the rat was recorded as the end of the test. This span of time was recorded for all animals for 5 consecutive days and the average time was calculated for each group.

Statistical analysis

The final values of the data are expressed in Mean \pm SD. The statistical analysis was done for all the groups using one way analysis of variance (ANOVA) and the results were compared with Group I (untreated animals).

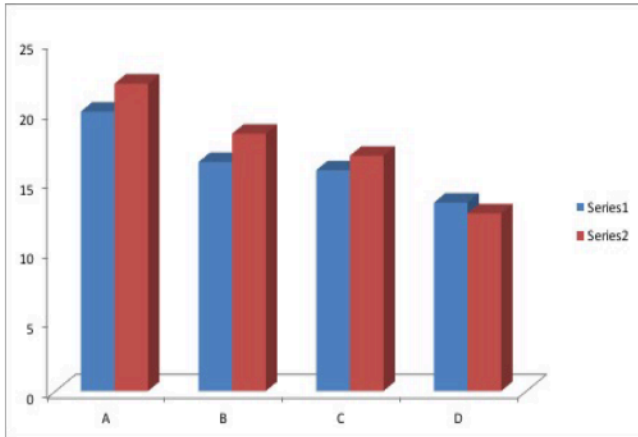


Figure 1: Rota Rod Test (Bar chart showing the female and male wistar rat responses for all groups)
 A = Normal Saline
 B = Carbofuran 1mg/kg body wt
 C = Carbofuran 2.5 mg/kg body wt
 D = Carbofuran 5mg/kg body wt
 Series1 = Female Wistar rats
 Series 2 = Male Wistar rats
 Values are mean \pm SD of 5 animals/sex, $P < 0.01^{**}$

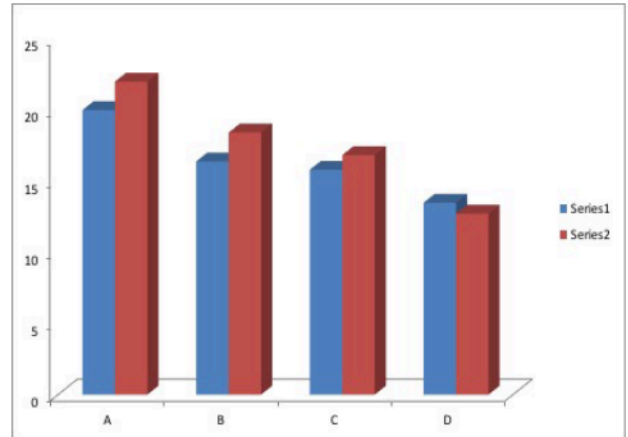


Figure 2: Pain Threshold Test (Bar chart showing the female and male wistar rat responses for all groups)
 A = Normal Saline
 B = Carbofuran 1mg/kg body wt
 C = Carbofuran 2.5 mg/kg body wt
 D = Carbofuran 5mg/kg body wt
 Series1 = Female Wistar rats
 Series 2 = Male Wistar rats
 Values are mean \pm SD of 5 animals/sex, $P < 0.01^{**}$

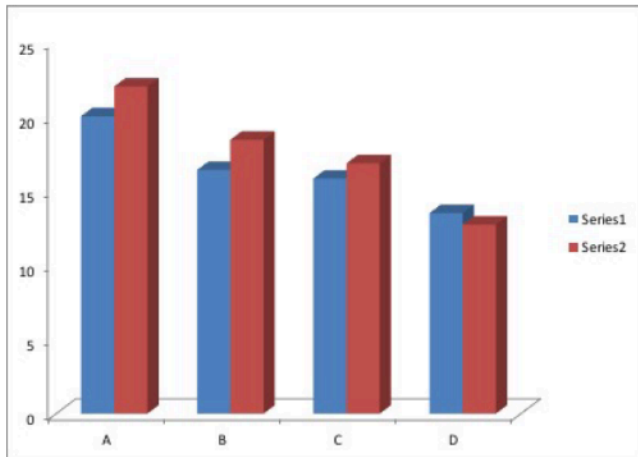


Figure 3: Grip Test Method (Bar chart showing the female and male wistar rat responses for all groups)
 A = Normal Saline
 B = Carbofuran 1mg/kg body wt
 C = Carbofuran 2.5 mg/kg body wt
 D = Carbofuran 5mg/kg body wt
 Series1 = Female Wistar rats
 Series 2 = Male Wistar rats
 Values are mean \pm SD of 5 animals/sex, $P < 0.01^{**}$

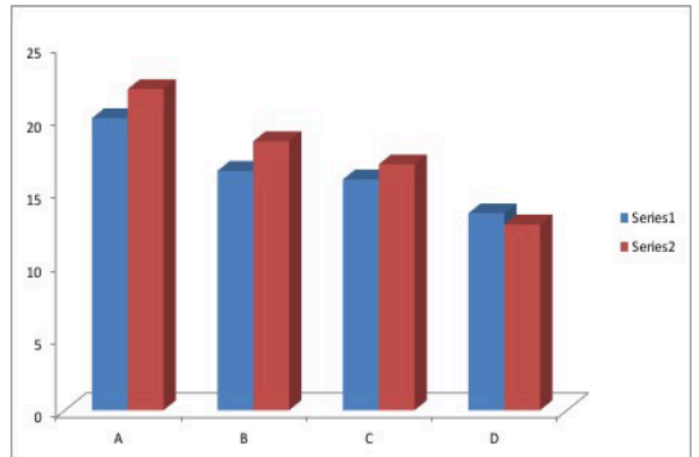


Figure 4: Water Consumption (Bar chart showing the female and male wistar rat responses for all groups)
 A = Normal Saline
 B = Carbofuran 1mg/kg body wt
 C = Carbofuran 2.5 mg/kg body wt
 D = Carbofuran 5mg/kg body wt
 Series1 = Female Wistar rats
 Series 2 = Male Wistar rats
 Values are mean \pm SD of 5 animals/sex, $P < 0.01^{**}$

Table 1: Effect of sub chronic administration of Carbofuran on behavioral study by using Rota Rod test

BEHAVIOURAL TEST	FEMALE (Mean \pm SD)	MALE (Mean \pm SD)
Rota Rod Test		
Normal	12.41 \pm 0.35	13.58 \pm 0.15
Carbofuran 1mg/kg body wt	6.11 \pm 0.21***	6.06 \pm 2.33***
Carbofuran 2.5mg/kg body wt	4.04 \pm 0.62***	4.09 \pm 0.48***
Carbofuran 5mg/kg body wt	3.8 \pm 0.81**	3.70 \pm 0.41***

Table 2: Effect of sub chronic administration of Carbofuran on behavioral study using Pain Threshold Test

BEHAVIOURAL TEST	FEMALE(Mean ± SD)	MALE(Mean ± SD)
Pain Threshold		
Normal	1.36±2.59	1.25±0.68
Carbofuran 1 mg/kg b.wt	3.56±0.88***	4.9±0.45***
Carbofuran 2.5 mg/kg b.wt	5.99±0.47***	5.4±0.11***
Carbofuran 5 mg/kg b.wt	14.78±0.4***	12.5±2.13***

Table 3: Effect of sub chronic administration of Carbofuran on behavioral study using Grip Test Method

BEHAVIOURAL TEST	FEMALE(Mean ± SD)	MALE(Mean ± SD)
Grip method		
Normal	27.67±5.44	29.86±6.74
Carbofuran 1 mg/kg b.wt	17.40±3.86***	18.42±9.88***
Carbofuran 2.5 mg/kg b.wt	13.89±3.69***	16.01±4.63***
Carbofuran 5 mg/kg b.wt	10.56±3.72***	11.47±4.96***

Table 4: Effect of sub chronic administration of Carbofuran on behavioral study using Water Consumption estimation

BEHAVIOURAL TEST	FEMALE(Mean ± SD)	MALE(Mean ± SD)
Water Consumption (in ml)		
Normal	20±5.8	22±8.14
Carbofuran 1 mg/kg b.wt	16.39±2.28***	18.34±2.69***
Carbofuran 2.5 mg/kg b.wt	15.81±3.21***	16.84±7.42***
Carbofuran 5 mg/kg b.wt	13.49±1.32***	12.72±4.82***

RESULTS AND DISCUSSION

Sub chronic toxicity

The general conditions when observed for Carbofuran administered animals was found to show salivation, muscle twitching, tremors in the body and clonic tonic convulsions for a duration of thirty minutes and they persisted for approximately one hour. The data's are shown in the table.

Body weight

The Carbofuran administered animals showed a fall in weight. The major fall was observed in animals with high doses of Carbofuran. They were showing a great difference in weight as compared to the untreated animals with the progressive days.

Food and water consumption

Similarly the food and water consumption was reduced in cases of the Carbofuran treated rats as compared to the untreated animals. Both the male and females rats were observed to show visible food and water consumption. The effect on the water consumption in variable groups is collected and represented in Table 4 and Figure 4 which has shown a progressive decrease in water intake of the rat.

Effect of Carbofuran on grip strength test

The Carbofuran treated animals were showing a proper decrease ($P < 0.01$) in grip strength in case of females as well as males when compared to the untreated animal group. The data's are explained in Table 3 and the comparative study can be easily seen in figure 3.

Behavioral study by Rota rod test

There was a significant impairment in the motor functions of the animal exposed to Carbofuran administration which showed a marked decrease ($P < 0.01$) in the retention time on rotating rod treadmill when compared with the untreated group of animals. A

comparative bar chart is well explained in figure 1 extracted from table 1.

Effect of Carbofuran on pain threshold test

The Carbofuran treated animals showed an immense increase in pain endurance ability ($P < 0.01$) when compared with the treated animals. The data's are well written in table 2 and figure 2.

The popularly used pesticide is being used in many countries as a regular pest control treatment in farms. Despite of the ban because of the toxicities it produces it is majorly being used. Carbofuran has been used in noticeable amounts and is reported to produce toxicities in air we breathe, drinking water, food and vegetables⁹.

The data from Rota rod test revealed that Carbofuran has decreased the behavioral activity of the rat as it was comparatively high in females rats reportedly 12.41 ± 0.35 which was progressively decreased by increasing quantities of Carbofuran and found to be 6.11 ± 0.21 during Carbofuran 1mg/kg body wt administration and 4.04 ± 0.62 for Carbofuran 2.5 mg/kg body wt administration while 3.8 ± 0.81 for Carbofuran 5mg/kg body wt administration. A similar pattern was observed in the male rats when a comparison was drawn from the standard group and the dosing groups.

CONCLUSION

The popularly used pesticide Carbofuran has shown to produce serious organ toxicities. Various researches have pointed out the oxidative stress and organ size reduction due to Carbofuran consumption¹⁰. Therefore we carried forward the experimentation to study the effect of this pesticide on neurological behavior of wistar rat. The results have shown that the wistar rats of control group (both male and female groups) have shown greater water consumption habits, grip strength and pain threshold as compared to the Carbofuran treated groups. The results are suggesting we have a clear interpretation that the neuro-behavior of the rats are being affected after being administered Carbofuran. If the neurological behavior of wistar rats is affected then it is a serious

possibility that the humans will face challenges if they consume crops and vegetables sprayed with Carbofuran.

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