Entomology

Effect of methanolic extract of *Lantana camara* on some physiological and histological aspects of the black rat, *Rattus rattus*, with field evaluation

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Abstract

Black rats, *Rattus rattus* (L.) (Rodentia: Muridae) (*R. rattus*), belong to the rodents that have a major negative impact on the agriculture economy in all aspects. The objective of the research was to determine the effect of a methanolic extract of *Lantana camara* leaves (*L. camara*), which was employed as a natural source of plant-derived compounds, on the control procedure against *R. rattus*. The results indicate that the LD_{50} and LD_{90} values for *lantana* extract were 894.43 and 2948.96 mg/kg body weight, respectively. Significant decrease in the test animals' body weight. As a biological response indicator, there is a significant increase in some biological parameters such as alnine transaminase (ALT), aspartate transaminase (AST), serum urea, creatinine, and lactate dehydrogenase (LDH). The liver, kidney, and heart sections had histopathological changes, including degeneration and necrosis in many hepatic regions with pyknotic nuclei. Certain convoluted tubules have vacuolar degeneration, and the proximal and distal convoluted tubules' structures are disorganized. The cardiac muscle fibers are widely spaced from each other and have certain necrotic areas. The methanol leaves extract included 11 compounds, according to GC/MS analysis. Field evaluation by employing bait consumption resulted in an acceptable reduction of 65.36% for *lantana* extract and an 84.72% reduction for the commercially rodenticide zinc phosphide.

Keywords: *rattus*, methanolic extract of *Lantana camara* leaves, toxicity, histology and field evaluation

Introduction

Rodents are a problem for agriculture and public health because of the big economic loss they may cause due to their variety, generalist food habits, and high reproduction rates (Singla and Babbar 2012; Herbreteau *et al*. 2012) [1,2]. Rodents are regarded as being among the main pests in Egypt and other regions, inflicting substantial damage to agricultural crops and poultry farms (Neena and Babbar, 2010) [3]. One of the most widespread and economically powerful rodents is the black rat, *Rattus rattus* (L.) (Rodentia: Muridae) (*R. rattus*), which not only causes severe damage to agricultural crops but also has negative value as a disease carrier (Witmer and Shiels, 2017) [4]. The environment, users, and farmers' health are all put in danger by the toxicity of traditional rodenticides, as a result, interest in developing pesticides with active constituents that originate from plants has increased due to their low cost and lack of adverse environmental pollution (Tripathi *et al*., 2008) [5]. The noxious ornamental weed *Lantana camara* Linn (Verbenaceae) (*L. camara*) has aromatic leaves (Mankani *et al*., 2005) [6]. A lot of *Lantana* species are poisonous to livestock. Due to the presence of active compounds, pentacyclic triterpenoids known as Lantadenes (A and B) *L. camara* is toxic to grazing animals. Lantadene type-A (rehmannic acid) is the most potent toxic principle. These toxins are absorbed across the entire gastrointestinal tract, particularly the small intestine, causing hepatotoxicity and jaundice in animals (Sharma *et al*., 2007) [7]. It has been used to treat a wide range of disorders all over the world (Pour and Sasidharan, 2011) [8]. *L. camara* leaf extracts have insecticidal, fungicidal, antimicrobial, nematocidal and larvicidal effects (Vadlapudi and Naidu, 2010; Saraf *et al*., 2011) [9, 10].

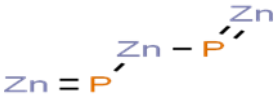
Therefore, the current study's objective is to evaluate the potential efficacy of methanolic extract of *Lantana camara* leaves as a rodenticide against the black rat, *Rattus rattus*, by examining the extract's effects on some physiological parameters, histological changes, and field assessments that determine the extract's ability to reduce rat populations in the field.

Materials and methods

1. Tested materials

1.1. Methanolic extract of *Lantana camara* leaves.

1.2. Zinc phosphide 80% DP was obtained from El-Nasr pesticides company, Egypt.



Zinc phosphide

2. Animals

In fields in Aga district of Dakahlia Governorate, Egypt, traps were used to catch the black rat, *R. rattus*. Rats were brought to the Plant Protection Research Institute branch in Mansoura, where each one of them was kept in a cage (50×30×30) for 2 weeks so they could become accustomed. They fed *ad libitum* on water and a standard diet of (65% crushed maize + 25% powdered wheat + 5% sugar + and 5% corn oil). Five adult and

healthy rats were selected for each group, which ranged in weight from 150 to 200 g, in addition to the corresponding group for the control.

3. Plant material and extraction

With a few minor modifications, the (Freedman *et al*. 1979) [11] technique was used for plant extraction. Fresh leaves of *L. camara* were obtained from Mansoura town, Egypt. The herbarium at the Faculty of Science, Mansoura University verified the plant's identity. Fresh leaves were air dried at room temperature and ground into a fine powder. Maceration of the dry leaves 1500g in 2.5 litters of methanol (solvent). Following 72 hours, the extract was sieved, and the liquid was filtered using a rotary evaporator while being vacuum-sealed at a temperature of no more than $50^{C^{o}}$ until completely dry. The crude extract was next put into little glass bottles and refrigerated until testing. The chromatographic analytical GC-MS was used to analyze the crude extract.

4. Laboratory experimentation

4.1. Toxicological test (LD_{50}) value

Serial doses of methanolic leaf *Lantana* extract 400, 800, 1000, and 2000 mg/kg body weight were given orally by stomach tube to *R. rattus*. Animals used as controls were given distilled water, while the rats ingested these doses after a 12-hour fast and were allowed food and water after two hours of treatment. The mortality percentage was monitored for up to seven days. LD_{50} and LD_{90} values were determined using Abbott's approach. (Abbott, 1925) [12] and the statistical method of probit analysis (Finney, 1971) [13].

4.2. Physiological analysis

The lethal dosage LD_{50} of *lantana* extract was examined as a physiological response. Animals were given oral intubation for 24 hours. After 3 days of treatment, the animals were sacrificed, and blood samples were collected from each rat and placed in sterile tubes. Biochemical parameters of serum specimens from the untreated and treated groups were estimated, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), which were measured using colorimetry (Huang *et al*. 2006) [14], serum urea using (Wang *et al*. 2022) [15], creatinine (Imasawa *et al*. 2021) [16], and lactate dehydrogenase (LDH) (Khan *et al*., 2020) [17]. At the start and end of the experiment, the weights of each rat in the treated and control groups were recorded. The percentage of body weight reduction was calculated.

4.3. Histological studies

Histological testing of the liver, kidney, and heart tissues to evaluate the effect of the lethal dosage LD_{50} of *Lantana* extract. Three days after the start of the treatment, the rats were sacrificed and dissected to obtain liver, kidney, and heart specimens from the control and treated groups. The specimens were immersed in 10% formalin for fixation. The samples were sent to the Faculty of Medicine's Histopathology Laboratory at Mansoura University for analysis. To demonstrate the histological examination, tissue sections with a thickness of 5µ were stained with hematoxylin and eosin (Creasy *et al*., 2021) [18].

4.4. Analysis of *lantana* extracts using gas chromatography-mass spectrometry (GC-MS)

Using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, Texas, USA), the chemical structures of the sample were determined with a capillary column TG-5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temp was maintained at 60°C, and then elevated by 6°C/min to 250°C with a 1-minute pause before flowing at 300 °C at 30 °C/min. The injector temperature was held at 270°C. Helium was applied as a carrier gas at a flow rate of 1 ml/min. The solvent latency was 4 min, and a diluted sample of 1 µl was injected using an Autosampler linked to the AS3000 with GC. EI mass spectra were taken at 70 eV ionization voltages over the range of m/z 50–650 in a high-speed scanning manner. The transfer line and ion source were both adjusted to 200 and 280 °C, respectively. The components were described by comparing their mass spectra with those of the Wiley and NIST mass databases (Abd El-Kareem *et al.*, 2016) [19].

5. Field evaluation

A field assessment of crushed maize bait containing 2% *lantana* extract and 1% zinc phosphide-treated groups was conducted in Aga district, Dakahlia Governorate. The black rat, *R. rattus*, has infested the whole area. A feddan-sized plot of each compound got treatment, while a plot of a similar size served as the control. By the food consumption technique, the rat population density was evaluated prior to and after the treatment. (Dubock, 1982) [20]. Each plastic bag weighed 2 kg and included 100g of the indicated bait. It was left in the selected plot for five days, and the amount of tested bait consumption was measured. The population reduction was calculated using the formula below:

$$\text{Population reduction \%} = \frac{\text{Pre-treatment consumed} - \text{post-treatment consumed}}{\text{Pre-treatment consumed}} \times 100$$

6. Statistical analysis

The LD_{50} values were stated in mg/kg body weight. All of the data, which were all displayed as mean ±SE, were subjected to a one-way analysis of variance (ANOVA) (St and Wold, 1989) [21]. The 95% simultaneous confidence intervals were calculated using Tukey's methodology. A 0.05 probability was considered significant. Using Cohort Software, each statistical evaluation was performed (Cho *et al*., 2004) [22].

Results and discussion

1. Acute oral toxicity LD_{50}

The toxicity of *lantana* extract against *R. rattus*, is shown in Table (1). The LD_{50} and LD_{90} values for *lantana* extract were 894.43 and 2948.96 mg/kg.b.w. respectively, with a slope of 2.47. There is a marked difference between the oral (60 mg/kg) and intravenous (1-3 mg/kg) doses of Lantadene A, which produced hepatotoxicity and cholestasis in sheep (Sharma *et al*., 2007) [7]. Hepatic necrosis rather than the characteristic cholestatic lesions of *lantana* poisoning were caused with an intravenous dosage greater than 3 mg/kg (Kumar *et al*., 2016) [23].

Table 1: The acute toxicity of *Lantana* extract against *R. rattus*

Treatment	LD_{50}	LD_{90}	Slope ±SE
Lantana extract	894.43	2948.96	2.47 ±1.29

LD_{50} and LD_{90} values expressed as mg/kg body weight

2. Body weight reduction

The data presented in Table (2) indicated that the *lantana* extract significantly reduced the body weight of the tested rats, the mean body weight of the treated rats lowering from 197.14 gm. to 115.71 gm., with a weight reduction percentage of - 41.30 gm. in comparison to the control group. The animals experience constipation immediately after consuming *lantana* leaves, and they stop eating after about two hours (Shyamkumar *et al*., 2021) [24].

Table 2: Effect of *Lantana* extract on body weight reduction % against *R. rattus*

Treatment	Mean Body weight (g)		
	Before	After	Reduction percentage
Lantana extract	197.14 ±3.76^b	115.71 ± 5.61^b	- 41.30
Control	216.43 ±4.59^a	274.29 ± 4.56^a	+ 26.73
P-Value	0.007	0.000	—

Values that express mean ± SE. within the same column that don't have the same letter (a or b) are significantly different.

Biochemical analysis

A number of biological parameters, including ALT, AST, serum urea, creatinine, and LDH, were measured and analyzed as a biological response to *Lantana* extract as a possible rodenticidal compound. The results are shown in Table (3). When *Lantana* extract was administered to *R. rattus* rats, a significant elevation in AST, ALT, serum urea, and LDH concentration was observed as compared to the control group. The liver, kidneys, and muscles all contain significant amounts of the transaminase enzyme known as AST. When these organs are damaged, their contents are released into the bloodstream; an increase in this release into the bloodstream indicates liver toxicity (Nirmal *et al*., 2021) [25]. When animals were exposed to LD_{50} value, AST, ALT, urea, creatinine, and LDH elevation values may be seen as a reflection of what occurs inside the body in terms of dissolution and malfunctioning and a direct effect of organ damage, especially liver and kidney damage (Elhamalawy *et al*. 2022) [26]. According to Asadu *et al*. (2015) [27], giving albino mice *Lantana camara* methanol leaf extract caused a significant ($p < 0.05$) increase in ALT, AST and ALP activities, urea and creatinine concentrations when compared to the control group.

Table 3: Effect of LD_{50} of *Lantana* extract on some biochemical parameters of *R. rattus*

Treatment	AST (U/ml)	ALT (U/ml)	Serum urea (mg/dl)	Creatinine (mg/dl)	LDH (U/L)
Lantana extract	45.2^a ± 0.22	46.04^a ±0.75	30.06^a ± 0.12	0.76^a ± 0.07	115.62^a ± 1.28
Control	40.24^b ± 0.58	31.12^b ± 0.40	25.3^b ± 1.03	0.66^a ± 0.05	98.44^b ± 0.41
P-value	0.00	0.00	0.019	0.026	0.00

Values that express mean ± SE. within the same column that don't have the same letter (a or b) are significantly different

3. Histological alterations

3.1 Liver

Examination of the liver's histological sections in the control *R. rattus* demonstrated that the normal hepatic lobule was comprised of masses of liver cells (hepatocytes), which were arranged in the shape of liver cords extending from the central vein. The hepatocytes were rounded in shape, with central and vesicular nuclei. The liver cords were separated from each one other with blood sinusoids bordered by endothelial cells and Von Kupffer cells (Figure 1A). Histological lesions of a liver from rats treated with *Lantana* extract showed pyknotic nuclei, necrosis, and degeneration in various hepatic areas (Figure 1B).

3.2 Kidney

Histological sections revealed the kidney's typical structure. The renal cortex is formed from Malpighian renal corpuscles and (proximal and distal convoluted tubules). Malpighian renal corpuscles consisted of a normal glomerulus with basement membrane, normal Bowman's capsules, normal cellularity surrounding tubules, and normal blood vessels (Figure 1C). The epithelial cells in some convoluted tubules showed vacuolar degeneration as a result of the *Lantana* extract treatment's effects on the kidney's histological alterations. Also, there is some necrosis, and the architecture of the proximal and distal convoluted tubules is disorganized (Figure 1D).

3.3 Heart

An examination of the control *R. rattus* heart's histological sections indicated that the myocardium was shown to be composed of branching and continuous cardiac muscle fibers moving in various directions. The cells that formed the cardiac muscle fibers were joined to each other by intercalated discs. Their cytoplasm seemed striated, and their nuclei were central and vesicular (Figure 1E). Compared to the control group, the histologic changes in the heart following treatment with *Lantana* extract indicated that the muscle fibers are widely separated from one another and there are some necrotic regions (Figure 1F).

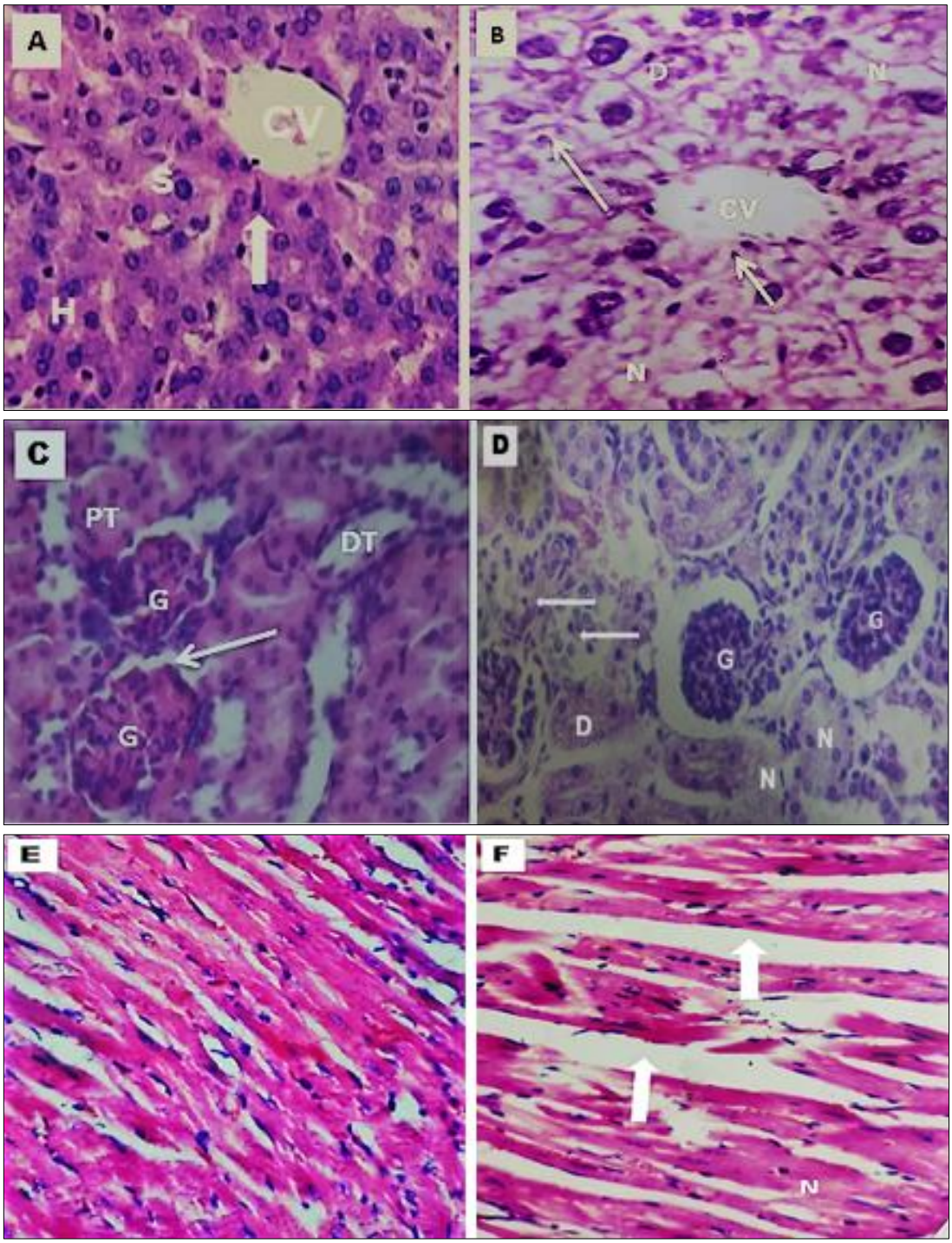


Fig 1: Photomicrographs of liver sections of *R. rattus*. [A]: Control, displaying the central vein (CV), Hepatocytes (H), blood sinusoids (S) and Kupffer cells (Arrow). [B]: After treated with *Lantana* extract, showing, necrosis (N), degeneration (D) and pyknotic nuclei (Arrow). Sections in kidney renal cortex [C]: Control, displaying Glomerulus (G), Bowman's capsules (Arrow), normal proximal (PT) and distal (DT) tubules. [D]: After treated with *Lantana* extract, showing, degeneration (D), necrosis (N) and disorganized PT and DT convoluted tubules (Arrows) (H & E X400). Sections in heart [E]: Control, showing normal arrangement of muscle fibers. [F]: After treated with *Lantana* extract, displaying widely separated of muscle fibers (Arrows) and some necrotic areas (N) (H & E X200).

The liver is generally considered to be the most important organ for the excretion of toxins or any other metabolites from the body. It is a vital organ that helps with food digestion, stores and synthesizes glucose, and regulates and eliminates dangerous substances from the body (Sun *et al*., 2021) [28]. In areas where *lantana* is common, grazing animals' consumption of plant leaves results in hepatotoxicity, which is a major cause of sheep illness and mortality (Sharma and Makkar, 1981) [29]. The livers of guinea pigs that had consumed *L. camara* showed swelling of the hepatic cells, increase of the bile canaliculi, vacuolation of the hepatocytes, pyknosis of the nuclei, and hydropic degeneration upon histopathological investigation (Sharma *et al*., 1992) [30]. The essential roles of the kidney are to remove waste and toxins as well as exogenous chemicals like pigments (Effendy *et al*. 2006) [31]. In Kumar *et al*., (2018) [32] research of the effects of *lantana* extract oral treatment on guinea pig kidneys; he observed hyaline cast accumulation, vacuolar degeneration of tubular epithelium and pyknosis of some convoluted tubules. *Lantana* administered to sheep showed patchy heart muscle fiber degradation, a few petechial

haemorrhages, and fragile myocardial fibers, in addition to pulmonary edoema and lung emphysema (Jadhav *et al*., 2017) [33].

4. Chemical components of *Lantana* extract

GC-MS analysis of *Lantana* methanol extract showed the presence of eleven compounds with wide molecular weight range as shown in Table (4) and Figure (2). Compounds were recognized based on their retention times, as follows: 2-propenoic acid, 2 ethyl hexyl ester, 1-ethylcyclohexanol, N-methyl-N-vinyl-2-methylpropanamide, 1-Tetradecanol, 8-Pentadecanone, 3-methyl-2-(2-methylallyl) furan, 1,6-Hexanediol, 1-Hexadecene, Phytol, Myristic acid and Neophytadiene. Studies on the phytochemistry of many *Lantana* species revealed the presence of alkaloids, steroids, terpenoids, flavonoids, and glycosides, which are known to have a variety of biological effects (Bashir *et al*., 2019) [34].

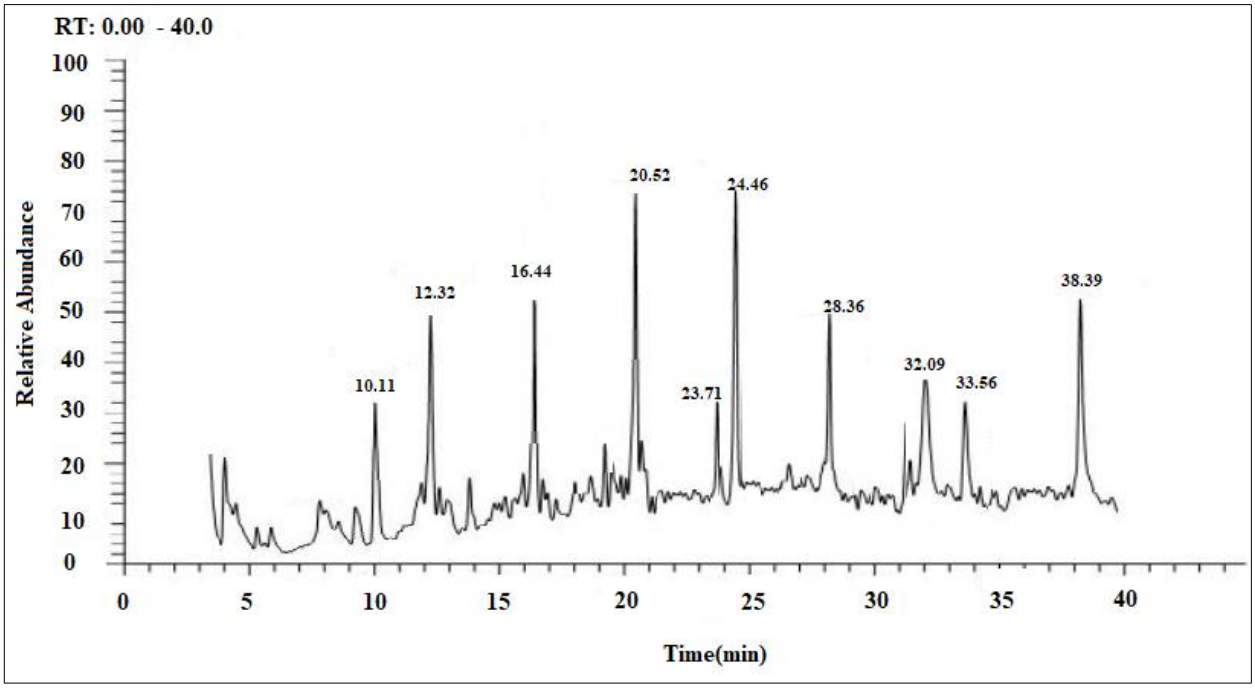


Fig 2: GC/MS analysis of *lantana* extract

Table 4: Major components of *lantana* extract identified by GC/MS

No.	Compound	Retention Time (min)	% Abundance	Molecular Formula
1	2-propenoic acid, 2 ethyl hexyl ester	8.13	8.07	$C_{11}H_{20}O_2$
2	1-ethylcyclohexanol	10.11	6.58	$C_8H_{16}O$
3	N-methyl-N-vinyl-2-methylpropanamide	12.32	8.06	$C_7H_{13}NO$
4	1-Tetradecanol	16.44	7.13	$C_{14}H_{30}O$
5	8-Pentadecanone	20.52	8.55	$C_{15}H_{30}O$
6	3-methyl-2-(2-methylallyl) furan	23.71	4.73	$C_9H_{12}O$
7	1,6-Hexanediol	24.46	6.68	$C_6H_{14}O_2$
8	1-Hexadecene	28.36	7.02	$C_{16}H_{32}$
9	Phytol	32.09	8.08	$C_{20}H_{42}O$
10	Myristic acid	33.56	7.89	$C_{14}H_{28}O_2$
11	Neophytadiene	38.39	8.64	$C_{20}H_{38}$

5. Field evaluation

Results in Table (5) showed that, using the bait consumption method in the field, the effectiveness of *Lantana* extract and the suggested rodenticide zinc phosphide El-Nasr proved effective against the black rat, *R. rattus*. *Lantana* extract and zinc phosphide pre-treatment mean consumption of untreated crushed maize was 255.34 g and 277.28 g, respectively, whereas post-treatment consumption was 88.44 g and 42.38 g for both. Zinc phosphide bait reduced the population by 84.72 percent, and *lantana* extract reduced it by 65.36 percent. In field application, the Norwegian rat population was decreased by 66.5% with Oshar plant extract and by 78.4% with zinc phosphide, respectively (Eisa and Yassin, 2016) [35].

Table 5: Field evaluation of *Lantana* extract against the black rat, *R. rattus*, at EL- Dakahlia Governorate

Compound	Bait consumption (g /Feddan)			
	Pre-treatment	Treatment	Post-treatment	Reduction percentage
Lantana extract	255.34±11.32^a	186.18±37.03^a	88.44±6.82^b	65.36
Zinc phosphide	277.28±8.09^a	155.2±16.40^a	42.38±3.67^c	84.72

Control	283.74±12.21[a]	-	271.2±4.81[a]	-
P-Value	0.186	0.466	0.000	-

Values that express mean ± SE. within the same column that don't have the same letter (a or b) are significantly different

Conclusion

The current study demonstrates the toxicity of methanolic leaf *lantana* extract against the black rat, *Rattus rattus*, as a potential rodenticide component. The link between the *lantana* extract, biochemical measures, and histological examination of the liver, kidney, and heart was studied. The physiological and histological parameters changed, according to the laboratory testing. In comparison to the conventionally recommended rodenticide, zinc phosphide, field conditions indicated that *lantana* methanol extract achieved satisfactory results in terms of reduction percentage and controlling the rat population.

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