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دراسات سمية لمادة الكلوريد بين  
على الفئران البيضاء\*

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تم خلال هذه الدراسة تحديد الجرعة متوسطة السمية وكانت تساوى ١٦ر٥ ملجرام لكل كيلوجرام من وزن الحيوانات . وباختيار المادة عن طريق الاختبارات اللونية فثبت أن اختيار الاليزارين الأحمر يعطى لونا أصفر مع مادة الكلوريدين كذلك تم خلال هذه الدراسة تحديد طرق الكشف عنها فى الانسجة ومدى توزيعها وقد ثبت استعمال التحليل اللونى الطيفى الرقيق وجود المادة فى المخ والكبد بنسبة عالية ثم القناة الهضمية والكلية كذلك ثبت عبور مادة الكلوريدين خلال السخد فى الأمهات الحوامل الى الاجنة . كذلك تم خلال هذا البحث دراسة التأثير الهستوثولوجى فى حالة اعطاء جرعات مختلفة فى الاناث الحوامل والغير حوامل وقد ثبت خلال هذه الدراسة أن هذه المادة لها تأثير سرطانى على خلايا المعدة .

TOXICOLOGICAL STUDIES ON CHLORODINE IN ALBINO RATS  
(WITH 4 TABLES AND 4 FIGURES)

BY  
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SUMMARY

The present study investigated some toxicological aspects of chlorodene in abino rats. The study included determination of LD<sub>50</sub>, ultraviolet spectrophotometric analysis distribution of the insecticide in the different organs of the rats and lastly the histopathological changes produced in rats after oral administration of a single dose of 0.025 mg/Kg BWT chlorodine.

INTRODUCTION

Chlorodine is one of insecticide which is used in Egypt for control of cotton pest owing to its ovidical and larvicidal activity. It is recommended also as an acaricide due to its excellent ovidical, larvicidal and adulticidal properties. Field observations showed that this compound may cause toxicological hazards either through arial application to cotton fields or from eating contaminated feed or drinking polluted water. This was noticed through the several accidental hazards which occurred in Egypt from misuse or eating food contaminated with insecticides (SHAABAN, 1972). In this study toxicological investigation, analytical and histopathological studies of chlorodine were carried out.

MATERIALS AND METHODS

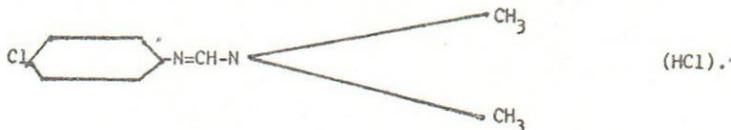
Determination of roal LD<sub>50</sub> dose: Female albino rats 5 months average age were used. Food and water were given ad libitum.

Preliminary trials for oral LD<sub>50</sub> determination to determine the zero % and 100% mortality were carried out on six group (4 animals each), the 1st., 2nd., 4th. and 5th. groups administered 2.5, 5, 6.25 and 12.50 mg/Kg., respectively, while the 6th. group served as a control.

The actual determination of LD<sub>50</sub> was carried according to LICHTFIELD and WILCOXON (1949).

Animals survived from the LD<sub>50</sub> dose were sacrificed after 11 months and their organs were subjected to histological examination.

Insecticides: Chlorodine was supplied by Ciba Giegy, technical office in Egypt. The compound has the following structure and empirical formula:



N-(2-methyl 1-4 chlorophenyl) N-N dimethyl formamidine.

For qualitative and quantitative analysis of chlorodine:

- 1- Colour tests were performed by adding drops of the test reagents (Table, 2) to aliquots contained chlorodine.
- 2- Thin layer chromatographic analysis (TLC) to investigate the behaviour of chlorodine on silica gel G chromatoplatest of 0.3 mm thickness, using 8 different solvent systems (Table, 3). Different spraying agents were tried (Table, 4). The RF value of each spot was recorded (FIFIELD and KEARLY, 1975) for each solvent system.

General Director of Forensic Med. Dept. Minstrg of Justing CAIRO.

3- Quantitative estimation of chlorodine using automatic recording ultraviolet unicum Sp. 1800 with sensitivity of  $10^{-6}$  and the absorption spectra were recorded, against a blank. A standard curve was plotted between absorbance and concentration (DELAHAY, 1956).

Infra-red absorption spectrophotometry using automatic recording infra-red unicum spectrophotometer Sp. 1800 using potassium bromide disc technique.

The compound was given via intragastric intubation. To investigate tissue distribution and transplacental transmission the  $LD_{50}$  dose were given to 40 pregnant female rats at late stage of pregnancy (18-19 days) and just after delivery (2 days later), off spring were taken and mother sacrificed and their organs kept frozen till estimation and determination of tissue abundance in mothers and presence of the insecticide in the foetus.

Stass otto method was used to precipitate the protein in tissue samples. The protein free residue was extracted in alkaline medium by chloroform, the chloroform layer was separated and evaporated to dryness, the residue was extracted in acidic medium using chloroform. Detection and quantitative determination of chlorodine in different tissue extract were carried out by colour tests thin layer chromatography, ultraviolet and infra-red spectrophotometric technique.

## RESULTS

Preliminary trials for  $LD_{50}$  determination showed that the zero % & 100% mortality were 0.0125 and 0.4 mg./Kg., respectively. The oral  $LD_{50}$  of chlorodine to albino rats was found 16.5 mg/Kg. with 19/20 confidence limits of 10.78 - 25.24. Results are shown in Table (1) and Fig. (1).

Colour test of chlorodine showed that it gave yellow colour with alizarine red while it was negative with marquis test, bromocresol green, Frode's test, Vitalin test and Lieberman's test.

The different reagents used for visualization of the spots of chlorodine on silica gel developed colours either directly or under ultraviolet light or no coloured developed (Table, 2). The RF value using different eluents are shown in Table (3).

The ultraviolet spectrophotometric analysis showed that chloridine has absorption maxima at 268 nm wave length (Fig. II). The standard curve plotted showed a linear relationship between chlorodine concentration and absorbance. The previous relation was used to determine the unknown concentration of chlorodine.

Distribution of the insecticide in different organs showed its presence in brain, liver, gastrointestinal tract and kidneys (Table, 4). Analysis of tissue from embryo prenatally exposed to the compound by combined T.L.C. and spectroscopic techniques proved the transplacental transmission (Fig. IV).

Chlorodine gives infra-red spectrum (Finger print region,  $2000-650\text{ cm}^{-1}$ ) three major bands 2830, 1710 and 2730. The bands have been labelled a, b and c in decreasing order of intensity (Fig., III). Detection of the compound in different organs and foetal tissues was also confirmed by infra-red spectroscopy.

Histopathological changes produced in Agoza strain female rats after oral administration of single dose of 0.025 mg/Kg. B.Wt. of chlorodine and slaughtered after 11 months post administration were:

### Stomach:

The gastric mucosa was ulcerated and haemorrhagic, the inflammatory cells were lymphocytes and macrophages with few neutrophils.

Squamous papilloma (Fig. 1) was seen in the non glandular gastric mucosa and hyperplasia of the gastric glands of the glandular portion. Hyaline degeneration was seen in the muscle fibres of the muscular coat.

### Intestine:

The epithelial lining was partially hyperplastic, ulcerated and showed squamous metaplasia; the mucosa and submucosa were infiltrated with lymphocytes, macrophages and neutrophils. Haemorrhagic areas were seen in the mucosa, and the submucosa was oedematous, the muscular coat was degenerated and inflamed. The subserosa was congested and contained eosinophils, macrophages and fibrin threads.

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Liver:

The central and portal veins were congested, and surrounded with lymphocytes, macrophages and fibroblasts. The hepatic cells showed focal areas of necrosis invaded by lymphocytes and macrophages in addition to peribular vacuolar degeneration.

Spleen:

Some Malpighian bodies were necrotic and others were hyperplastic.

Lungs:

Fibrinous pneumonia was seen with focal alveolar emphysema and hemorrhage. There was peribronchial lymphocytic hyperplasia with hyperplasia and desquamation of the bronchial epithelium.

Heart:

Subpericardial haemorrhage was seen, myomalacia and non suppurative myocarditis (Fig. 2).

Kidneys:

They were congested, haemorrhagic and showed non suppurative nephritis. Some retrogressive changes were seen in the renal tubules which included cloudy swelling, vacuolar degeneration and hyaline casts inside the renal tubules. Other renal tubules were cystic, some glomeruli were collapsed and others were enlarged, ischemic and highly cellular. Areas of coagulative necrosis were present (Fig. 3). The renal pelvis was congested, haemorrhagic and infiltrated with lymphocytes its epithelial lining was partially hyperplastic and desquamated (Fig. 4). The ureter showed hyperplastic epithelial cells which formed finger like projections. Submucosal congestion and haemorrhage.

Brain:

The meningeal, cerebrum and cerebellum were congested and haemorrhagic, the choroid plexus was congested, (Fig. 5). The cerebellum and cerebrum showed neurophagia and gliosis.

Histopathological changes in offsprings three months old after prenatal administration of chlorodine:Stomach:

It showed acanthosis of the non glandular portion and catarrhal gastritis of the glandular stomach with erosion.

Intestine: There was catarrhal enteritis with submucosal oedema and degeneration of the muscular coat.

Liver:

It showed degenerative changes represented by cloudy swelling and vacuolar degeneration in addition to focal areas of coagulative necrosis. There was an increase in the number of bile ducts (hyperplasia) which were surrounded with proliferating fibroblasts.

Spleen: The lymphocytes in the Malpighian corpuscles were either hyperplastic or necrotic.

Lungs:

Bronchopneumonia was evident, some bronchioles showed bronchioleostasis and contained few neutrophils. Aggregations of hyperplastic lymphocytes were seen around the bronchioles and the blood vessels partially replaced the air vesicles.

Heart: There was myomalacia cordis and haemorrhage.

Placenta:

The placenta showed congestions, haemorrhage and oedema specially at the communicating zone between placenta foetalis and placenta maternalis.

Degeneration of the chorionic trophoblasts and decidual cells were seen. Fibrin threads and leucocytic infiltration mainly macrophage and lymphocytes were present in placenta foetalis.

## DISCUSSION

In the present study, the estimation of chlorodine by ultraviolet spectrophotometer gave satisfactory results. As the standard curve plotted showed a linear relationship between the concentration and absorbance, so this relation used to determine the unknown concentration of the compound in quantitative estimation and also in toxicological analysis. The T.L.C. has a distinct advantages in the detection of chlorodine in toxicological analysis, since simple performance is met with T.L.C. as the run of the compound completed within 30 minutes, a period of time which allows no fluctuation in the room temperature. Since the time factor is of importance in the medicolegal practice, the separation of the compound after extraction from tissue organs in chromatoplates is completed in half an hour. The detection of the compound from tissue organs of embryo was proved by T.L.C. thus confirming the transplacental transmission from the mother to the foetus.

Our results indicated that placental disturbances were produced by chlorodine which were manifested by the circulatory disturbances and degenerative changes in decidua cells and chorionic trophoblasts in addition to leucocytic infiltration and presence of fibrin threads in placenta foetalis. It is probable therefore, that the disturbance in the placental function may play a part in the production of the embryopathic effects. Detection of chlorodine in foetus may also causes the pathological changes found in the foeti. The estimation of tissue abundance of the insecticide facilitate the organ of choice in toxicological cases and may through a light on its toxodynamic effects.

The effect of chlorodine on adult female rats survived for eleven months after oral administration of LD<sub>50</sub> dose showed papilloma in the forestomach in addition to other pathological changes. The tumorigenic effect of the compound has been proved previously in albino rats (SACHESSE, 1976).

## REFERENCES

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Table (1): Solution of the dose effect curve of chlorodine in female albino rats.

Dose (mg/kg)	Response	Observed %	Expected %	Observed minus expected	Contribution to (Chi) <sup>2</sup>
0/8	0(0.3)	0.3	1.0	0.7	0.005
0/8	0(1.15)	1.15	3.5	2.35	0.016
1/8	12.5	12.5	6.0	6.5	0.017
4/8	50.0	50.0	35.0	15.0	0.100
8/8	100(99.7)	99.7	99.5	0.2	0.008
				Total	0.146

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Table (2): The reaction of different spot locating agents with chlordine (10 ug on chromatoplates).

Method of visible light	Locating agent			
	Nitric acid/ propanol	H <sub>2</sub> SO <sub>4</sub> / propanol	HCl/ propanol	H <sub>2</sub> SO <sub>4</sub> / ethanol
A- Visible light	-ve	-ve	-ve	-ve
	Vanilline/ HCl	Vanilline/ H <sub>2</sub> SO <sub>4</sub>	Ferric chloride/ HCl	
	-ve	-ve	Brown	
B- Ultraviolet light	Rosin	Fluorecine	Acridflavin	
	-ve	Violet	-ve	

Table (3): The RF values of chlordine on silica gel G chromatoplates using mixtures of some aromatic hydrocarbon compounds.

Eluent	C-M	C-E	C-M	B-M	B-E
Ratio	4:1	9:1	4:1	9:1	4:1
RF	0.89	0.6	0.93	0.18	0.16

C : Chloroform .

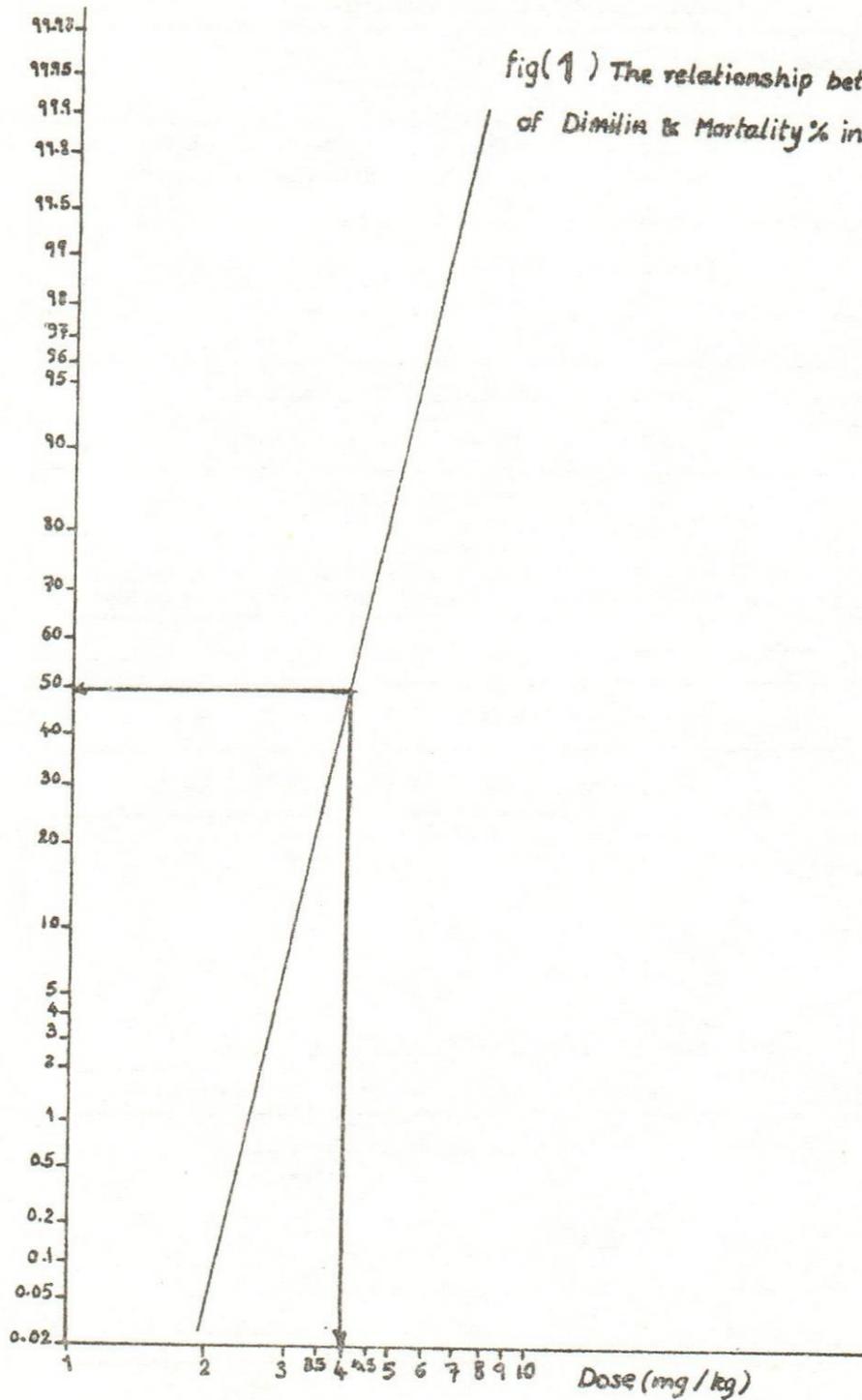
M : Methanol .

E : Ethanol .

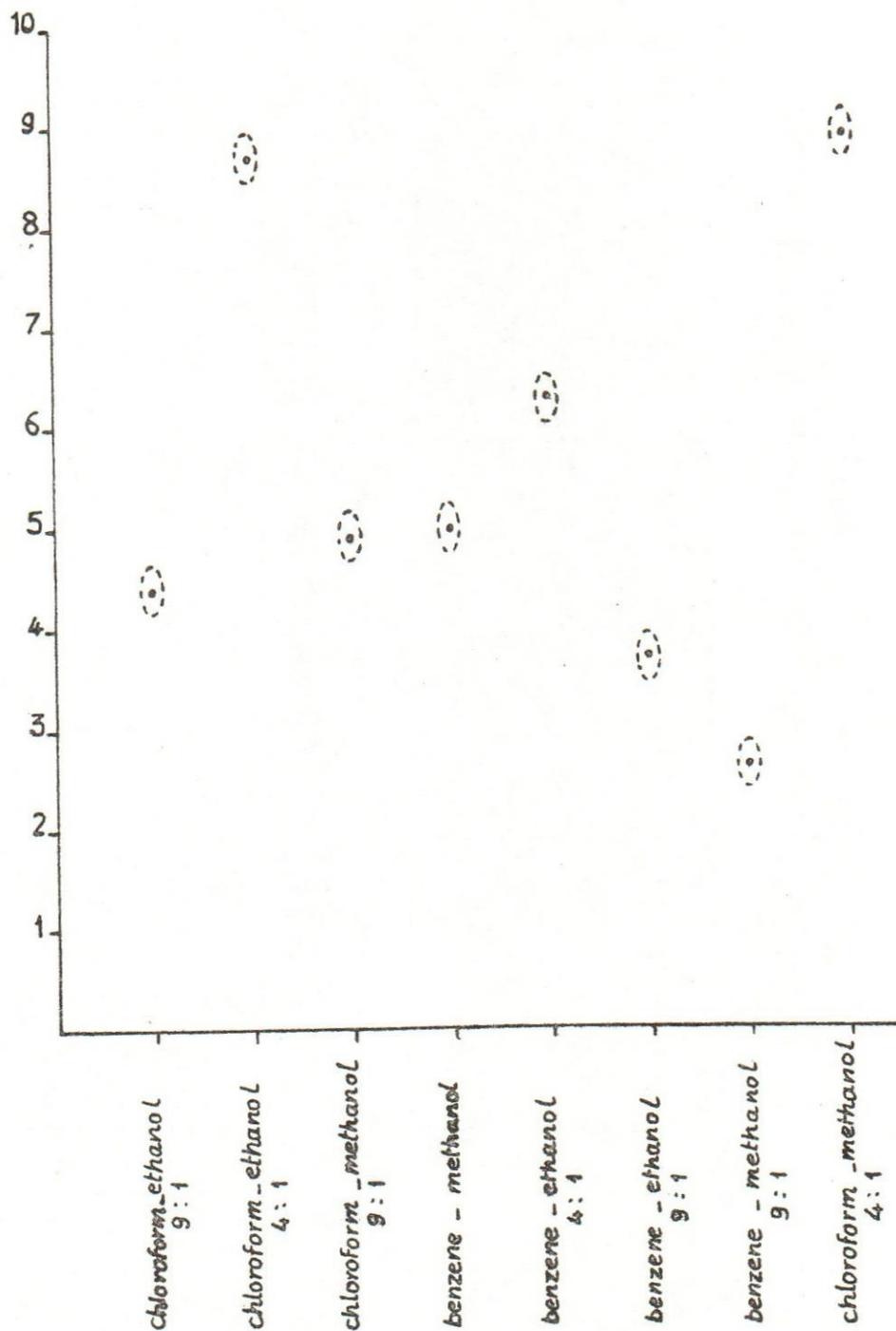
B : Benzene .

Table (4): Distribution of chlordine in different organs.

Sample No.	Amount of chlordine (ug/g. of tissues)			
	Brain	Kidneys	Liver	Stomach and intestine
I	0.27	0.36	0.47	1.5
II	0.22	0.32	0.40	1.2
III	0.18	0.40	0.50	1.0
Mean±S.E.	0.22±0.05	0.36±0.04	0.46±0.05	1.23±0.28



fig(1) The relationship between doses of Dimilin & Mortality% in Rats.



fig(2) The Rf values of Dimiline on Silica gel G Chromatoplates using mixtures of some aromatic hydrocarbones.

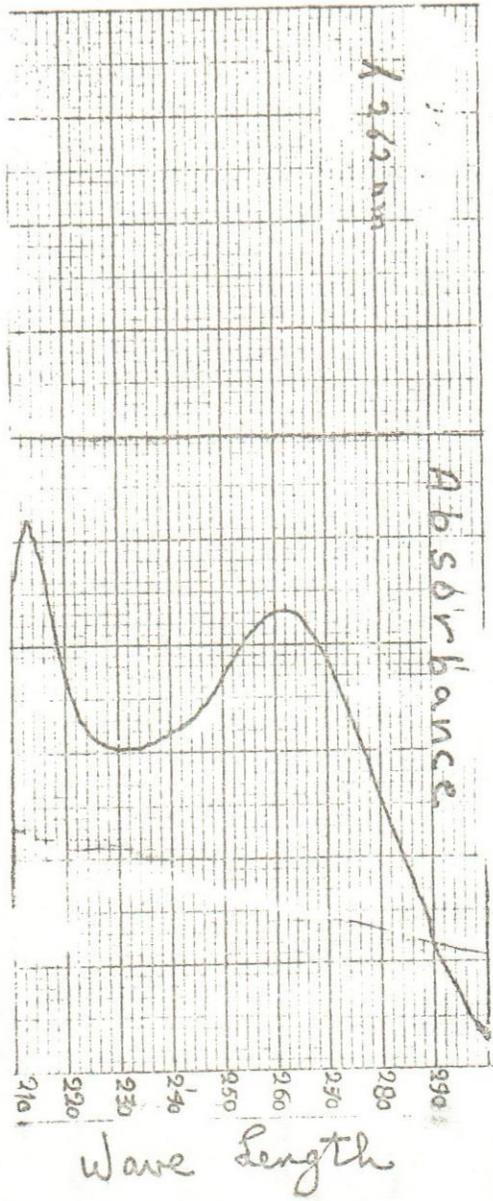


Fig ( 3 ) Absorption spectra of Dimilin  
in ethanol ( conc. 180 ).

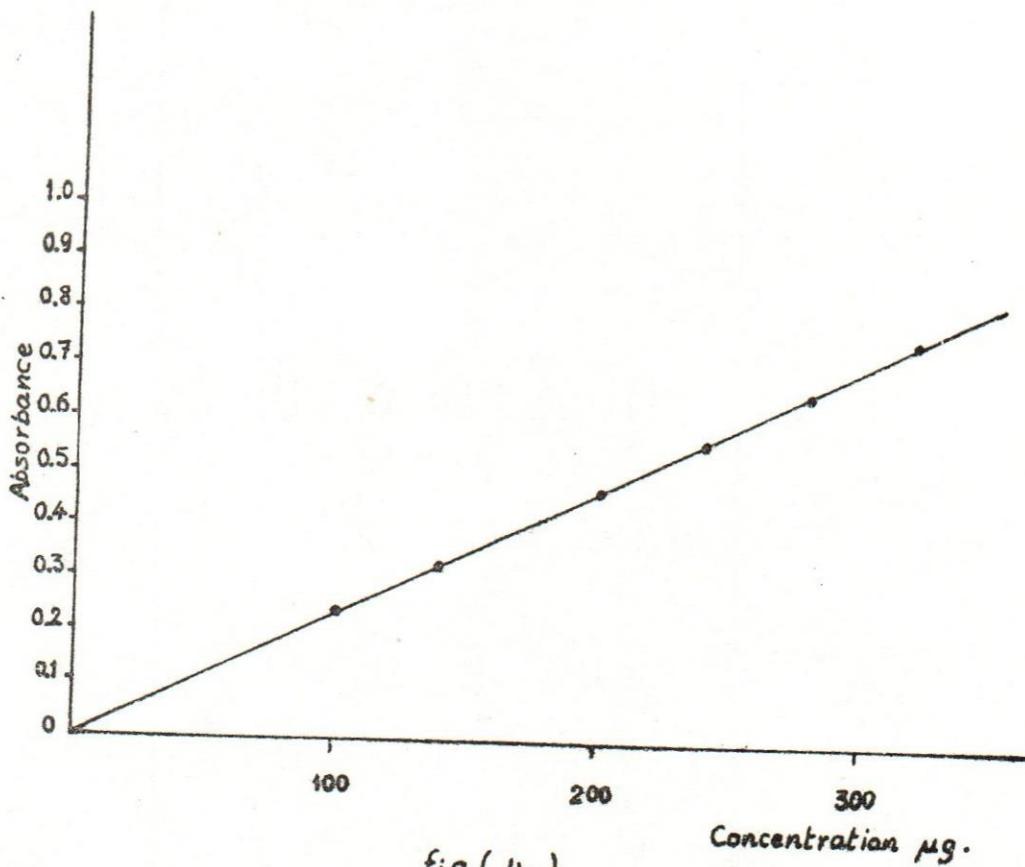


fig (4)

Standard curve of Dimiline in ethanol  $\lambda$  262 nm

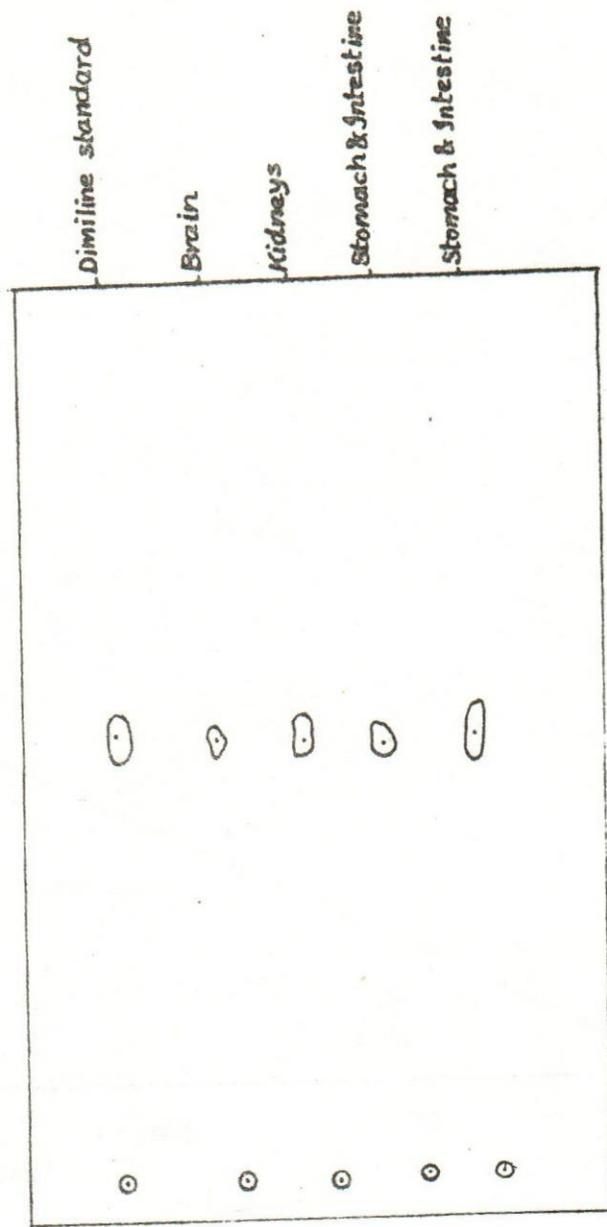


fig ( 5 )

TLC of extracted Dimilene from different organs .

System : benzene - ethanol  
4 : 1