A pharmacological profile of the Auyrvedic Drug Chaturbhuj Ras

This dissertation is submitted to the department of pharmacy, East West University, Mohakhali, Dhaka for the partial fulfillment for the degree of Bachelor of Pharmacy.

East West University
Mohakhali
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Submitted by
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Dedicated to my beloved father and mother

Certificate

This is to certify that, the pharmacological profile of the auyrvedic drug Chaturbhuj Ras (CVR), submitted to the department of pharmacy, East West University Mohakhali Dhaka, for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B Pharm) was carried out by Yamin Tauseef Jahanngir, ID: 2006-1-70-010 under my guidance and supervision and that no part of this thesis had been submitted for any other degree. I further certify that all the sources of information and laboratory facilities availed of this connection is duly acknowledged.

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Abstract

Pharmacology study of Chaturbhuha Ras (CVR) an Ayurvedic preparation was used and experiments were carried out utilizing small laboratory animals. Here different types of studies were done to see different pharmacological effect of CVR. Oral administration of CVR was found to decrease the activity of the animals. The test of Hypoxia is usually carried out to see the hypothermia or hyperthermia effect. In hypoxia test been carried out, survival time has increased without any significant effect. In formalin induced paw licking the drug exerted a mild anti inflammatory effect and showed decrease in analgesic activity compared to control group. The study carried out to see the neuropharmacological effects / side-effects like-open field, hole cross, hole board showed an decrease in ambulatory effect in generally and some of the data were significant. In neuropharmacological studies drug treated mice exerted decrease in emotional defecation.

Acknowledgement

In my one and half year of research I have learnt the methods of carrying out the pharmacology experiments at Jahangirnagar University located at Savar. In this duration of my work I have found several factors to be interesting and my interest has driven me to be more dedicated and devoted towards my work.

First of all I would like to thank the Department of Pharmacy of East West University to provide me with such interesting courses which made pharmacy an important part of my life. I am glad that I had this priviledge to work under our Chairperson of Department of Pharmacy, Dr. Chowdhury Faiz Hossain and would like to thank him for his relentless efforts and valued advices, which helped me to get this far of the journey. His contribution to this research paper was undoubtedly of high appreciation.

I would also like to take this opportunity to thank the Dean of Pharmacology in the Department of Pharmacy at Jahangirnagar University Dr. Shabuddin K. Chaowdhury, for providing all the facilities required to carry out the experiments successfully and in favourable conditions as well.

I would like to thank my friends and family who has supported me in every step of my life and especially in making this paper. They have been my strength and I am glad to have them by my side always.

Lastly, I would like to thank Allah, for His blessings and giving me hope and courage to perform and as I had faith in Him, the paths got better as I start to walk on it.

I hope this research work will help the future students to know a great deal about Pharmacology.

Thank you,
Yamin Tauseef Jahangir

Terms used in the research

CVR	Chaturbhuj Ras
mg	milligram
Kg	kilogram
Ctrl	Control
Min	Minutes
t	mean value
p	standard error

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Chapter - 1

Introduction

1.1 Historical Background

Natural products research continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry. While microbial products have been the mainstay of industrial natural products discovery, in recent years phytochemistry has again become a field of active interest. Drug discovery programmes based on microbial products and phytochemicals have been discussed and contrasted. The exploration of structural chemical databases comprising a wide variety of chemotypes, in conjunction with databases on target genes and proteins, will facilitate the creation of new chemical entities through computational molecular modelling for pharmacological evaluation.

In natural products drug discovery it is important to follow systems-theory and systems-biology applications to facilitate the process. Routine random efforts are not likely to increase the desired success rate of discovery, while experience indicates that a modified collection policy offered better chances for the discovery and development
of agents for treatment of AIDS and cancer. Numerous drugs have entered the international pharmacopoeia through ethnobotany and traditional medicine. There are many similarities in traditional systems of medicine as well as ethnomedicines being connected to each other as 'great traditions and little traditions'. All botanical drugs will have to fulfil the international requirements on quality, safety and efficacy.

1.2 Ayurveda- the ancient science of life

Ayurveda remains one of the most ancient and yet living traditions practised widely in India, Sri Lanka and other countries and has a sound philosophical and experiential basis. Atharvaveda (around 1200 BC), Charak Samhita and Sushrut Samhita26 (1000–500 BC) are the main classics that give detailed descriptions of over 700 herbs.

A scholarly description of the legacy of Caraka in contemporary idiom, best attempted with a commentary from modern medicine and science viewpoint, gives some glimpses of ancient wisdom. Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions. India has about 45,000 plant species; medicinal properties have been assigned to several thousands. About 2000 are found in the literature; indigenous systems commonly employ about 500–700. Some recent work in drug development relates to species of Commiphora (used as a hypolipidaemic agent), Picrorhiza (which is hepatoprotective), Bacopa (memory enhancer), Curcuma (antiinflammatory) and Asclepias (cardiotonic). This is based upon centuries old observation, rich in traditional wisdom and with its own strong basic principles and philosophy as its skeleton and body. As per ayurvedic concepts, every material of earth is made up of 5 basic elements, which are prithvi (earth), jal (water), tej (fire), vayu (air), aakash (space). This is true for both plants as well as human beings providing their interface.

1.3 Safety

Aims and objectives of Ayurveda are:

1. To preserve and promote the health of a healthy person

2. To alleviate the disease in a patient.

Ayurveda, therefore, followed a holistic approach to tackle any day-to-day health promotive, protective and disease related issues. The traditional usage and wide ranging concurrent usage through out India by millions of people on daily basis there is a perfect case of providing Ayurvedic medicines a status of Generally Recognized As safe (GRAS). This is especially applicable to the pure herbal formulations. Strict compliance of GMP should be able to take care the safety of metallic, mineral or their combination products by monitoring stringently for application of textual methods for manufacturing these medicines. In certain cases, however, there is a scope of more stringent regulatory guidelines to conduct toxicity studies of Ayurvedic products, though such instances may be few. Lack of such data, however, should not be used as non-tariff barrier in trade by any country.

1.4 Tradirtional use

Ayurvedic medicines have been traditionally used for thousands of years in India. In 1998 as per statistics of Govt. of India, there were 609,400 Physicians of Indian Systems of Medicine and Homeopathy in India out of which, more than half belong to Ayurveda stream. Global resurgence of Ayurveda specially its herbal component has led to the need of its scientific validation both in terms of efficacy and safety. Few recent events published in international journals have refocused the attention on safety aspects of Ayurvedic products. About 80% of the population in India depends on traditional medicine. Out of which almost 70-75% depend on Ayurvedic medicines in one form or the other. That means if approx. 2,50,000 Ayurvedic physicians see on an average 10 patients per day it converts to 2.5 m patients per day. Almost equal number of people does not go to physician and use these medicines on their own. That means, almost 5 m people use Ayurvedic medicines on daily basis in India. Forth estate enjoys full freedom in India and even then media reported incidents of side effects are almost nil. This is the best evidence of safety of Ayurvedic medicine going by their traditional usage pattern.

1.6 Formulation

CHATURBHUJA RAS

(Rasendrasarasangraha, Unmadacikitasa, 20-21½.)

1. Mrta suta (rasa sindura)	2 parts.
2. Hema (svarna) bhasma	1 part.
3. Sila (suddha manahsila)	1 part
4. Kasturika	1 part
5. Tala (rasa manikya)	1 part.
6. Kanya (kumari svarasa)	Q.S. (for mardana)
7. Eranda patra (Lf.)	Q.S. (for avestana)

Special method of preparation

After covering with leaves of eranda it is to be kept with in a heap of dhanya (grains) for 3 days.

Dosage

125 mg.

Anupana

triphala kvatha, honey, brahmi svarasa.

Important therapeutic use

jvara *(pyrexia)*; sannipatajvara *(typhoid fever)*; dhatuksaya *(neurasthenia, impairment of memory, impotency)*; sirahkampa *(shaky head)*; apasmara *(epilepsy)*; unmada *(insanity)*; paksaghata *(haemiplegia, paralysis)*.

Chapter - 2

Statement of Purpose

2.1 Aims of Study

The main objective of this study includes:

- To find out the hypothermia or hyperthermia test of CVR by performing the Hypoxia test.
- To determine the analgesic and anti inflammatory studies of CVR by performing Formalin Induced Paw Licking test.
- To find out neuropharmacological effect of CVR by doing the Hole Cross test, Hole Board test and Open Field test.
- To monitor the psychopharmacological effect of CVR by performing the Climbing Out test.

Chapter - 3

Materials & Methods

MATERIALS AND METHOD

For the pharmacological study, CHATURBHUJA RAS (CVR) was collected from Sree Kundeswari Aushadhalaya Ltd, Chittagong, Bangladesh.

3.1. Doses Used In Different Experiments

Serial no	Name of the experiment	Doses
1	Hypoxia Test	100mg/kg body weight
2	Formalin Test	100mg/kg body weight
3	Hole Cross Test	100, 200, 400 mg/kg body weight
4	Hole Board Test	100, 200, 400 mg/kg body weight
5	Open Field Test	100, 200, 400 mg/kg body weight

3.2 Administration of Drug

For the pharmacological experiment, the powdered tablets were made into a solution using distilled water and administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For all the pharmacological studies the drugs were administered per oral route at a dose of 100mg/kg body weight.

3.3 Experimental Animals

Male and Female mice (Swiss-Webster strain, 20-40 gm body weight) bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiments. They were kept in cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages.

The animals were provided with standard laboratory food and tap water '*ad libitum*' and maintained at natural day night cycle. They were fed with "mouse chow" (prepared according to the formula developed at BCSIR, Dhaka).

Before starting an experiment the animals were carefully marked on different parts of their body, which was later used as identification mark for a particular animal, so that the response of a particular mouse prior to and after the administration could be noted separately.

3.4 Controls

Two groups of equal number of mice were simultaneously employed in the experiment. Six to ten mice were taken for each group for both the control and the experiment group. As the drug treated group, other group is treated with distilled water and this group served as the control.

3.5 PHARMACOLOGICAL STUDY WITH ANIMALMODELS:

3.5.1 Hole Cross Test:

In this experiment, the method of Takagi et al (1971) was employed. In a box having dimension of 30 X 20 X 14 cm, a hole of 3 cm in diameter at a height of 4.5 cm from the floor was constructed on the dividing wall. Spontaneous movement of the animals through the hole from one chamber to the other was counted for a period of 2 minutes. The observation was conducted 30, 60, 120 and 240 minutes after oral administration of test drugs and was compared with control animal administered with normal saline. (Takagi *et al* 1971). [9]

3.5.2 Hole Board Test:

The hole-board test has been conceived to study the behavior of the mouse confronted with a new environment (head plunging stereotype) according to the method devised by Boissier and Simon in 1964, Boissier, Simon and Lwoff in 1964 and Boissier and Simon in 1967. [1]

This experiment was carried out by the following method of Nakama et al, 1972. [5] A total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters. Each of the animal was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of fecal boluses excretion was recorded for a period of 2 minutes at pre 30 minutes and post 30, 60, 120 and 240 minutes intervals and were compared with the control animals administered with distilled water (Nakama et al)

3.5.3 Formalin Test:

Formalin 1% was administered to mice and immediately the licking time was registered for 5 min (first phase, neurogenic). Twenty minutes after the beginning of the experiment (second phase, inflammatory) the licking time was registered for other 5 min. Experimental drug was administered 60 min (p.o.) before the formalin injection. (Tjolsen et al., 1992). [7]

3.5.4 Open Field Test:

In this experiment, the method of Gupta (1971) was employed. The floor of an open field of half square meter was divided in to a series of squares, each alternatively colored black and white. The apparatus had a wall of 40 cm. The number of squares, traveled by the animal, was recorded for a period of two minutes. The open field test is designed to measure behavioral responses such as locomotor activity, hyperactivity, and exploratory behaviors. Open field is also used as a measure of anxiety. (Gupta et al 1971). [8]

3.5.5 Hypoxia Test:

Three set of ten mice per groups were used. 2 hr after the treatment, the hypoxia time was recorded individually for all the animals. The animals were placed in an empty glass jar of 300 mL capacity jar and the jars were made air tight with greased glass stoppers and the time until the onset of convulsion was recorded.

Caillard C was described this model to test hypoxia of some anticonvulsant drug in 1975. [4]

3.5.6 Statistical Analysis

Data were presented as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS™ (Ver. 14) was applied for the analysis of data. $p = 0.05$ was taken to be the level of significance, $p = 0.01$ was taken to be the level of highly significance, $p = 0.001$ was taken to be the level of very highly significance.

P-value determines the appropriateness of rejecting the null hypothesis in a hypothesis test. P-values range from 0 to 1.

Chapter -4

Result & Discussion

4.1 HYPOXIA TEST:

This experiment was designed to determine the drug's property to modify the survival time of mice under conditions of hypoxia. The hypoxia induced convulsion onset time is inversely proportionate to the brain oxygen demand. The experimental results are analyzed below:

Statistical Finding:

At dose 100mg/Kg, CVR treated male mice slightly increased the survival time compare with the control group. But the result was not statistically significant.

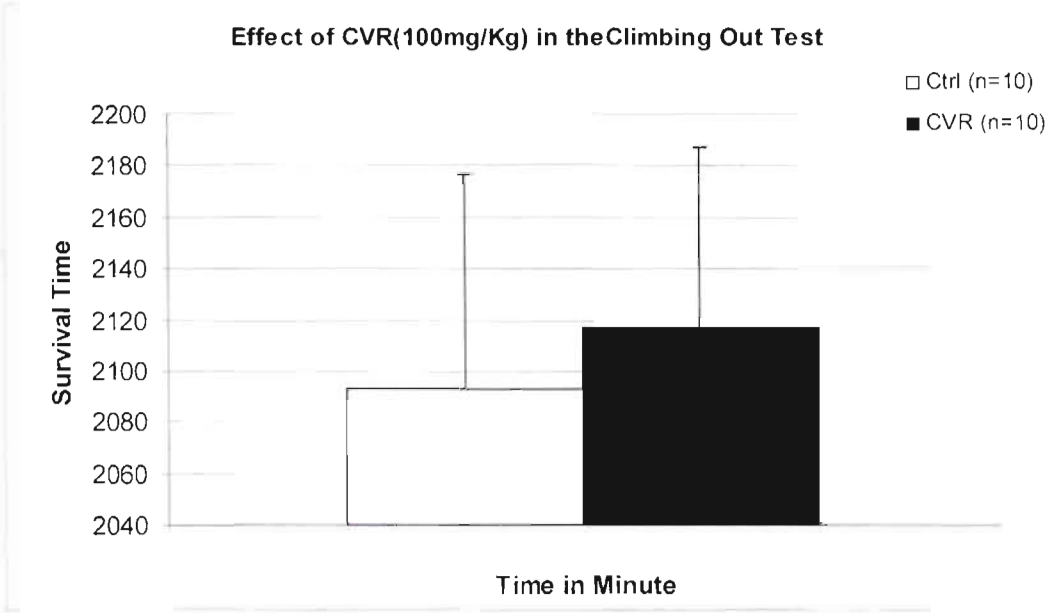
4.1 Tabular and Graphical presentation of the effect of CVR (100mg/kg) on the Hypoxia Test utilizing Male mice.

Table: 4.1. The effect of CVR (100mg/kg) in the Hypoxia Test.

Group		Survival Time(sec)
Ctrl(n=10)		2092.90 ± 74.176
CVR(n=10)		2116.80 ± 70.337
t/p		-0.003/0.998
95% confidence interval	Lower	-215.060
	Upper	214.460

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 4.1. Graphical Presentation of the effect of CVR (100mg/kg) in Hypoxia Test.



4.2. FORMALIN INDUCED PAW LICKING (ANALGESIC + INFLAMMATION) TEST:

The formalin pain test is very useful for evaluating the mechanism of pain and analgesia (Tjolsen et al., 1992). Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of pain in this model while peripherally acting drugs such as aspirin are indomethacin, only inhibit the late phase. (Santos et al., 1994).

Statistical Finding:

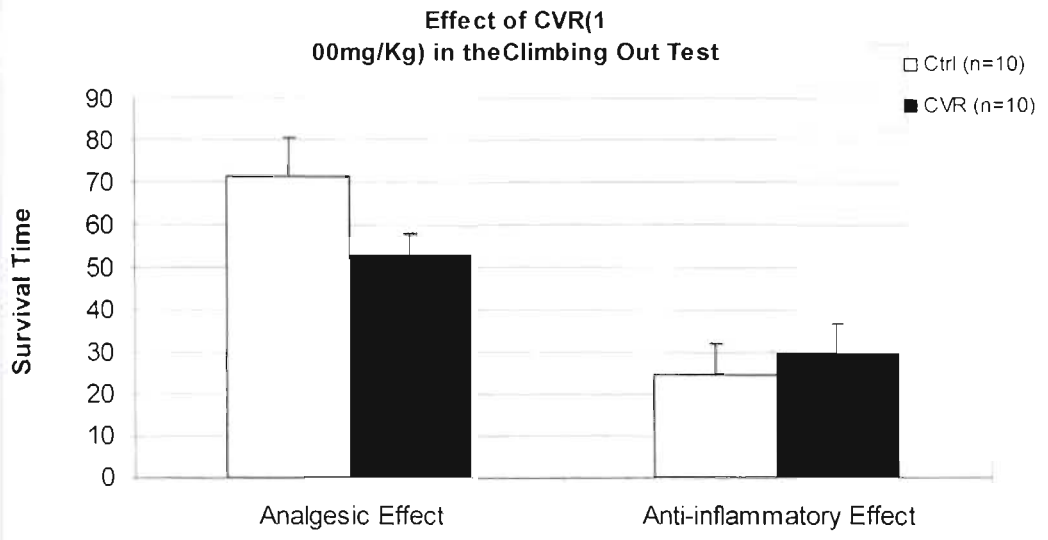
CVR at dose 100mg /kg exerted an decrease in analgesic activity and very mildly exerted anti- inflammatory activity in male mice compared to the respective control group but none of the results were statistically significant.

4.2. Tabular presentation of the effect of CVR(100mg/kg) on the Formalin Induced Paw licking (Analgesic + Inflammation) Test utilizing Male mice.

Group		Analgesic (1st Phase)	Inflammation (2nd Phase)
Ctrl(n=10)		71.10±9.379	24.50±7.403
CVR(n=10)		53.00±5.099	29.60±7.142
t/p		1.695/0.107	-0.496/0.626
95% confidence interval	Lower	-4.328	-26.711
	Upper	40.528	16.511

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 4.2. Graphical Presentation of the effect of CVR (100mg /Kg) in the Formalin Induced Paw licking (Analgesic) Test and Anti- Inflammatory Effect.



4.3 HOLE CROSS TEST:

As spontaneous movements of the animals include, by definition, both the propulsive and non-propulsive movements of the animal, and as the fluctuating and multifarious nature of many overt movements patterns impossible, to accurately measure the effects of a drug on the spontaneous motor activity of animals by using a single experimental procedure, the hole cross test was performed (Robbing, 1977).

Statistical finding:

CVR treated female mice at three dose levels (100 mg/kg, 200 mg/kg, and 400 mg /Kg) exerted overall mixed response in hole cross activity.

At dose of 400mg /Kg at min 120 ($p=0.046^*$) the result of increase was found to be statistically significant. Although there was difference in the response none of the other results were statistically significantly different from the corresponding control animals.

4.1 Tabular and Graphical presentation of the effect of CVR (100 mg/kg, 200 mg/kg, 400 mg/kg) on the Hole Cross Test utilizing Female mice.

Table: 4.1 The effect of CVR (100mg/kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		1.171 ± 0.477	1.000 ± 0.365	1.67 ± 0.919	1.00 ± 0.516	0.50 ± 0.342	1.17 ± 0.477
CVR(n=6)		1.000 ± 0.516	1.33 ± 0.615	1.17 ± 0.543	0.50 ± 0.342	1.000 ± 0.632	0.33 ± 0.211
t/p		0.237/ 0.817	-0.466/ 0.653	0.469/ 0.649	-0.808/ 0.438	-0.696/ 0.503	1.597/ 0.141
95% confidence interval	Lower	-1.400	-1.977	-1.878	-0.880	-2.102	-0.329
	Upper	1.733	1.310	2.878	1.880	1.102	1.996

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 4.1: Graphical Presentation of the effect of CVR (100mg/Kg) in Hole Cross Test

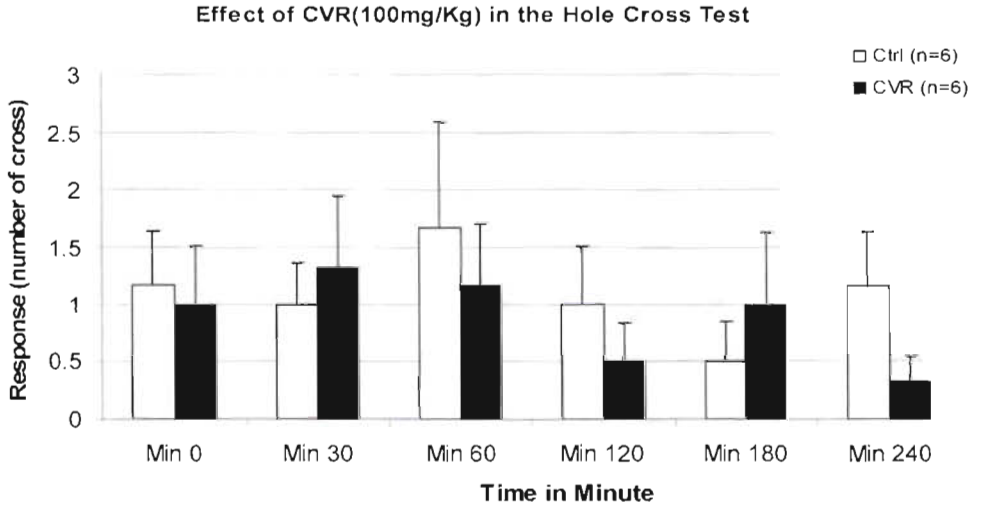


Table: 4.2. The effect of CVR (200mg/kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		3.33 ± 0.558	3.50 ± 1.285	2.17 ± 0.910	2.17 ± 0.792	2.00 ± 0.632	2.67 ± 0.715
CVR(n=6)		4.83 ± 1.990	4.33 ± 1.202	3.67 ± 0.667	2.17 ± 0.703	1.50 ± 0.619	1.17 ± 0.477
t/p		-0.726/ 0.496	-0.474/ 0.646	-1.330/ 0.213	0.00/ 1.00	0.565/ 0.585	1.745/ 0.112
95% confidence interval	Lower	-6.605	-4.753	-4.013	-2.360	-1.472	-0.415
	Upper	3.605	3.086	1.013	2.360	2.472	3.415

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 4.2. Graphical Presentation of the effect of CVR (200mg/Kg) in Hole Cross Test.

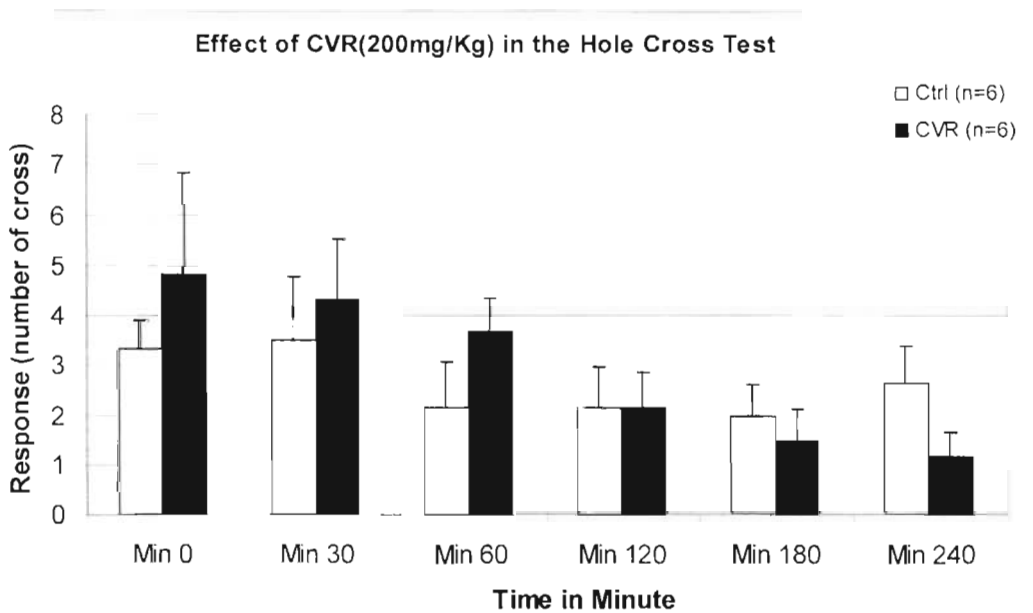
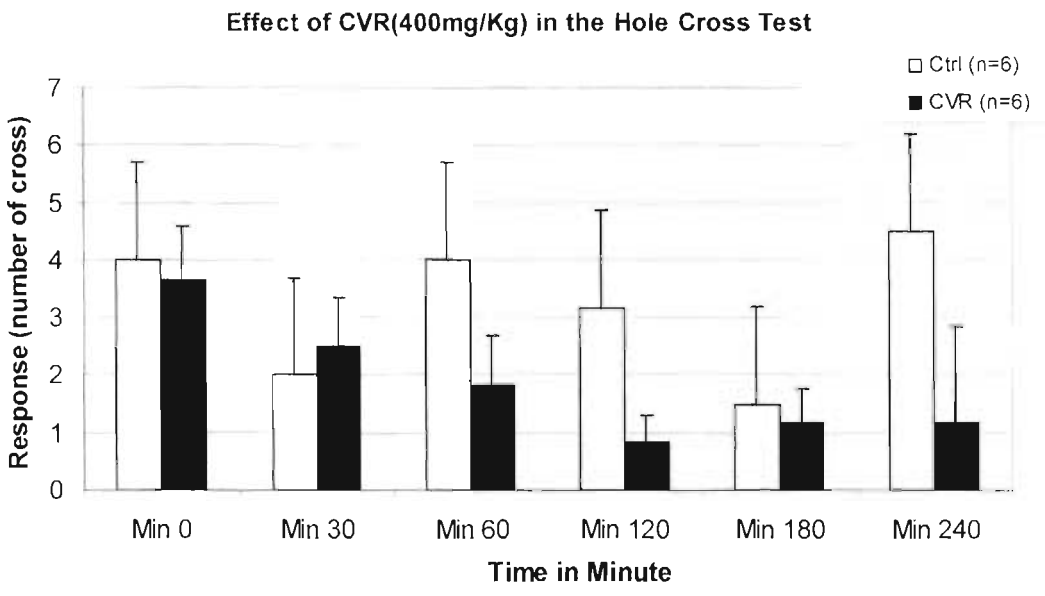


Table: 4.3. The effect of CVR (400mg /kg) in the Hole Cross Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		4.00 ± 1.693	2.00 ± 1.238	4.00 ± 1.506	3.17 ± 0.910	1.50 ± 0.671	4.50 ± 2.566
CVR(n=6)		3.67 ± 0.919	2.50 ± 0.847	1.83 ± 0.872	0.83 ± 0.477	1.17 ± 0.601	1.17 ± 0.477
t/p		0.173/ 0.866	-0.333/ 0.746	1.245/ 0.241	2.271/ **0.046***	0.370/ 0.719	1.277/ 0.230
95% confidence interval	Lower	-3.959	-3.482	-1.710	0.044	-1.673	-2.482
	Upper	4.626	2.842	6.044	4.623	2.340	9.148

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 4.3: Graphical Presentation of the effect of CVR (400mg/Kg) in Hole Cross Test.



4.4 HOLE-BOARD TEST

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior characterized by spontaneous ambulatory activity, exploratory activity and emotional defecation of the animals. This experiment presents with a different and more complex environment to explore. For this experiment female mice weight range 25- 30 g was used.

Statistical Finding:

Ambulation

CVR treated group, at dose 100 mg/Kg and 200 mg/Kg, showed an overall increase in ambulatory activity in all through out the experimental study period when compared to the corresponding control group. But none of the results were statistically significant. The exception when treated with dose 100 mg/kg at min 30 *(p=0.057*)* when decrease was noted at statically significant level.

At dose 200mg/Kg, CVR group exerted an increase in ambulatory activity at min 30, min 120 and min 180. But interestingly ambulatory activity decreased at min 240.

But, none of these effects of ambulation was statistically significant.

Head Dipping

At dose 100mg/Kg, CVR treated group showed an overall increase in head dipping activity with few exceptions in the 200mg/Kg and 400mg/Kg in all through out the experimental study period when compared to the corresponding control group.

At dose 100mg/Kg, there was an overall increasing effect. The results were not statistically significant.

On the contrary, at dose 200 mg/kg, CVR treated group showed a similar increasing effect in head dipping activity through the experimental period compare with the control group but there were exceptions at min 0 and min 240. Min 0 showed no activity with the drug group whereas in min 240 the activity decreased.
However, none one of the results were statistically significant.
At dose 400mg/Kg the activity began to decrease at min 60, min 120, min 180 and min 240. The result was not statically significant.

Emotional Defecation

At dose 100mg/Kg CVR treated group showed an increase in emotional defecation in the experimental study period and exception included no change in activity at min 60 but activity decreased at min 120 and min 240 when compared to the corresponding control group. But at a higher dose of 200 mg/Kg, CVR treated group showed an overall decreasing effect of emotional defecation and increase only occurred in min 30 and min 240 whereas in min 180 no activity was observed. None of the results were statistically significant.

On the contrary, at dose 400 mg/Kg, CVR treated group showed an unchanged effect in emotional defecation from min 30, min 120 and min 240. But the noticeable change was at min 180 when there was very noticeable increase in emotional defecation. But none of the results were significant statically.

4.4 Tabular and Graphical presentation of the effect of CVR (100 mg/kg, 200 mg/kg, 400 mg/kg) on the Hole Board Test utilizing Male mice.

Table: 4.4 The effect of CVR (100 mg/kg) in the Ambulation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		20.50 ± 6.152	18.50 ± 6.692	20.17 ± 6.730	26.67 ± 7.069	17.50 ± 5.104	12.17 ± 5.486
CVR(n=6)		40.67 ± 8.921	43.67 ± 9.597	36.17 ± 4.771	31.83 ± 4.564	22.00 ± 1.155	17.33 ± 3.323
t/p		1.861/ 0.092	2.151/ **0.057***	1.939/ 0.081	0.614/ 0.553	-0.86/ 0.426	-0.806/ 0.439
95% confidence interval	lower	-44.312	-51.236	-34.381	-23.916	-17.585	-19.458
	Upper	3.978	0.903	2.381	15.582	8.585	9.125

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.4. Graphical Presentation of the effect of CVR (100 mg/Kg) in Ambulation of Hole Board Test

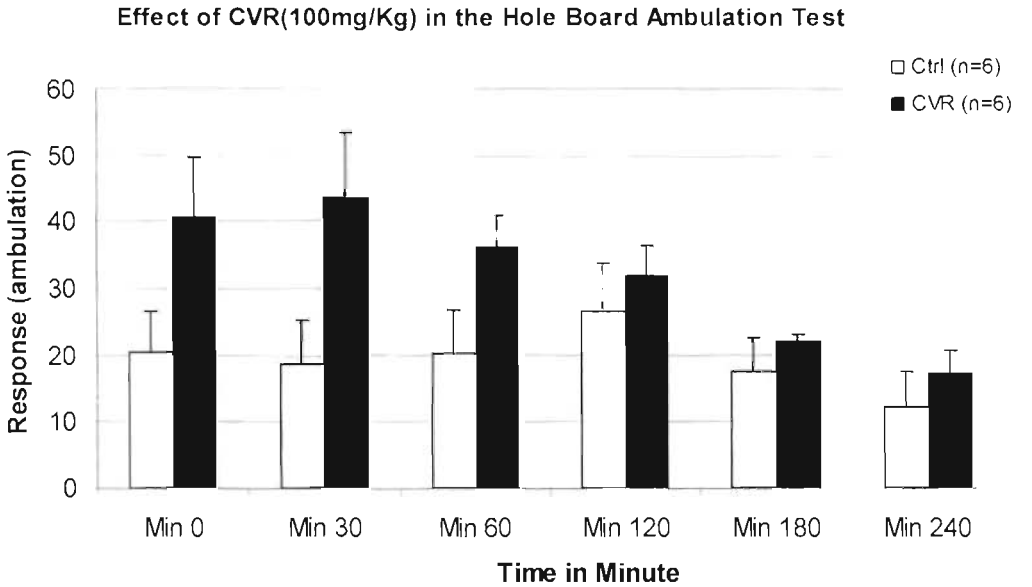


Table: 4.5. The effect of CVR (200 mg/kg) in the Ambulation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min 180	Min240
Ctrl(n=6)		20.33 ± 7.482	20.50 ± 8.831	15.17 ± 4.061	23.67 ± 9.193	20.67 ± 7.915	18.17 ± 6.306
CVR(n=6)		22.17 ± 6.680	28.50 ± 10.132	32.33 ± 8.237	28.00 ± 10.829	22.00 ± 10.162	11.17 ± 3.135
t/p		-0.183/ 0.859	-0.595/ 0.565	-1.869/ 0.091	-0.305/ 0.767	-0.104/ 0.920	0.994/ 0.344
95% confidence interval	lower	-24.182	-37.946	-37.629	-35.984	-30.033	-8.690
	Upper	20.515	21.946	3.296	27.317	27.367	22.690

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.5. Graphical Presentation of the effect of RR (200 mg/Kg) in Ambulation of Hole Board Test

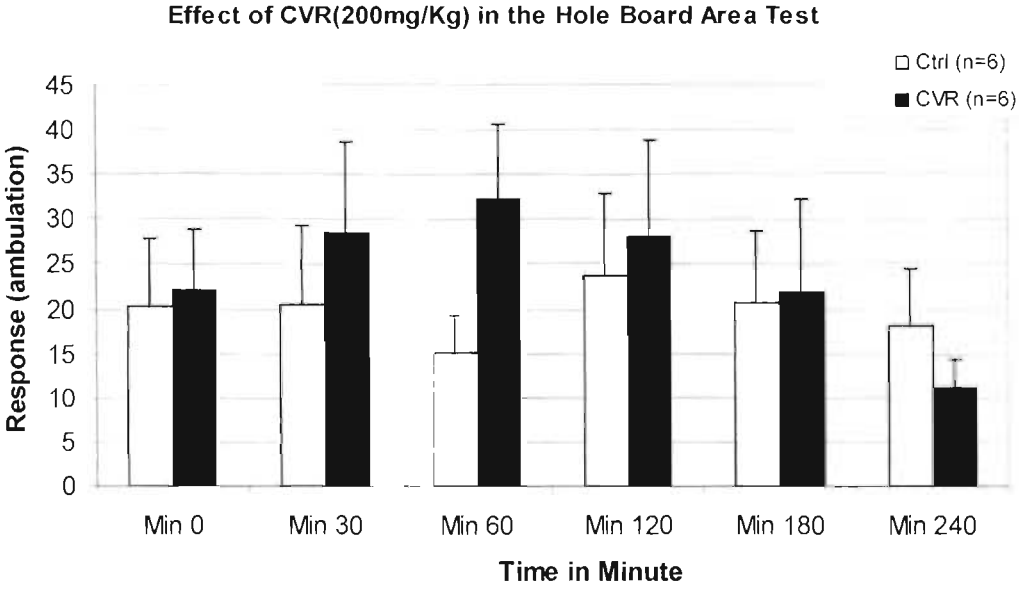


Table: 4.6. The effect of CVR (400 mg/kg) in the Ambulation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		20.50 ± 4.766	20.33 ± 3.490	23.83 ± 3.600	22.17 ± 3.005	22.83 ± 3.250	28.50 ± 2.604
CVR(n=6)		14.00 ± 3.367	21.83 ± 2.428	31.50 ± 2.977	23.00 ± 1.862	27.00 ± 3.152	31.17 ± 2.535
t/p		1.114/ 0.291	-0.353/ 0.732	-1.637/ 0.133	-0.236/ 0.818	-0.920/ 0.379	-0.734/ 0.480
95% confidence interval	lower	-6.502	-10.972	-18.104	-8.709	-14.254	-10.765
	Upper	19.502	7.972	2.771	7.043	5.920	5.432

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 04.3: Graphical Presentation of the effect of CVR (400 mg/Kg) in Ambulation of Hole Board Test.

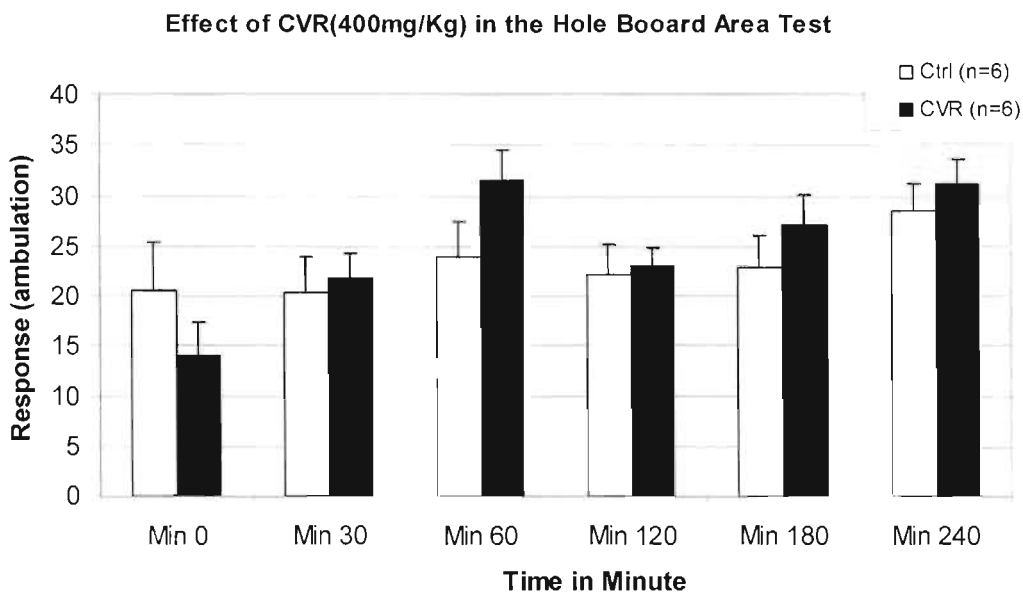


Table: 4.7. The effect of CVR (100 mg/kg) in the Head dipping of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		3.00 ± 2.017	5.67 ± 4.112	3.67 ± 1.520	1.50 ± 0.719	2.50 ± 0.764	1.33 ± 0.494
CVR(n=6)		4.17 ± 1.740	4.67 ± 3.499	6.50 ± 2.110	5.50 ± 2.729	7.67 ± 3.148	4.33 ± 2.140
t/p		0.438/ 0.671	0.185/ 0.857	-1.090/ 0.301	-1.417/ 0.209	-1.595/ 0.142	-1.366/ 0.202
95% confidence interval	lower	-7.101	-11.031	-8.627	-10.999	-12.385	-7.893
	Upper	4.768	13.031	2.960	2.999	2.051	1.893

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.7. Graphical Presentation of the effect of CVR (100 mg/Kg) in Head dipping of Hole Board Test.

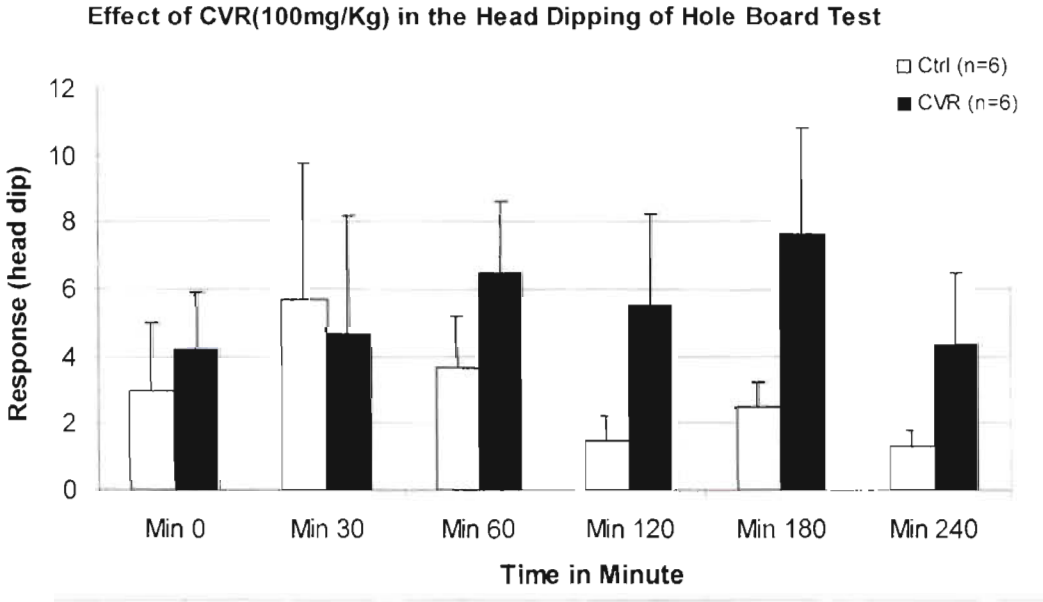


Table: 4.8. The effect of CVR (200 mg/kg) in the Head dipping of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		0.33 ± 0.333	0.17 ± 0.167	1.83 ± 0.601	0.83 ± 0.833	0.17 ± 0.167	1.33 ± 0.667
CVR(n=6)		0.00 ± 0.00	1.83 ± 1.138	2.50 ± 1.118	1.00 ± 0.632	0.33 ± 0.211	0.33 ± 0.211
t/p		1.000/ 0.363	-1.449/ 0.205	-0.525/ 0.611	-0.159/ 0.877	-0.620/ 0.549	1.430/ 0.203
95% confidence interval	lower	-0.524	-4.586	-3.495	-2.498	-0.765	-0.712
	Upper	1.190	1.253	2.161	2.164	0.432	2.712

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.8. Graphical Presentation of the effect of CVR (200 mg/Kg) in Head dipping of Hole Board Test.

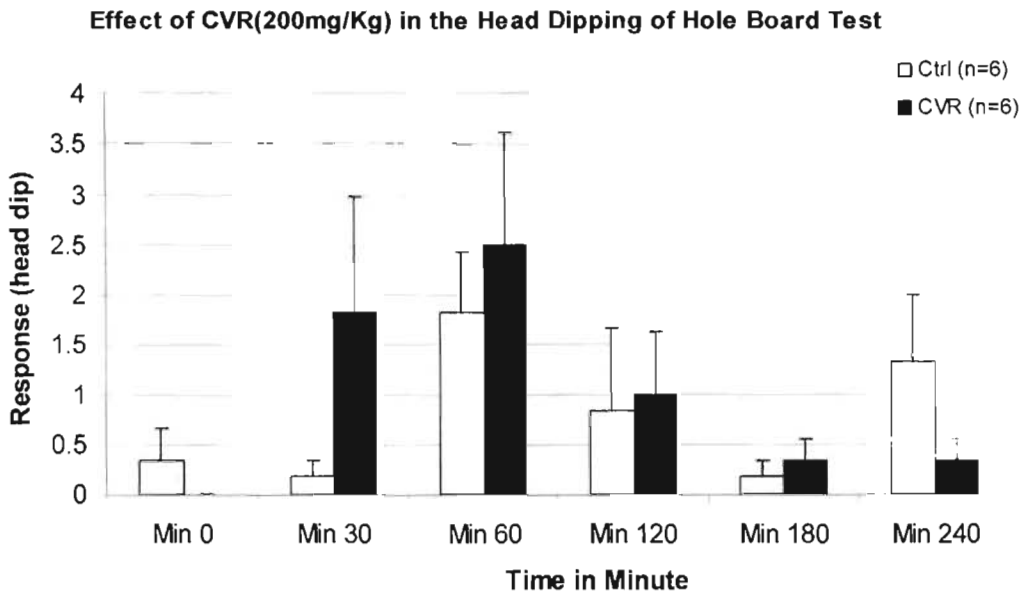


Table: 4.9. The effect of CVR (400 mg/kg) in the Head dipping of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		3.00 ± 1.033	3.00 ± 1.211	4.50 ± 1.607	5.00 ± 1.826	5.00 ± 1.125	4.83 ± 0.703
CVR(n=6)		4.17 ± 1.537	4.67 ± 1.282	3.83 ± 0.401	2.17 ± 0.601	2.33 ± 0.843	3.00 ± 1.065
t/p		-0.630/ 0.543	-0.945/ 0.367	0.402/ 0.702	1.474/ 0.190	1.896/ 0.087	1.437/ 0.181
95% confidence interval	lower	-5.292	-5.597	-3.454	-1.857	-0.467	-1.009
	Upper	2.595	2.263	4.787	7.523	5.800	4.676

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.9. Graphical Presentation of the effect of CVR (400 mg/Kg) in Head dipping of Hole Board Test

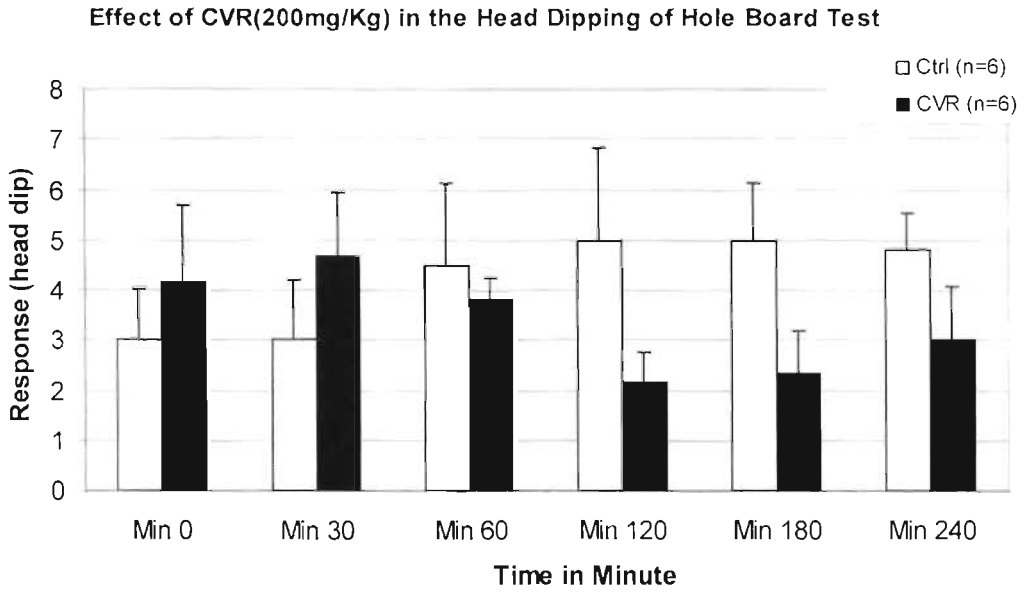


Table: 4.10. The effect of CVR (100 mg/kg) in the Defecation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		1.17 ± 0.543	0.67 ± 0.333	0.67 ± 0.422	0.50 ± 0.224	0.17 ± 0.167	0.83 ± 0.307
CVR(n=6)		1.33 ± 0.715	1.17 ± 0.477	0.67 ± 0.333	0.33 ± 0.211	0.50 ± 0.342	0.33 ± 0.211
t/p		-0.186/ 0.856	-0.859/ 0.411	0.000/ 1.000	0.542/ 0.599	-0.877/ 0.401	1.342/ 0.209
95% confidence interval	lower	-2.166	-1.797	-1.198	-0.518	-1.180	-0.330
	Upper	1.833	0.797	1.198	0.851	0.513	1.330

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.10. Graphical Presentation of the effect of CVR (100 mg/Kg) in Defecation of Hole Board Test.

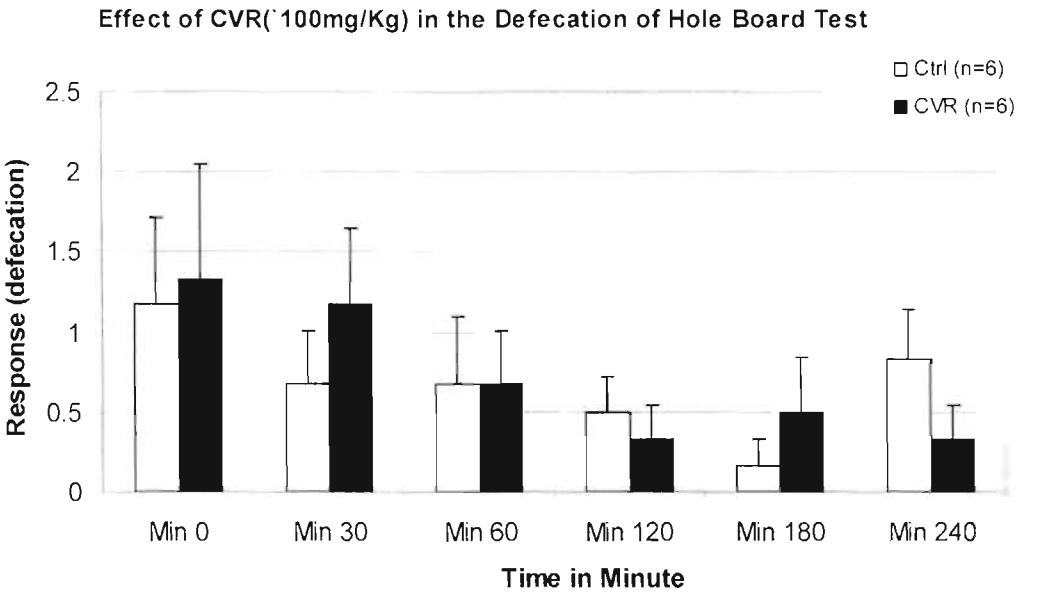


Table: 4.11. The effect of CVR (200 mg/kg) in the Defecation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		2.33 ± 0.760	0.50 ± 0.342	0.83 ± 0.307	0.50 ± 0.224	0.33 ± 0.211	0.17 ± 0.167
CVR(n=6)		0.17 ± 0.167	2.00 ± 0.577	0.33 ± 0.211	0.33 ± 0.333	0.00 ± 0.00	0.33 ± 0.333
t/p		2.784/ 0.035	-2.236/ 0.049	1.342/ 0.209	0.415/ 0.687	1.581/ 0.175	0.447/ 0.664
95% confidence interval	lower	0.218	-2.995	-0.330	-0.728	-0.209	-0.997
	Upper	4.115	-0.005	1.330	1.061	0.875	0.664

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.11.Graphical Presentation of the effect of CVR (200 mg/Kg) in Defecation of Hole Board Test.

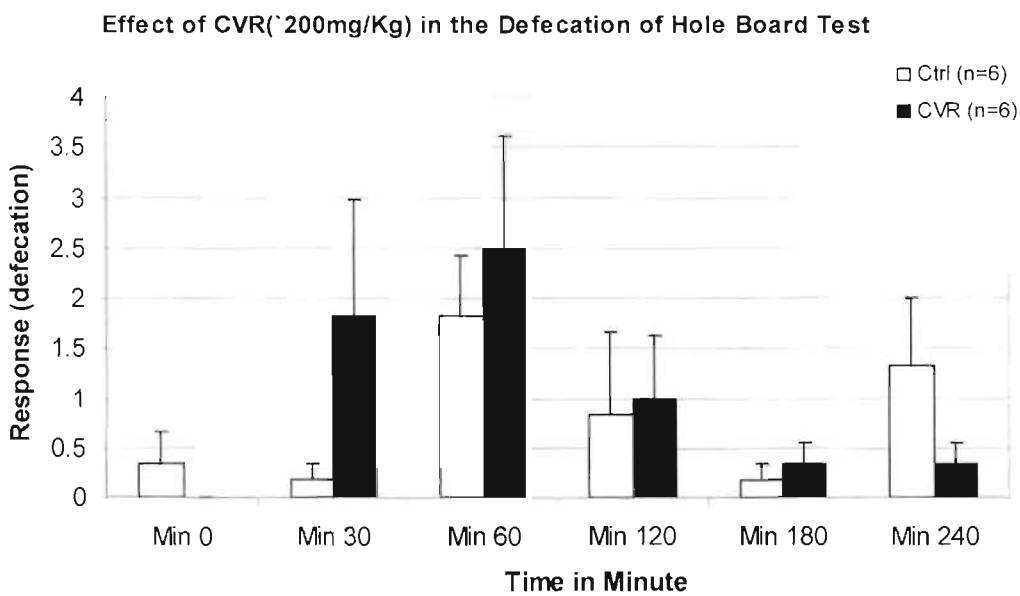
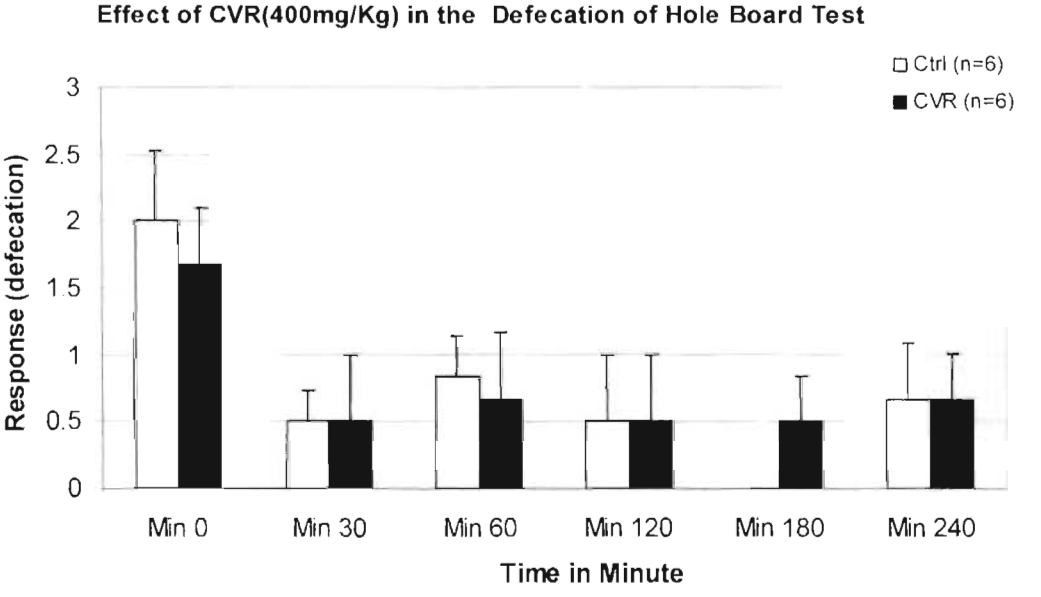


Table: 4.12. The effect of CVR (400 mg/kg) in the Defecation of Hole Board Test.

Group		Min0	Min30	Min60	Min120	Min180	Min240
Ctrl(n=6)		2.00 ± 0.516	0.50 ± 0.224	0.83 ± 0.307	0.50 ± 0.50	0.00 ± 0.00	0.67 ± 0.422
CVR(n=6)		1.67 ± 0.422	0.50 ± 0.50	0.67 ± 0.494	0.50 ± 0.50	0.50 ± 0.342	0.67 ± 0.333
t/p		0.500/ 0.628	0.00/ 1.00	0.286/ 0.780	0.00/ 1.00	-1.464/ 0.174	0.00/ 1.00
95% confidence interval	lower	-1.152	-1.228	-1.130	-1.576	-1.261	-1.198
	Upper	1.819	1.220	1.464	1.576	0.261	1.198

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant

Figure: 4.12. Graphical Presentation of the effect of CVR (400 mg/Kg) in Defecation of Hole Board Test.



5.0 OPEN FIELD TEST

The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of behavior. This experiment presents with a different and more complex environment to explore.

Statistical findings

Total Ambulation

CVR treated male mice at dose 100 mg/Kg levels exerted overall decrease in ambulation. At dose 100 mg/Kg, exerted overall decrease in ambulation compare with the control group. The decreasing effect at min 180 (p=0.014*) was significant statically. None other results were significant statically. At higher dose of 200 mg/Kg only at min 0 and min 240 when ambulation decrease was observed. Other then that all the time during the experimental period the ambulation decreased. At min 240 there was a significant decrease in ambulation (p=0.006*). At the highest dose 400mg/Kg significant decrease were observed in min 120, 180 and 240 respectively having p= 0.004, 0.018, 0.021.

Total Ambulation in Center region

CVR treated male mice at dose levels 100 mg/Kg exerted overall decrease in total movement in the center region. The effect was same at dose levels 200 mg/Kg except in min 60 the result was almost similar. However, at dose levels 400 mg/Kg the effect increased and only at min 60 there was a significant decrease where p= 0.017*.

Total standing up behavior

At dose 100 mg/Kg except at min 60 the effect had decreased compare with the control group. At min 120 there was a significant decrease where p=0.032*. Similarly at dose 200 mg/kg in all through out the experimental period, CVR treated mice exerted an decrease in the standing up behavior in comparison to that of control group. But the decrease was statistically significant only at min 60, 120 and 180 when p=0.32*, 0.024**, 0.33*. The effect was highly significant at min 120.However, at dose levels 400 mg/Kg there was an overall decrease in the behavior pattern. But none of the results were statistically significant.

Total Emotional Defecation

CVR at doses (100 mg/Kg and 200 mg/Kg, 400 mg/kg) showed decrease in effect, but the exception has been at dose levels 400 mg/kg, where there was an increase in the defecation.. None of the results were significant statistically.

5.1 Tabular and Graphical presentation of the effect of CVR (100 mg/kg, 200 mg/Kg, 400 mg/Kg) on the Open Field Test utilizing Male mice.

Table: 5.1 The effect of CVR (100 mg/kg) on Ambulation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		126.17 ± 10.358	100.00 ± 9.748	71.00 ± 10.621	69.50 ± 5.596	45.00 ± 1.807	36.67 ± 8.053
CVR (n=6)		113.33 ± 7.504	78.83 ± 6.925	66.67 ± 7.817	29.17 ± 4.959	25.00 ± 5.532	30.67 ± 3.712
t/p value		1.003/ 0.339	1.771/ 0.107	0.329/ 0.749	0.00/ 0.590	3.437/ **0.014***	0.677/ 0.514
95% confidence interval	Lower	-15.666	-5.468	-25.050	23.678	5.792	-13.757
	Upper	41.333	47.801	33.717	56.989	34.208	25.757

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.1 Graphical Presentation of the effect of CVR (100 mg/Kg) on Ambulation in the open field test.

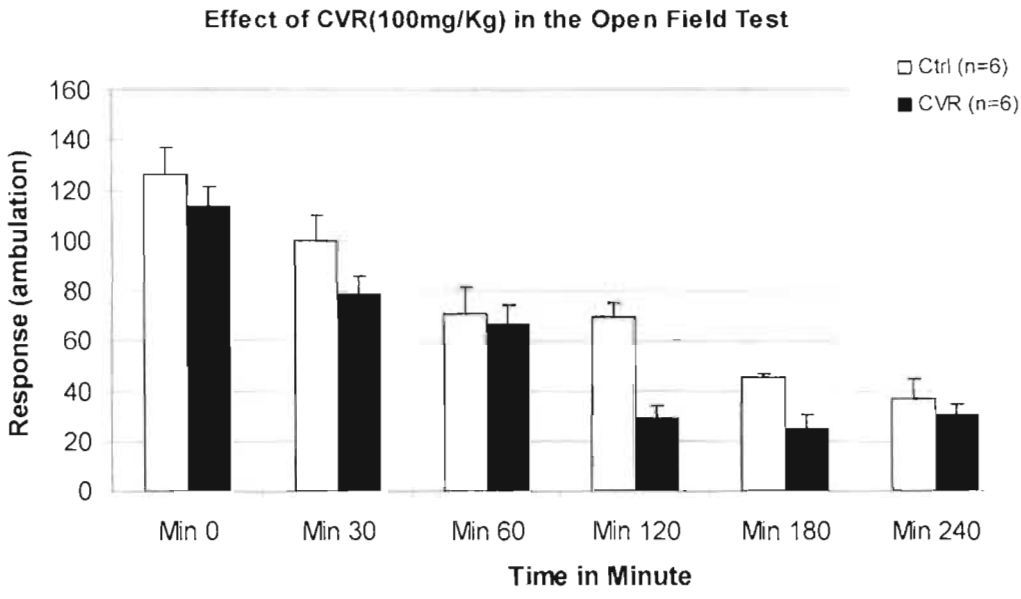


Table: 5.2 The effect of CVR (200mg/kg) on Ambulation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		114.33 ± 8.160	98.17 ± 10.566	69.83 ± 9.938	58.17 ± 8.064	45.00 ± 1.807	40.67 ± 3.283
CVR (n=6)		81.00 ± 6.476	79.50 ± 11.445	57.33 ± 8.488	36.83 ± 6.258	32.17 ± 5.062	25.17 ± 3.092
t/p value		3.20/ **0.009****	1.199/ 0.258	0.956/ 0.361	2.090/ 0.063	2.387/ 0.053	3.437/ **0.006***
95% Confidence interval	Lower	10.123	-16.024	-16.620	-1.410	-0.191	5.451
	Upper	56.544	53.358	41.620	44.077	25.858	25.549

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.2 Graphical Presentation of the effect of CVR (200 mg/Kg) on Ambulation in the open Field test.

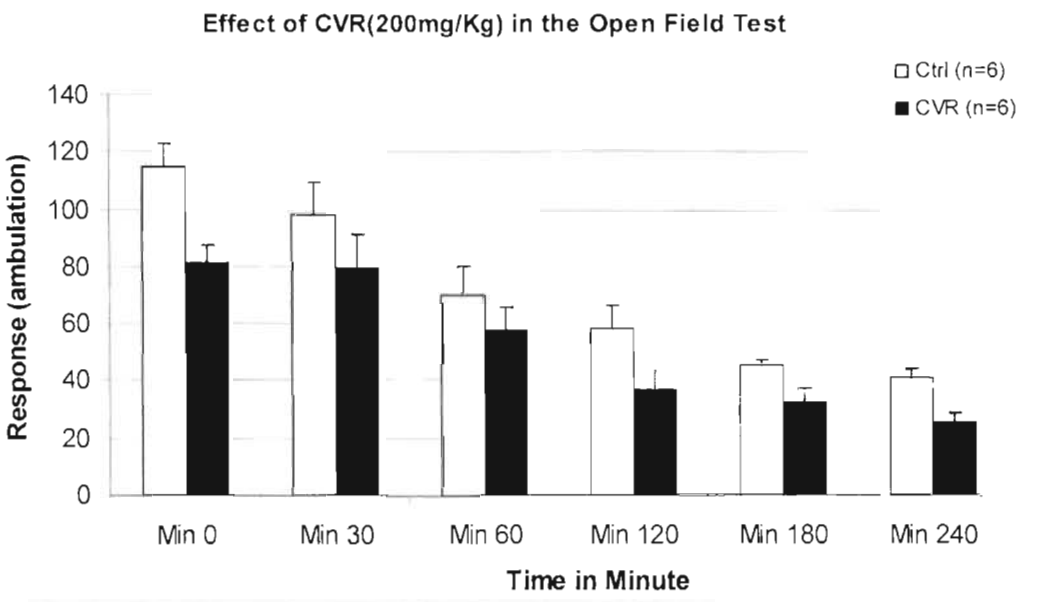


Table: 5.3 The effect of CVR (400mg/kg) on Ambulation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		125.17 ± 11.831	122.33 ± 14.207	111.17 ± 14.525	79.17 ± 9.502	79.00 ± 11.213	72.50 ± 8.065
CVR (n=6)		81.33 ± 9.917	88.67 ± 13.583	74.17 ± 13.775	40.83 ± 3.928	43.33 ± 5.909	47.17 ± 4.475
t/p value		2.925/ **0.015***	1.713/ 0.118	1.848/ 0.094	3.728/ **0.004****	2.814/ **0.018***	2.747/ **0.021***
95% confidence interval	Lower	10.446	-10.129	-7.603	15.423	7.426	4.782
	Upper	77.221	77.463	81.603	61.243	63.907	45.885

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.3 Graphical Presentation of the effect of CVR (400 mg/Kg) on Ambulation in the open field test.

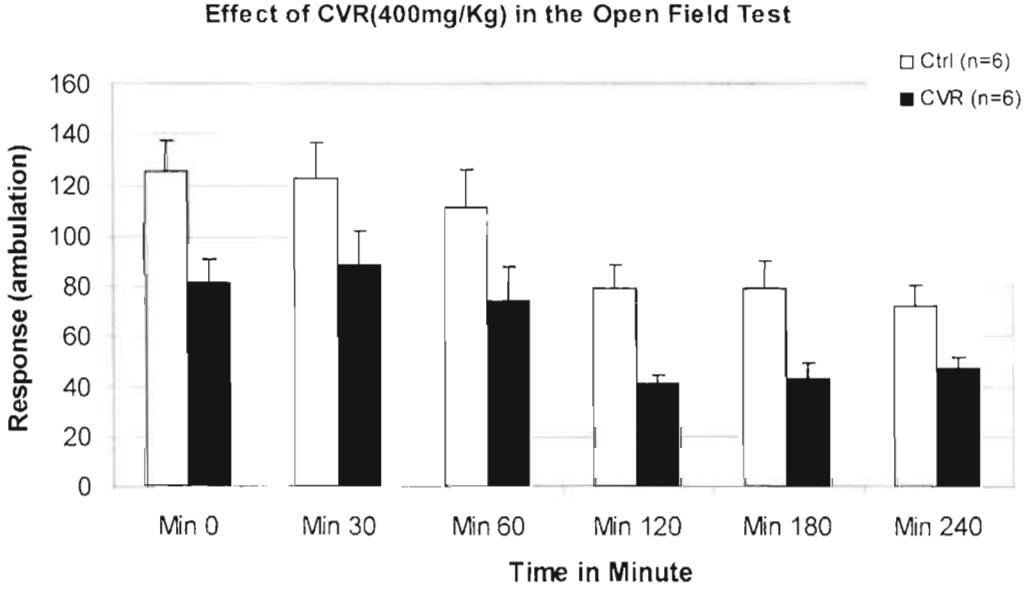


Table: 5.4 The effect of CVR (100 mg/kg) on the center ambulation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		2.50 ± 0.428	1.17 ± 0.401	1.33 ± 0.494	0.33 ± 0.211	0..17 ± 0.167	0.00 ± 0.00
CVR (n=6)		1.00 ± 0.365	1.00 ± 0.365	0.50 ± 0.342	0.17 ± 0.167	0.00 ± 0.00	0.00 ± 0.00
t/p value		2.666/ 0.024	0.307/ 0.765	1.387/ 0.196	0.620/ 0.549	1.00/ 0.341	0.00/ 0.00
95% confidence interval	Lower	0.246	-1.042	-0.506	-0.432	-0.205	0.00
	Upper	2.754	1.376	2.172	0.765	0.538	0.00

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.4 Graphical Presentation of the effect of CVR (100 mg/Kg) on the center ambulation in the open field test.

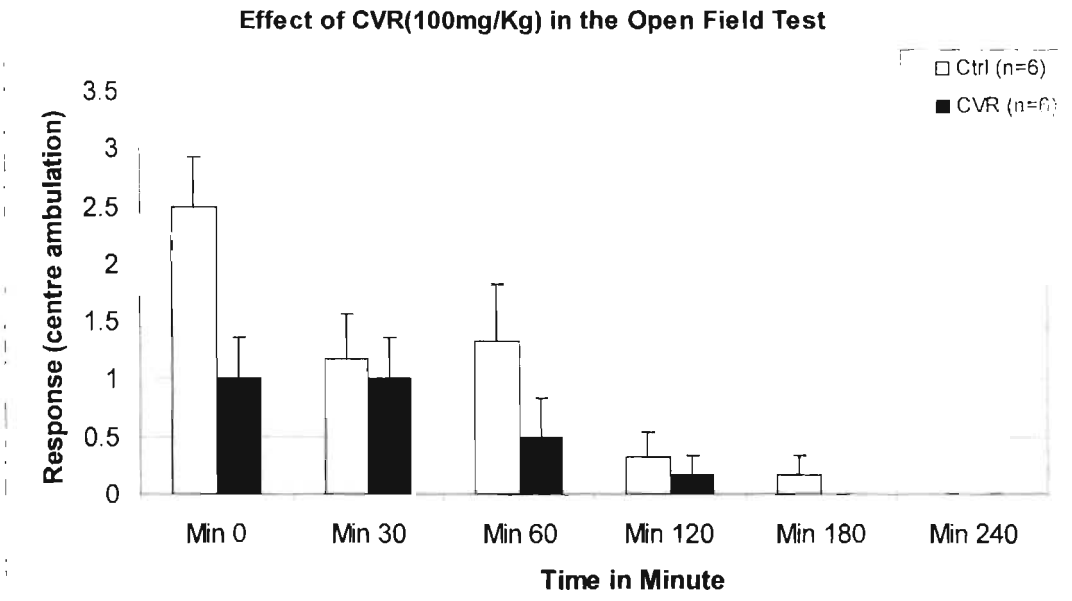


Table: 5.5 The effect of CVR (200 mg/kg) on the center ambulation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		2.33 ± 0.715	1.33 ± 0.494	1.00 ± 0.516	2.00 ± 0.258	1.50 ± 0.428	1.67 ± 0.333
CVR (n=6)		0.83 ± 0.401	1.00 ± 0.365	1.00 ± 0.516	1.00 ± 0.447	0.67 ± 0.422	1.17 ± 0.401
t/p value		1.830/ 0.97	0.542/ 0.599	0.00/ 1.00	1.936/ 0.089	1.387/ 0.196	0.958/ 0.360
95% confidence interval	Lower	-0.327	-1.036	-1.627	-0.191	-0.506	-0.663
	Upper	3.327	1.703	1.627	2.191	2.172	1.663

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.5 Graphical Presentation of the effect of CVR (200 mg/Kg) on the center ambulation in the open field test.

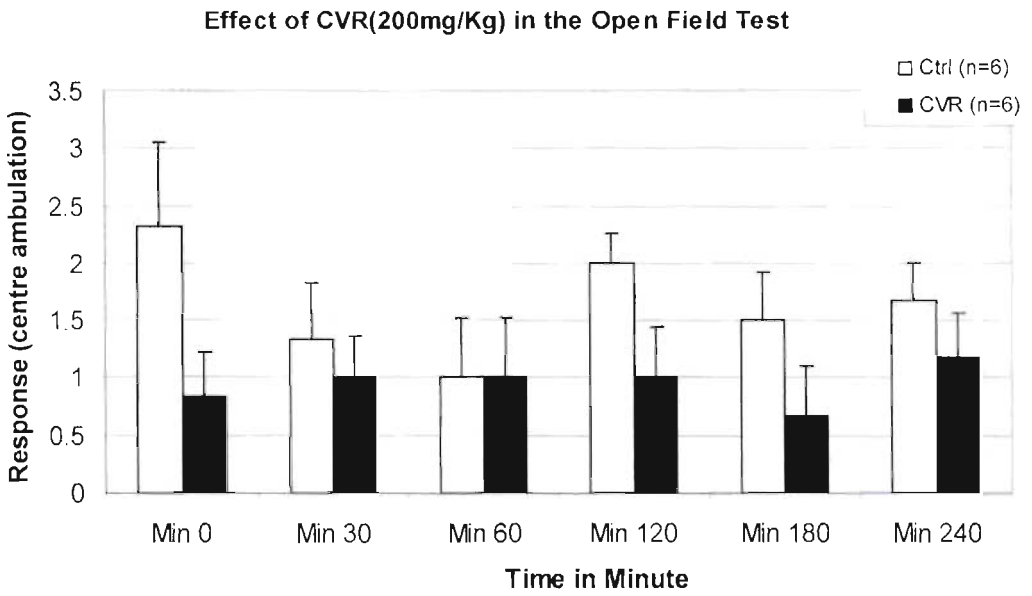


Table: 5.6 The effect of CVR (400 mg/kg) on the center ambulation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		0.33 ± 0.211	0.83 ± 0.401	0.00 ± 0.00	1.50 ± 0.516	0.67 ± 0.422	0.83 ± 0.401
CVR (n=6)		1.33 ± 0.494	1.00 ± 0.365	1.50 ± 0.428	0.50 ± 0.224	1.33 ± 0.422	0.83 ± 0.401
t/p value		-1.861/ 0.092	-0.307/ 0.765	-3.503/ **0.017***	0.889/ 0.395	-1.118/ 2.90	0.00/ 1.00
95% confidence interval	Lower	-2.198	-1.376	-2.601	-0.754	-1.995	-1.265
	Upper	0.198	1.042	-0.399	1.754	0.662	1.265

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.6 Graphical Presentation of the effect of CVR (400 mg/Kg) on the center ambulation in the open field test.

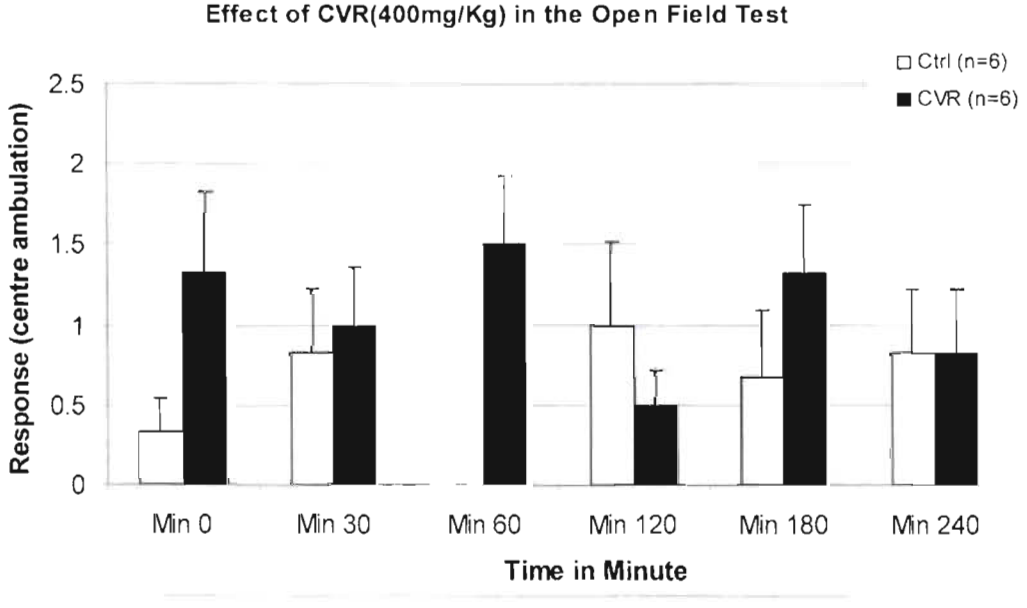


Table: 5.7 The effect of CVR (100 mg/kg) on the standing up behavior in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		8.17 ± 1.249	7.83 ± 1.662	6.00 ± 1.366	4.83 ± 0.601	2.17 ± 0.401	2.33 ± 0.333
CVR (n=6)		7.17 ± 0.792	7.50 ± 1.118	8.83 ± 2.600	1.83 ± 1.046	2.83 ± 0.703	2.50 ± 0.224
t/p value		0.676/ 0.514	0.166/ 0.871	-0.965/ 0.357	2.487/ **0.032***	-0.823/ 0.429	-0.415/ 0.687
95% confidence interval	Lower	-2.297	-4.129	-9.378	0.312	-2.471	-1.061
	Upper	4.297	4.796	3.711	5.688	1.137	0.728

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure: 5.7 Graphical Presentation of the effect of CVR (100 mg/Kg) on the standing up behavior in the open field test.

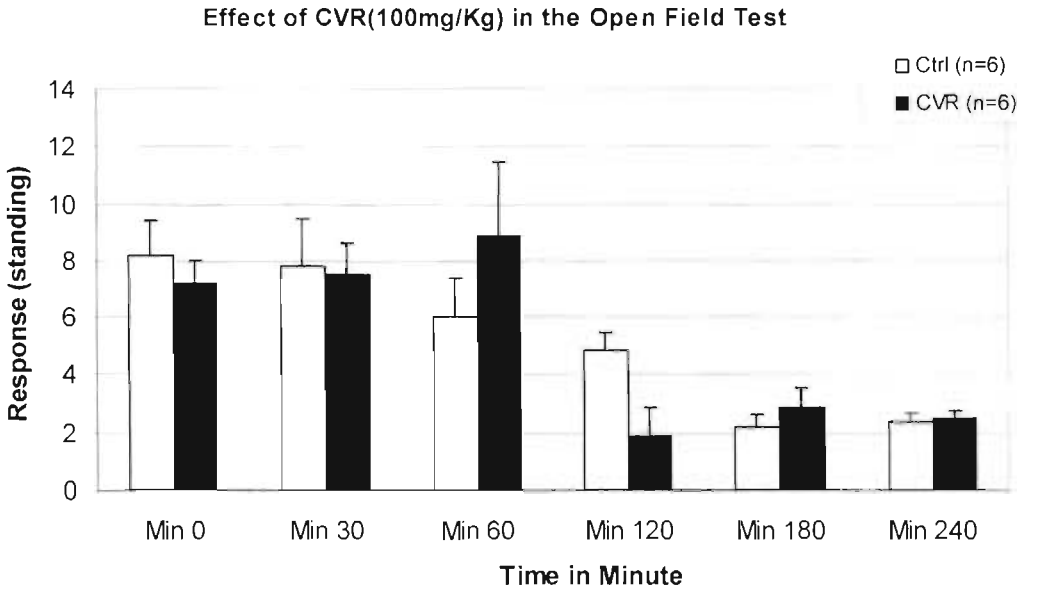


Table: 5.8 The effect of CVR (200 mg/kg) on the standing up behavior in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		7.50 ± 1.335	8.17 ± 1.470	4.17 ± 0.654	6.00 ± 1.125	6.50 ± 0.922	7.83 ± 1.778
CVR (n=6)		6.00 ± 1.317	7.50 ± 1.478	2.33 ± 0.333	2.33 ± 0.333	3.17 ± 0.980	4.00 ± 0.577
t/p value		0.800/ 0.442	0.320/ 0.756	2.497/ **0.32***	2.650/ **0.024***	2.477/ **0.033***	2.051/ 0.086
95% confidence interval	Lower	-2.678	-3.978	0.198	0.504	0.335	-0.733
	Upper	5.678	5.311	3.469	5.829	6.322	8.400

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure 5.8 Graphical Presentation of the effect of RR (200 mg/Kg) on the standing up behavior in the open field test.

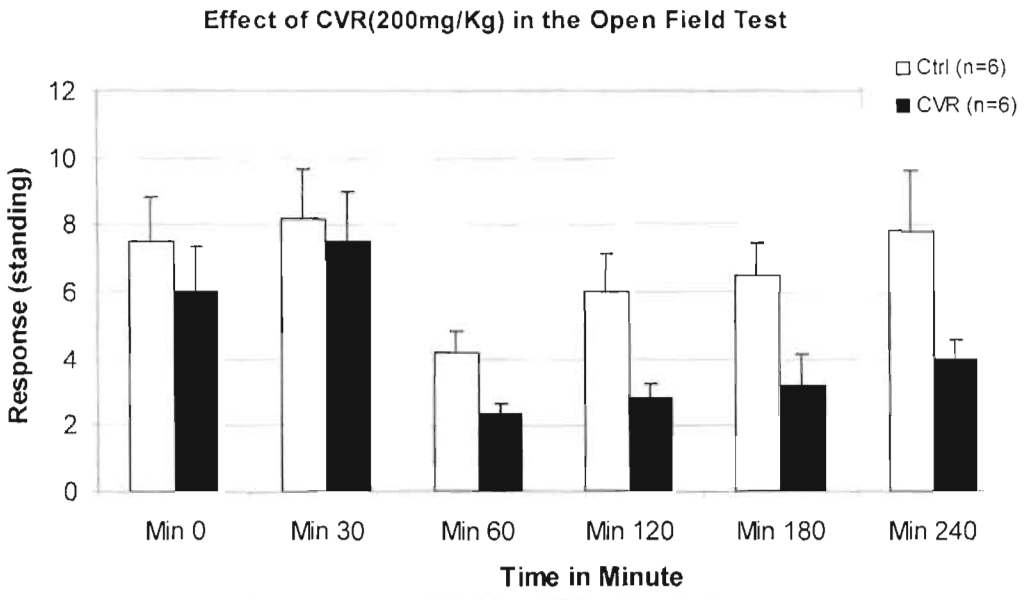


Table 5.9 The effect of CVR (400 mg/kg) on the standing up behavior in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		6.83 ± 1.470	5.67 ± 0.882	5.17 ± 0.833	7.00 ± 1.125	5.67 ± 1.498	6.33 ± 1.33
CVR (n=6)		4.83 ± 0.601	6.50 ± 1.118	4.67 ± 1.022	5.50 ± 0.885	3.50 ± 0.764	3.33 ± 0.615
t/p value		1.259/ 0.250	-0.585/ 0.571	0.379/ 0.712	1.048/ 0.319	1.288/ 0.227	2.043/ 0.068
95% confidence interval	Lower	-1.799	-4.006	-2.438	-1.690	-1.580	-0.271
	Upper	5.799	2.340	3.438	4.690	5.914	6.271

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure 5.9 Graphical Presentation of the effect of CVR (400 mg/Kg) on the standing up behavior in the open field test.

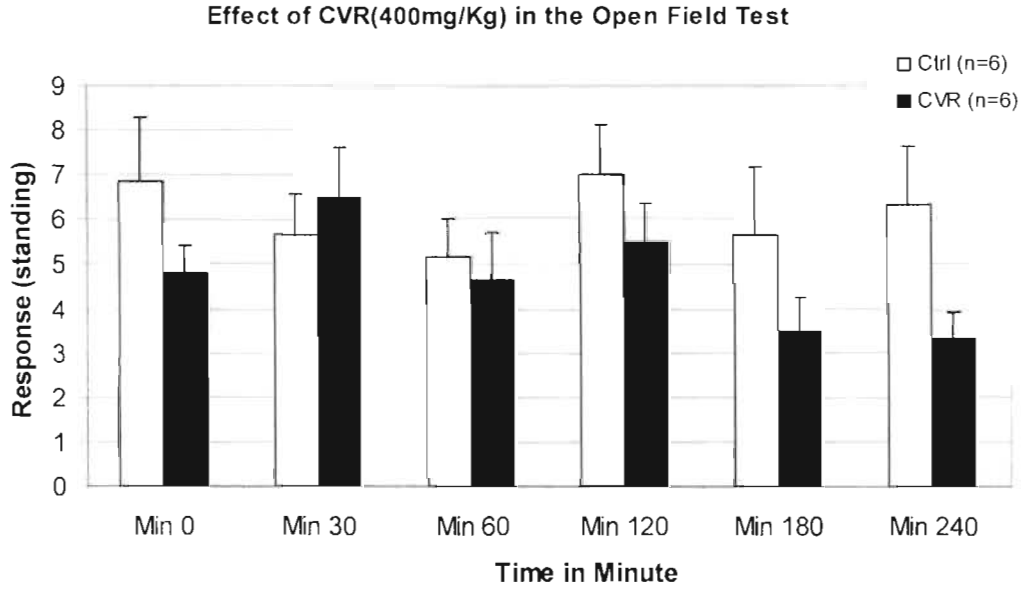


Table 5.10 The effect of CVR (100 mg/kg) on the Emotional defecation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		0.17 ± 0.167	0.00 ± 0.00	0.33 ± 0.211	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.00
CVR (n=6)		0.00 ± 0.00	0.83 ± 0.654	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
t/p value		1.00/ 0.363	-1.274/ 0.259	1.581/ 0.175	0.00/ 0.00	0.00/ 0.00	0.00/ 0.00
95% confidence interval	Lower	-0.262	-2.515	-0.209	0.00	0.00	0.00
	Upper	0.595	0.848	-0.875	0.00	0.00	0.00

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure 5.10 Graphical Presentation of the effect of CVR (100 mg/Kg) on the Emotional defecation in the open field test.

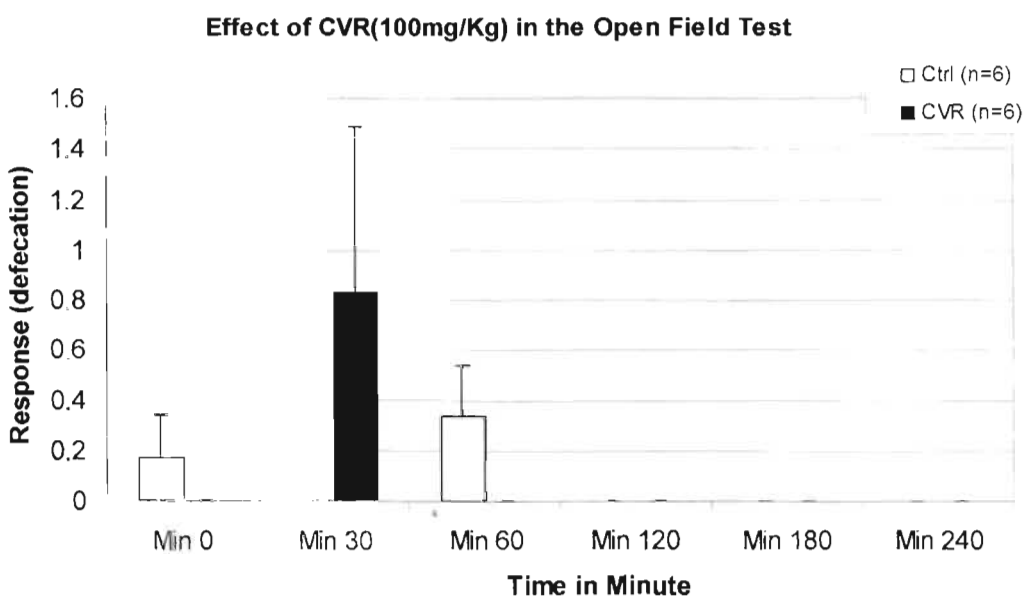


Table 5.11 The effect of CVR (200 mg/kg) on the Emotional defecation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		0.50 ± 0.342	0.33 ± 0.211	0.67 ± 0.667	0.67 ± 0.422	0.67 ± 0.333	0.5 ± 0.342
CVR (n=6)		0.83 ± 0.543	1.00 ± 0.447	0.67 ± 0.333	0.83 ± 0.477	0.67 ± 0.667	0.833 ± 0.401
t/p value		-0.520/ 0.614	-1.348/ 0.219	0.00/ 1.00	-0.262/ 0.799	0.00/ 1.00	-0.632/ 0.541
95% confidence interval	Lower	-1.762	-1.832	-1.661	-1.586	-1.661	-1.508
	Upper	1.095	0.499	1.661	1.252	1.661	0.841

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure 5.11 Graphical Presentation of the effect of CVR (200 mg/Kg) on the Emotional defecation in the open field test.

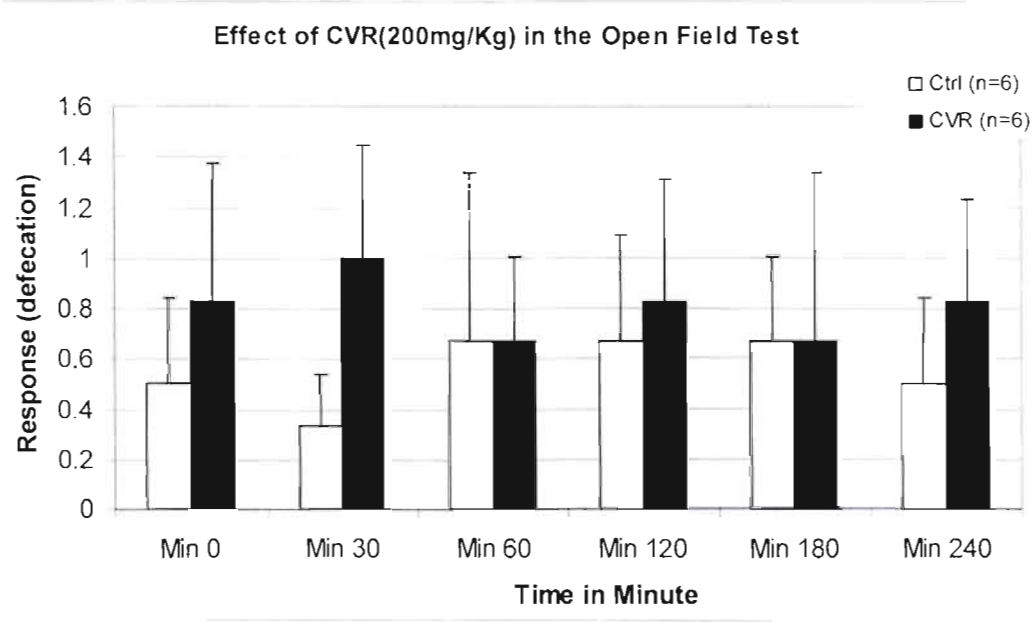
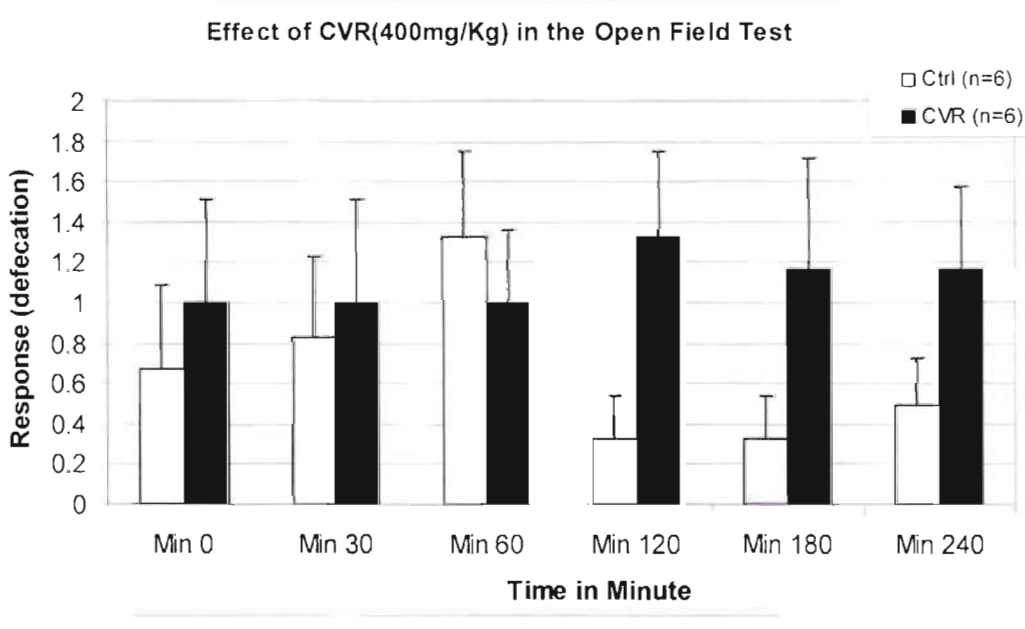


Table 5.12 The effect of CVR (400 mg/kg) on the Emotional defecation in the open field test.

Group		Min 0	Min 30	Min 60	Min 120	Min180	Min 240
Control (n=6)		0.67 ± 0.422	0.83 ± 0.401	1.33 ± 0.422	0.33 ± 0.211	0.33 ± 0.211	0.50 ± 0.224
CVR (n=6)		1.00 ± 0.516	1.00 ± 0.4516	1.00 ± 0.365	1.33 ± 0.422	1.17 ± 0.543	1.17 ± 0.401
t/p value		0.500/ 0.628	-2.55/ 0.804	0.598/ 0.0.563	-2.121/ 0.60	-1.431/ 0.199	- 1.451/0. 186
95% confidence interval	Lower	-1.819	-1/624	-0.909	-2.050	-2.233	-1.730
	Upper	1.152	1.291	1.576	0.050	0.566	0.397

N.B :*(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Figure 5.12 Graphical Presentation of the effect of CVR (400 mg/Kg) on the Emotional defecation in the open field test.



Conclusion:

This ayurvedic drug (CVR) has shown depressive activity. After carried out the experiments we got significant results in the Hole Cross test, Open Field test and also in the Hole Board experiment. It indicates that this drug has anti-stimulant property. We also got significant result in Hypoxia test, and the survival time has increased. We also carried out the Formalin induced paw licking test and Climbing Out test. But we haven't got any significant result from these experiments. So we may say that the drug CVR is effective as depressant and has no psychopharmacological side effect.

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