Trends in Agricultural Sciences

OPEN ACCESS

Behavioral Changes in Male Gerbils Following Subacute Exposure to K-Optimal 35 EC Pesticide Formulation

[1]Faustin Pascal Tsagué Manfo, [2]Asamaou Ayinuih Sahfua, [1]Rene Bilingwe Ayiseh, [3]Nadège Emégam Kouémou, [4]Edouard Akono Nantia and [5]Fidelis Cho-Ngwa

[1]Laboratory for Drugs and Molecular Diagnostics Research (ANDI Centre of Excellence for Onchocerciasis Drug Research), Biotechnology Unit, Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Buea, Cameroon

[2]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P.O. Box 63 Buea, Cameroon

[3]Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P.O. Box 63 Buea, Cameroon

[4]Department of Biochemistry, Faculty of Science, University of Bamenda, P.O. Box 39 Bambili, Cameroon

[5]Laboratory for Drugs and Molecular Diagnostics Research (ANDI Centre of Excellence for Onchocerciasis Drug Research), Biotechnology Unit, University of Buea, Buea, Cameroon

ABSTRACT

Background and Objective: Despite their benefits in agriculture, pesticides can be hazardous to non-target organisms, including animals and humans. The present study assessed the subacute toxicity of K-Optimal, an agro-pesticide formulation containing lambda-cyhalothrin and acetamiprid. **Materials and Methods:** Three groups of male gerbils were given either the vehicle or K-Optimal at doses 8.75 and 17.5 mg kg^{-1}, 6 days a week for 4 weeks. The animal's body weight was recorded every 4 days and behavioral tests were carried out from day 20-25 of the experiment to assess anxiety, exploration and motor coordination. The gerbils were sacrificed on day 29 and serum was used for assessment of cholinesterase and aminotransferase activities, uric acid, total antioxidant capacity (TAC) and testosterone levels. Brain nitric oxide (NO) levels were also determined while catalase activity, glutathione and thiobarbituric reactive substances levels were assessed in the liver, testis and brain. **Results:** Exposure to K-Optimal resulted in anxiety-like behavior characterized by decreased suspension time in the hanging wire test and decreased grooming frequency and time spent at the center in the open field test. The K-Optimal induced increased liver weight while decreasing body weight. There were decreased brain NO levels as well as increased TAC, though the latter occurred only in the animals exposed to 8.75 mg kg^{-1} pesticide. **Conclusion:** Subacute exposure to K-optimal induced anxiety-like behavior and impaired motor coordination in the gerbils, which may result from decreased brain NO levels. The pesticide also alters body and liver weights and should therefore be considered a potential health hazard for humans and other animals.

KEYWORDS

Anxiety, brain, gerbils, K-Optimal, nitric oxide, pesticide toxicity

ISSN: 2957-9449 (Online)
ISSN: 2957-9430 (Print)
https://doi.org/10.17311/tas.2023.249.260

Received: 14 Jan. 2023
Accepted: 06 Jun. 2023
Published: 30 Sep. 2023

INTRODUCTION

Pesticides are either chemical or biological agents, widely used in agriculture that deter, incapacitate, kill, or otherwise prevent pests from affecting crops[1,2]. Pesticides are increasingly used in tropical regions and elsewhere to control biotic problems of insects, weeds and pathogens which interfere with crops and livestock production. Unfortunately, pesticides also cause severe environmental pollution and health hazards in nontarget organisms including humans[3-5]. Pesticide toxicity from repeated low-level exposures (mainly through food/drinks) in non-target animals may be acute, chronic or even not manifest for decades[6]. Pesticides may induce oxidative stress, that is, the imbalance between pro-oxidant molecules (reactive oxygen and nitrogen species) and antioxidant defence mechanisms. The toxicity of pesticides may also induce hepatocellular disorders and kidney and reproductive dysfunctions. Altered neurological function with cholinesterase inhibition by pesticides was also reported[5,7-9].

The K-Optimal 35 EC is a pesticide formulation frequently used in the South-West Region of Cameroon to kill insects that interfere with crop production. It contains two active ingredients, acetamiprid and lambda-cyhalothrin in a ratio of 20:15. Acetamiprid is a neonicotinoid, a relatively new family of pesticides. It has been used to kill Lepidoptera and Hemipteran insects such as aphids, on crops like fruits, tea and vegetables, which have gained resistance to organophosphates, carbamates and synthetic pyrethroid[10,11]. Lambda-cyhalothrin on the other hand is a type II synthetic pyrethroid, used to control a wide range of insects and pests both in food and in non-food crops. Pyrethroids disrupt the normal functioning of sodium channels thereby causing a longer delay in sodium channel inactivation and inhibiting sodium channel-dependent activity of cortical neurons[6]. However, these 2 active ingredients of K-Optimal 35 EC have been shown to induce dysfunction in mammals including humans. Acetamiprid may affect the level of cellular oxidative stress in mice. Acute poisoning with acetamiprid results in vomiting and convulsions in humans[12,13]. Lambda-cyhalothrin alters reproductive function in male rats. It increases lipid peroxidation and inhibits antioxidant enzymes in human erythrocytes. The DNA damage was also reported in human lymphocytes and mice exposed to lambda-cyhalothrin, suggesting induction of the adverse immune effects by the chemical[14-16]. Despite the aforementioned effects of the respective active ingredients, information on the subacute effects of the formulation of these two ingredients K-Optimal 35 EC, on animal behavior and other vital functions, has not been-over-emphasized. Thus, the present study was conducted to assess the behavioral changes in male gerbils following exposure to K-Optimal 35 EC on the liver, kidney, brain and reproductive function.

MATERIALS AND METHODS

Study area: The study was carried out at the Laboratory for Drugs and Molecular Diagnostics Research, Biotechnology Unit, University of Buea, Cameroon. The study was conducted in June to November, 2019.

Materials: The K-Optimal 35 EC (lot N° 20180816) containing lambda-cyhalothrin (15 g L^{-1}) and acetamiprid (20 g L^{-1}), manufactured by Mano-China and distributed by Louis Drefus Commodities (Z.I Bonaberi BP 2368) in Douala (Cameroon), was purchased from a local agro-pesticides shop in Muea (Buea, Cameroon). Experimental animals were 18 adult laboratory male Mongolian gerbils (*Meriones unguiculatus*, aged 7-8 months old, weighing on average 55-60 g), raised in the animal house of the Laboratory for Drugs and Molecular Diagnostics (Biotechnology Unit, Faculty of Science, University of Buea). The gerbils were housed in clean cages with sawdust as their beddings and fed with standard animal feed consisting of ground corn, soybeans, premise and bones. Animal ethical consideration regarding the safe use of experimental animals was obtained from the University of Buea-Institutional Animal Care and Use Committee (UB-IACUC) approved under permit number UB-IACUC No 020/2019.

Methods

Experimental design and procedure: Male gerbils were divided into three groups (six animals per group). The control group received the vehicle (distilled water) while other groups were treated with K-Optimal at doses 8.75 and 17.5 mg kg^{-1}. The pesticide/vehicle was given to gerbils orally through

gavage, once a day, 6 days a week for 4 weeks. The oral median lethal dose 50 (LD_{50}) values of acetamiprid and lambda-cyhalothrin in mice or rats were ≤198 and ≤79 mg kg^{-1}, respectively. Therefore, the highest dose of K-pptimal administered, 17.5 mg kg^{-1}, corresponds to LD_{50}/19.8 of acetamiprid and LD_{50}/10.5 of lambda-cyhalothrin[16,17]. The animals were observed once daily, for detection of morbidity and mortality. Behavioral and motor coordination were assessed using a hanging wire test, open field test and elevated plus maze test, conducted on day 20, day 22 and day 25 of the experiment, respectively. The gerbils were sedated with ketamine/xylazine and sacrificed on day 29 and serum obtained from blood was stored at -20°C for further biochemical analyses. The liver, brain, kidneys and testes were dissected out and weighed and homogenates of the liver and brain were prepared in sodium phosphate buffer (100 mM, pH 7.4) for further biochemical analyses.

Behavioral Assessment and biochemical analyses

Hanging wire test: The hanging wire test was conducted after a two days' trial session (days 18 and 19) on the ability of animals to hang with only the forepaws to a wire cable of 2 mm in diameter taut between two stands at 55 cm apart and 50 cm above a layer of bedding. Each gerbil has suspended five times with a resting time of one minute apart and the longest suspension time within five minutes was recorded[9].

Open-field test: To assess locomotor activity, an open field with the dimensions (40 cm width×40 cm length×45 cm height) was fabricated locally and used. The open field had lines dividing its floor into 16 smaller squares of equal dimensions (10×10 cm). The assay was initiated by placing an individual gerbil at the center of the apparatus. Then, the gerbil was observed for five minutes to record the number of lines crossed with all four paws, frequency of rearing, rearing on the hind limbs, stretch attempt posture, grooming and the number of defecations. The exploratory activities were also assessed through the record of the number of center square entries, duration in the center square and total distance traveled[9]. The apparatus was thoroughly cleaned with 70% ethanol after testing each gerbil.

Elevated plus maze test: In order to evaluate the exploratory and emotionality behavior of the gerbils, a wood-made plus maze elevated to a height of 35 cm was used. The apparatus consisted of two open arms (30×8 cm, in length by width, respectively) and two closed arms (30×8×20 cm in length, width by height, respectively), that extended from a common central platform (8×8 cm) arranged so that both open and closed arms faced each other. The test was initiated by placing a gerbil at the center area of the maze facing an open arm. The animal was carefully observed (by a person situated 2 m far from the maze) for record of the time spent at the center, exploring the open and closed arms, the number of entries into the open and closed arms, the number of head dipping and grooming for the duration 5 min test session. Entry into an open or closed arm was counted only when all four paws were placed into the corresponding arm. Also, a greater frequency of open-arm activity and head dipping, indicated a greater level of exploration for the animals[18,19]. Emotionality or fear behaviors include closed-arm activity, stretch attempts, grooming, freezing and defecation[9]. The maze was thoroughly cleaned with 70% ethanol after testing each gerbil.

Biochemical analyses

Assessment of serum biochemical parameters: The ferric reduction antioxidant power (FRAP) method was used to determine serum total antioxidant capacity (TAC)[20]. This method was adapted to a 96-well plate[5]. Summarily, 100-1000 µM standard solutions of $FeSO_4.7H_2O$ were prepared. Five microliters of each standard solution or sample were pipetted into 96 well plates containing 65 µL of acetate buffer (300 mM, pH 3.6). A freshly prepared FRAP reagent was added to all wells (100 µL in each well) and the absorbance was read after 4 min at 595 nm. The TAC value for each sample was extrapolated from a standard curve and expressed as µMFe(II) equivalent[5]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured in serum using kits from Chronolab Systems S.L. (Barcelona, Spain), as per manufacturer's instructions. A kit from Chronolab Systems was also used for the assessment of the uric acid levels, as reported earlier[7]. For the assessment of testosterone levels, a

competitive ELISA kit from Calbiotech was used and the assay was conducted as described by Manfo *et al*.[5]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were determined spectrophotometrically in both serum and brain homogenate as described by Jońca *et al*.[21] with slight modification[5].

Determination of biochemical parameters in brain, liver and testis homogenates: Nitrite ions were measured in brain homogenate as an indicator of nitric oxide (NO) production by Griess reagent[22]. Briefly, the Griess reagent (1% sulphanilamide and 0.1% naphthylethylenediamide, v/v) was mixed either with sodium nitrate standard solution (0.00625- 0.2 mM, prepared by dilution of a 0.2 mM stock in phosphate buffer pH 7.4) or homogenates. Absorbance was read at 546 nm and concentrations of nitrite extrapolated from the standard curve. For catalase activity, H_2O_2 was used as substrate and its degradation was monitored for 90 sec (30, 60 and 90 sec) using a spectrophotometer at the wavelength of 240 nm[23]. Reduced glutathione and thiobarbituric reactive substances (TBARS) levels were measured using Ellman's reagent 2,2'-dinitro-5,5'-dithio-dibenzoic acid and 2-thiobarbituric acid, respectively[24,25]. All these parameters were normalized to the protein concentration of the tissue homogenate[26].

Statistical analysis: Body weight variation and relative organ weights were calculated for the experimental animals. All data obtained including biochemical parameters were expressed as Mean±Standard Error of Mean (SEM) and analyzed using MedCalc vs. 14.8.1 software. The data were tested for normality using the Kolmogorov-Smirnov Test. This was followed by the Analysis of Variance (one-way ANOVA test) and the Student-Newman-Keuls Test for normally distributed data, or the Kruskal-Wallis Test for skewed data. Differences were considered to be significant at $p<0.05$.

RESULTS

Clinical signs, relative body and organ weights

Clinical signs: Various clinical symptoms were manifested by treated gerbils from day 4 till the end of the experiment. As presented in Table 1, test group gerbils receiving the highest dose of K-Optimal (17 mg kg^{-1}) showed a tendency of mouth smacking and salivation, tremors, loose fur and respiratory depression. However, the group exposed to the lower dose showed respiratory depression only (Table 1).

Relative body weights: Outcomes of the relative body weights (%) were shown in Fig. 1. A K-Optimal induced a significant decrease ($p<0.05$) in weights of gerbils at the doses of 8.75 and 17.5 mg kg^{-1}, when compared with the vehicle group, from day 4 till the end of the experiment ($p<0.05$).

Relative weight of organs: The relative weights of organs (g/100 g body weight of the animal) of gerbils are presented in Fig. 2. Treatment with K-Optimal at the higher dose, 17.5 mg kg^{-1}, significantly increased liver weight by about 30% ($p<0.05$) when compared to the vehicle group. However, other organs assessed were not altered following exposure to the pesticide formulation ($p<0.05$).

Hanging wire, open field and elevated plus maze results

Hanging wire: A Hanging wire test was carried out during the experiment and the results were presented in Table 2. As shown by the duration, K-Optimal dose-dependently induced a decrease in the time of suspension of gerbils on the wire. The decrease in time of suspension was significant ($p<0.05$) when the animals were exposed to the highest dose of pesticide, 17.5 mg kg^{-1}, when compared to the vehicle group.

Open-field data: The treatment of gerbils with K-Optimal resulted in a decrease in the number of grooming and total time spent at the center of the open field by gerbils compared to the control. The group treated with 17.5 mg kg^{-1} showed a significant decrease ($p<0.01$) in the number of grooming and total time spent at the center when compared to the control group. However, the number of crossings, rearing and fecal mass of the gerbils were not affected by intoxication with K-Optimal as shown in Table 2.

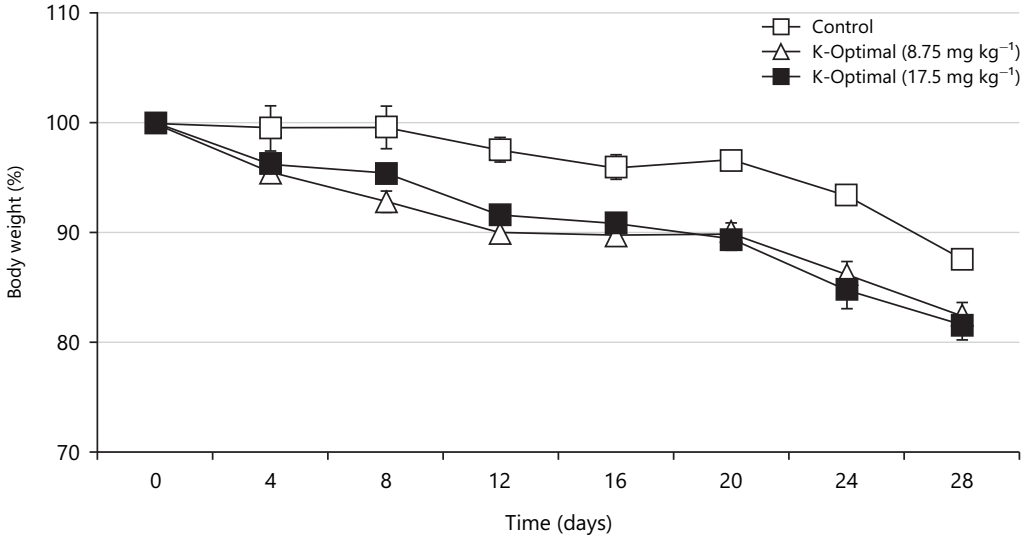

Fig. 1: Variation of the relative body weight of gerbils during exposure to K-Optimal

Values represent means and SEM values for six gerbils per group. The significant difference when compared to the vehicle group treated with distilled water, at $p<0.05$ (Student-Newman-Keuls Test)

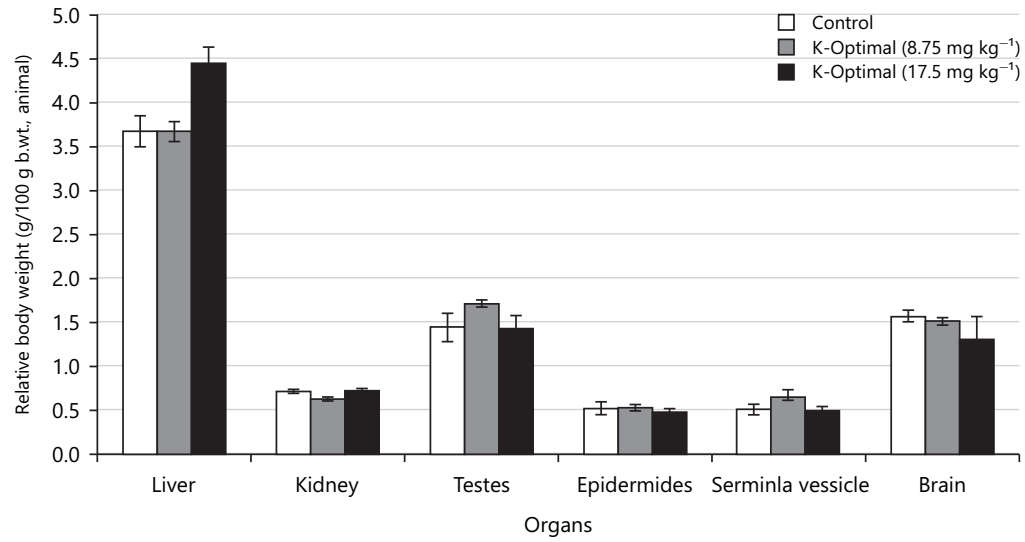

Fig. 2: Relative organs weights of gerbils after exposure to K-Optimal

Values represent means and SEM values for six gerbils per group. The significant difference when compared to the vehicle group treated with distilled water, at $p<0.05$ (Student-Newman-Keuls Test)

Table 1: Clinical signs and symptoms exhibited by treated gerbils compared to the vehicle group

K-Optimal (mg kg^{-1})	Mouth smacking	Salivation	Tremor	Loose fur	Respiratory depression
0	-	-	-	-	-
8.75	-	-	-	-	+
17.5	+	+	+	+	+

+, -: Presence of clinical signs and symptoms, respectively

Elevated plus maze test: The K-Optimal formulation prolonged ($p<0.05$) the time spent by gerbils at the center of the elevated plus maze, irrespective of the dose tested (Table 3). However, pesticide exposure did not affect the number of entries into the open and closed arms, the time spent in the open and closed arms, the number of head deeps, rearing and the fecal mass of the gerbils.

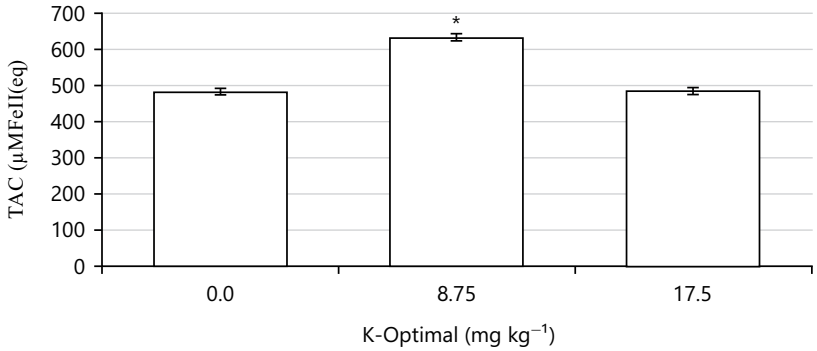

Fig. 3: Serum total antioxidant capacity levels in gerbils at the end of the treatment

Values represent means and SEM values for 6 gerbils per group. Significant difference when compared to the vehicle group treated with distilled water, at *p<0.05 (Student-Newman-Keuls Test)

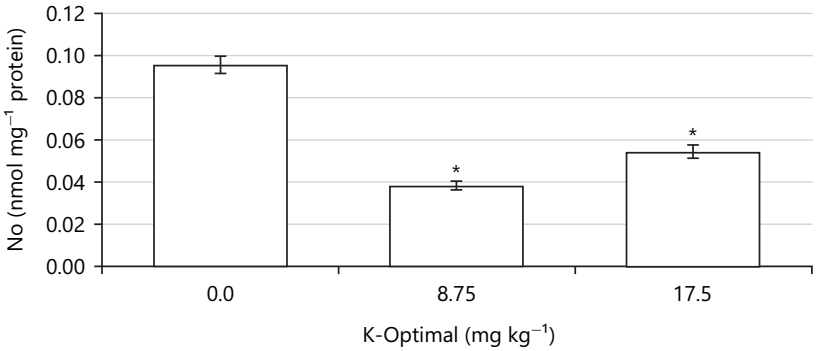

Fig. 4: Brain nitric oxide levels in gerbils following exposure to the pesticide formulation K-Optimal for 28 days

Values represent means and SEM values for 6 gerbils per group. Significant difference when compared to the vehicle group treated with distilled water, at *p<0.05 (Kruskal-Wallis Test)

Table 2: Average length of suspension time and open-field test parameters of animals

	Hanging wire Test	Open-field Test				
K-Optimal (mg kg^{-1})	Suspension time (sec)	Time spent at the center (min)	Crossing (counts)	Grooming (counts)	Rearing (counts)	Fecal mass (g)
0	6.03±1.62	16.65±2.14	167.17±7.77	9.67±0.95	20.33±2.52	0.01±0.01
8.75	2.76±0.50	18.38±1.91	145.50±14.11	6.50±1.20	16.33±1.05	0.03±0.01
17.5	1.65±0.34*	7.55±2.00**	148.17±8.93	4.67±0.76**	19.50±2.14	0.04±0.03

Values represent means and SEM for six gerbils per group. The significant difference when compared to the vehicle group treated with distilled water at *p<0.05 and **p<0.01 (Student Newman-Keuls Test)

Serum total antioxidant capacity (TAC): The results of the TAC levels measured in serum are summarized in Fig. 3. As shown by the figure, the TAC levels in gerbils showed a non-monotonic variation, with a significant increase in the animals exposed to 8.75 mg kg^{-1} when compared to the vehicle group ($p<0.05$).

Serum ALT, AST, cholinesterase enzymes, uric acid and testosterone levels: Aminotransferase (ALT and AST) and cholinesterase enzymes (AChE and BuChE) activities, uric acid and testosterone were measured in serum and the results were presented in Table 4. When compared to the vehicle group, none of these parameters were affected by the intoxication of the gerbils with the pesticide formulation ($p<0.05$) (Table 4).

Nitric oxide levels in the brain and liver: Brain NO levels in gerbils were analyzed and the results are presented in Fig. 4. The K-Optimal formulation induced a significant ($p<0.05$) difference in the NO levels in the brain at both investigated doses, when compared to the control group in Fig. 4.

Table 3: Elevated plus maze test parameters of gerbils

K-Optimal (mg kg^{-1})	Entry into the open arm (counts)	Entry into the close arm (counts)	Time spent in open arm (min)	Time spent in the closed arm (min)	No head deeps (counts)	Time spent at the center (min)	No. of rearing (counts)	Fecal mass (g)
0	29.00±3.25	21.33±2.03	2.74±0.22	1.94±0.30	43.67±7.30	0.32±0.02	26.50±3.05	0.004±0.004
8.75	28.50±2.09	32.50±2.85	1.76±0.21	2.18±0.14	30.17±4.64	1.23±0.07*	25.00±2.44	0.017±0.017
17.5	26.33±1.98	24.67±3.19	1.83±0.32	2.12±0.27	37.83±4.51	0.98±0.17*	21.17±3.18	0.019±0.012

Values represent means and SEM of six gerbils per group. The significant difference was when compared to the vehicle group treated with distilled water at *p<0.05 (Kruskal-Wallis Test), serum and organ biochemical parameters

Table 4: Serum activities of aminotransferase and cholinesterase enzymes, uric acid and testosterone levels in pesticide-exposed groups and vehicle group

K-Optimal (mg kg^{-1})	ALT (U L^{-1})	AST (U L^{-1})	Serum AChE (IU) and values (%)	Serum BuChE (IU) and values (%)	Uric acid (µmol L^{-1})	Serum testosterone (ng mL^{-1})
0	56.39±3.77	90.71±4.17	3726.10±32.42 (100±0.870)	2697.63±28.10 (100±1.048)	204.54±2.57	0.70±0.12
8.75	48.13±2.72	124.15±4.11	4384.20±29.20 (117.66±0.784)	3172.88±24.39 (117.62±0.904)	215.28±3.38	1.18±0.05
17.5	68.35±3.75	116.28±3.27	4409.40±102.02 (118.34±2.738)	3365.93±96.65 (124.77±3.583)	246.06±3.36	0.75±0.07

Values in the table represent Means±Standard Error of Means (SEM) of six gerbils per group. No significant difference when compared to the vehicle group at p<0.05 (One Way ANOVA, Student-Newman-Keuls and Kruskal-Wallis Test)

Table 5: Brain, liver and testis catalase, glutathione and thiobarbituric reactive substances

Organs	K-Optimal (mg kg^{-1})	Catalase (IU mg^{-1})	Reduced glutathione (mmoles mg^{-1} protein)	TBARS (nmoles mg^{-1} protein)
Brain	0.00	444.05±109.92	0.27±0.03	1.24±0.14
	8.75	407.66±39.62	0.34±0.02	3.56±0.56
	17.5	295.84±125.62	0.53±0.33	1.55±0.57
Liver	0.00	209.37±72.70	0.09±0.02	0.79±0.18
	8.75	269.69±38.23	0.06±0.01	0.42±0.06
	17.5	224.21±48.64	0.04±0.009	0.44±0.15
Testis	0.00	768.49±176.64	0.11±0.03	1.98±0.44
	8.75	1062.84±75.07	0.19±0.03	3.79±0.71
	17.5	524.37±98.30	0.17±0.04	3.82±0.79

TBARS: Thiobarbituric reactive substances. Values in the table represent Means±Standard Error of Means (SEM) of six gerbils per group. No significant difference when compared to the vehicle group at p<0.05 (Kruskal-Wallis Test)

Oxidative stress biomarkers: Catalase, reduced glutathione and TBARS levels were measured in brain, liver and testis tissues and results were presented in Table 5. When compared to the vehicle group, none of these parameters were affected by the intoxication of the gerbils with the pesticide formulation (p>0.05).

DISCUSSION

This study was designed to assess the subacute toxicity of K-Optimal, a pesticide formulation containing two active ingredients, acetamiprid and imidacloprid and inert ingredients intended to improve the physical characteristics of the product. This pesticide formulation is currently used by farmers in Cameroon, despite limited information on its health effects on humans. Adverse effects of each of these active ingredients have been reported by authors[5,7,13,27,28]. However, there is a scarcity of information on the potential toxicity of the mixture of the two chemicals. The inert ingredients have also been shown to potentiate the toxicity of the pesticide formulation. As K-Optimal is currently applied by farmers in Cameroon and elsewhere to control insects, toxicological data on the formulation may be more relevant to current exposure scenarios among humans. The Mongolian Gerbil model was used for the study.

Exposure to the pesticide formulation induced respiratory depression, profuse salivation and mouth smacking in gerbils. Such signs have been observed in rodents orally treated either with acetamiprid or imidacloprid[28,29]. This was suggestive of an altered nervous system, which was further assessed through behavioral tests including hanging wire, open-field and elevated plus maze tests.

The hanging wire test is a functional method for the evaluation of forelimbs strength and has been used to investigate the effects of xenobiotics on neuromuscular disorders[9]. There was a decrease in the ability of the K-Optimal-exposed gerbils to hang on the wire, suggesting reduced muscle strength and altered coordination in the gerbils. Impairment in grip strength following exposure to pesticides has been reported by Kada *et al*.[9] and may indicate muscle weakness. The open field test revealed a decrease in the total time spent at the center in the gerbils exposed to 17.5 mg kg^{-1} K-Optimal. This was indicative of lesser exploratory and high anxiety-like behaviors. The latter was further supported by the reduction of grooming in the open-field test[18,19]. This study corroborates the observations of Kada *et al*.[9], who reported the anxiogenic effects of Parastar pesticide formulation in Wistar albino rats.

In the elevated plus maze test, entries in both the open and closed arms parameters are often used as indicators of behavioral changes of anxiety origin, with their decreased frequency interpreted as evidence for the anxiogenic-like effect of some drugs[30]. In the current study, exposure to K-Optimal did not alter these parameters. However, the increased time spent at the center of the maze further supports the anxiogenic-like effect observed in the gerbils exposed to the pesticide formulation. Changes in behavior may result from altered physiology within the brain, biochemical changes, or related to general health conditions in the gerbils intoxicated with the pesticide. Therefore, body and organ weight as well as selected biochemical parameters were assessed including cholinesterase activities, NO levels, oxidative stress indicators and main androgen.

The enzyme AChE abbreviates neurotransmission in neuromuscular and neuro-neuronal synapses. The physiological function of BuChE remains unknown, though it serves as a biomarker for exposure to cholinesterase inhibitors. Cholinesterase inhibitors include the K-Optimal active ingredients lambda-cyhalothrin and acetamiprid[8,31]. Surprisingly, exposure to K-Optimal did not affect the activity of both enzymes, suggesting a different action mechanism of the pesticide formulation on the nervous system. Noteworthy, the freely diffusible gaseous compound NO serves as an important messenger in the central nervous system. It is produced by neurons and glial cells in the brain and the decreased levels of brain NO in the gerbils exposed to K-Optimal observed in this study corroborated behavioral changes. Nitric oxide plays an important role in many brain functions, such as sexual behavior and long-term potentiation. Long-term potentiation involves persistent strengthening of synapses leading to a long-lasting increase in signal transmission between neurons[32].

The K-Optimal elicited a reduction in the gerbil's body weight, concomitantly with increased liver weight, whereas weights of the main excretory and reproductive organs were unchanged. The decrease in body weight is in agreement with previous observations in rats exposed to one of the active ingredients of K-Optimal, acetamiprid when given at a dose of 30 mg kg^{-1} for 35 days[13]. The liver is well known as a key xenobiotic-metabolizing organ that also governs body energy metabolism. The increase in its relative weight may be indicative of hepatocellular disorders and could be a result of edema, hepatocellular hypertrophy or cell damage leading to lipid and glycogen accumulation[13]. Biochemical liver biomarkers of toxicity were assessed to determine the extent of the effects of the pesticide formulation on the gerbils and the integrity of the excretory and reproductive functions was also examined.

Alanine aminotransferase (ALT) is primarily localized in the liver but AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver[33]. These enzymes are normally present in the cytosol of the cell, hence, higher than normal serum levels are indicative of hepatocellular damage. Though damages in other tissues like the heart and muscle can lead to increments, especially in AST activities, ALT increments are generally more specific to liver damage[32]. Activities of the aminotransferases did not show a significant variation following exposure to K-Optimal, although a 12-28% marginal increase

in aminotransferase activities was observed at a higher pesticide dose. However, previous toxicological studies with the two K-Optimal active ingredients, given to rats/mice at higher doses for longer periods, showed altered liver function. These include Zhang *et al.*[13], who reported increased ALT activity in male mice intoxicated with acetamiprid (30 mg kg^{-1}) for 35 days and Bhardwaj *et al*[29], who reported elevated ALT and AST in female rats intoxicated with imidacloprid (at doses of 10 and 20 mg kg^{-1}) for 90 days. Kidneys are among the most solicited organs for excretion of the xenobiotics and related residues. The excretory function was also examined through the measurement of uric acid, a multifactorial biomarker resulting mainly from protein degradation in mammals. It is generally excreted into the urine and increased levels in serum serve as an indicator of impaired mammalian kidney function. Progressive renal failure is generally accompanied by a relentless augment of serum uric acid secondary to a progressive decay in the capacity of the kidney to excrete urates[34,35]. In this study, there was no significant variation in the uric acid levels, suggesting non-altered kidney function following intoxication with K-Optimal. As for the reproductive function, assessment using serum testosterone levels as a biomarker indicated that it was not significantly affected by intoxication with K-Optimal. Testosterone is the main male androgen[36]. Although this observation suggests that the pesticide formulation does not alter steroidogenesis in the animals, it warrants further assessment of the reproductive function through the measurement of additional biochemical markers such as gonadotropins and sperm quality. Indeed, Kong *et al.*[37] demonstrated that repeated oral administration of a K-Optimal active principle acetamiprid, at doses of 10 and 30 mg kg^{-1} for 35 days resulted in decreased plasma testosterone levels in male rats.

Pesticides have been shown to alter cellular function through oxidative stress, which is a state characterized by an imbalance between pro-oxidant molecules (including reactive oxygen and nitrogen species) and antioxidant defenses. Oxidative stress plays a key role in the pathogenesis of the liver, kidneys, neurological and reproductive dysfunction[38]. The FRAP of plasma was increased in gerbils exposed to 8.75 mg kg^{-1} K-Optimal, suggesting increased total antioxidant power of plasma in the animals in response to the lower dose of the pesticide formulation. This non-monotonic effect on TAC could imply an increase in ROS in the animals exposed to the higher dose of the agrochemical formulation, 17.5 mg kg^{-1}, surpassing the antioxidant system. Indeed, Zhang *et al.*[13] reported that excess ROS led to a decrease in antioxidant levels. Also, the K-Optimal formulation had no significant effect on the activity of the antioxidant enzyme catalase as well as the levels of glutathione. The level of the lipid peroxidation marker, TBARS, was not significantly affected upon administration of the pesticide formulation. This was contrary to the observation of Ansari *et al.*[31], who reported decreased catalase and glutathione with increased TBARS in rats exposed to lambda-cyhalothrin, at 3.0 mg kg^{-1} for 15 days.

This study complements previous data on adverse health effects of the insecticides acetamiprid and lambda-cyhalothrin. Although the study enables appraisal of the toxicity of the two ingredients combined, it has some limitations worth mentioning. Reports on the toxicity of the pesticide ingredients in gerbils are scarce. Therefore, results presented herein were generally confronted with data in rats and mice, assuming that the latter rodents may share common behavior with the gerbils. The K-Optimal is a mixture of well-identified active ingredients but also inert substances for which the nature and concentration have not been disclosed by the manufacturer. This makes it difficult to properly interpret data with a delineation of the intrinsic adverse effect of each component.

CONCLUSION

Taken altogether, results from this study suggested that K-Optimal exposure induces behavioral changes, probably through reduced brain NO levels. The pesticide formulation also altered body and liver weight and should be therefore handled with care to limit exposure among potential users including farmers.

SIGNIFICANCE STATEMENT

Subchronic intoxication of gerbils by K-Optimal, a pesticide formulation containing acetamiprid and lambda-cyhalothrin, results in altered behaviors, as well as reduced brain nitric oxide. K-Optimal is currently used by farmers to kill insect pests on crops and may be detrimental to the farmer applying the chemical with inadequate/no protection. Farmers are therefore called upon to use pesticides properly, by good agricultural practices, to minimize/reduce exposure to K-Optimal.

REFERENCES

1. Arduini, F., F. Ricci, C.S. Tuta, D. Moscone, A. Amine and G. Palleschi, 2006. Detection of carbamic and organophosphorous pesticides in water samples using a cholinesterase biosensor based on Prussian blue-modified screen-printed electrode. Anal. Chim. Acta, 580: 155-162.
2. Gilden, R.C., K. Huffling and B. Sattler, 2010. Pesticides and health risks. J. Obstet. Gynecol. Neonatal Nurs., 39: 103-110.
3. Daniel, O.A. and A.L. Tajudeen, 2023. Tropical agriculture: Problems and possible solutions in Nigeria. Trends Agric. Sci., 2: 120-130.
4. Pajoumand, A., N. Jalali, M. Abdollah and S. Shadnia, 2002. Survival following severe aluminium phosphide poisoning. J. Pharm. Pract. Res., 32: 297-299.
5. Manfo, F.P.T., C.F. Suh, E.A. Nantia, P.F. Moundipa and F. Cho-Ngwa, 2021. Occupational use of agrochemicals results in inhibited cholinesterase activity and altered reproductive hormone levels in male farmers from Buea, Cameroon. Toxicol. Res., 10: 232-248.
6. Seenivasan, S. and N.N. Muraleedharan, 2009. Residues of lambda-cyhalothrin in tea. Food Chem. Toxicol., 47: 502-505.
7. Manfo, F.P.T., S.A. Mboe, E.A. Nantia, F. Ngoula, P.B. Telefo, P.F. Moundipa and F. Cho-Ngwa, 2020. Evaluation of the effects of agro pesticides use on liver and kidney function in farmers from Buea, Cameroon. J. Toxicol., Vol. 2020. 10.1155/2020/2305764.
8. Győri, J., A. Farkas, O. Stolyar, A. Székács, M. Mörtl and Á. Vehovszky, 2017. Inhibitory effects of four neonicotinoid active ingredients on acetylcholine esterase activity. Acta Biol. Hung., 68: 345-357.
9. Sanda, A.K., A.E. Nantia, T.F.P. Manfo, R.T. Toboh and R.E. Abende *et al*., 2022. Subchronic administration of parastar insecticide induced behavioral changes and impaired motor coordination in male Wistar rats. Drug Chem. Toxicol., 45: 426-434.
10. Mateu-Sánchez, M., M. Moreno, F.J. Arrebola and J.L.M. Vidal, 2003. Analysis of acetamiprid in vegetables using gas chromatography-tandem mass spectrometry. Anal. Sci., 19: 701-704.
11. Umina, P.A., O. Edwards, P. Carson, A. van Rooyen and A. Anderson, 2014. High levels of resistance to carbamate and pyrethroid chemicals widespread in Australian *Myzus persicae* (Hemiptera: Aphididae) populations. J. Econ. Entomol., 107: 1626-1638.
12. Imamura, T., Y. Yanagawa, K. Nishikawa, N. Matsumoto and T. Sakamoto, 2010. Two cases of acute poisoning with acetamiprid in humans. Clin. Toxicol., 48: 851-853.
13. Zhang, J.J., Y. Wang, H.Y. Xiang, M.X. Li and W.H. Li *et al*., 2011. Oxidative stress: Role in acetamiprid-induced impairment of the male mice reproductive system. Agric. Sci. China, 10: 786-796.
14. Zick, S.M., Z. Djuric, M.T. Ruffin, A.J. Litzinger and D.P. Normolle *et al*., 2008. Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. Cancer Epidemiol. Biomarkers Prev., 17: 1930-1936.
15. Al-Sarar, A.S., Y. Abobakr, A.E. Bayoumi, H.I. Hussein and M. Al-Ghothemi, 2014. Reproductive toxicity and histopathological changes induced by lambda-cyhalothrin in male mice. Environ. Toxicol., 29: 750-762.
16. Deeba, F., I. Raza, N. Muhammad, H. Rahman and Zia ur Rehman *et al*., 2017. Chlorpyrifos and lambda cyhalothrin-induced oxidative stress in human erythrocytes: *In vitro* studies. Toxicol. Ind. Health, 33: 297-307.

17. Terayama, H., K. Sakabe, D. Kiyoshima, N. Qu and T. Sato *et al*., 2022. Effect of neonicotinoid pesticides on Japanese water systems: Review with focus on reproductive toxicity. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms231911567.
18. Steimer, T. and P. Driscoll, 2003. Divergent stress responses and coping styles in psychogenetically selected roman high-(RHA) and low-(RLA) avoidance rats: Behavioural, neuroendocrine and developmental aspects. Stress, 6: 87-100.
19. Atrooz, F., K.A. Alkadhi and S. Salim, 2021. Understanding stress: Insights from rodent models. Curr. Res. Neurobiol., Vol. 2. 10.1016/j.crneur.2021.100013.
20. Benzie, I.F.F. and J.J. Strain, 1999. Ferric Reducing/Antioxidant Power Assay: Direct Measure of Total Antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. In: Methods Enzymology, Packer, L. (Ed.), Academic Press, Cambridge, Massachusetts, ISBN: 9780121822002, pp: 15-27.
21. Jońca, J., M. Żuk, B. Wasąg, A. Janaszak-Jasiecka and K. Lewandowski *et al*., 2015. New insights into butyrylcholinesterase activity assay: Serum dilution factor as a crucial parameter. PLoS ONE, Vol. 10. 10.1371/journal.pone.0139480.
22. Fotio, A.L., T. Dimo, T.B. Nguelefack, P.D.D. Dzeufiet and E.N. Lemba *et al*., 2009. Acute and chronic anti-inflammatory properties of the stem bark aqueous and methanol extracts of *Sclerocarya birrea* (Anacardiaceae). Inflammopharmacology, 17: 229-237.
23. Maehly, A.C., 1954. The Assay of Catalases and Peroxidases. In: Methods of Biochemical Analysis, Glick, D. (Ed.), Interscience Publishers, Inc., Geneva, Switzerland, ISBN: 9780471304265, pp: 357-424.
24. Chedjeu, D.Z., F.P.M. Tsague, E.A. Nantia, D. Zofou and J.C.N. Assob, 2021. Subchronic toxicity of a terbufos-based pesticide (counter 15FC) in adult male rats. J. Chem. Health Risks, 11: 169-180.
25. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.
26. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
27. Nieradko-Iwanicka, B. and M. Rutkowski, 2022. Effect of subacute poisoning with lambdacyhalothrin on vascular endothelial growth factor 2 receptor in mice kidneys. BMC Nephrol., Vol. 23. 10.1186/s12882-022-02828-1.
28. Mondal, S., S.K. Mukhopadhayay, S. Kumar, P.S. Mandal, S. Pradhan, S. Biswas and K. Purohit, 2015. Haematobiochemical profile in chronic acetamiprid exposure in sprague dawley rats. Adv. Anim. Vet. Sci., 3: 384-394.
29. Bhardwaj, S., M.K. Srivastava, U. Kapoor and L.P. Srivastava, 2010. A 90 days oral toxicity of imidacloprid in female rats: Morphological, biochemical and histopathological evaluations. Food Chem. Toxicol., 48: 1185-1190.
30. Rocha, V.M., C.M. Calil, R. Ferreira, M.J.C.S. Moura and F.K. Marcondes, 2007. Influence of anabolic steroid on anxiety levels in sedentary male rats. Stress, 10: 326-331.
31. Ansari, R.W., R.K. Shukla, R.S. Yadav, K. Seth and A.B. Pant *et al*., 2012. Cholinergic dysfunctions and enhanced oxidative stress in the neurobehavioral toxicity of lambda-cyhalothrin in developing rats. Neurotoxicol. Res., 22: 292-309.
32. Singh, R., S. Pervin, J. Shryne, R. Gorski and G. Chaudhuri, 2000. Castration increases and androgens decrease nitric oxide synthase activity in the brain: Physiologic implications. Proc. Nat. Acad. Sci. U.S.A., 97: 3672-3677.
33. Thapa, B.R. and A. Walia, 2007. Liver function tests and their interpretation. Indian J. Pediatr., 74: 663-671.
34. Ruilope, L.M. and J. Garcia-Puig, 2001. Hyperuricemia and renal function. Curr. Hypertens. Rep., 3: 197-202.
35. Tsahar, E., Z. Arad, I. Izhaki and C.G. Guglielmo, 2006. The relationship between uric acid and its oxidative product allantoin: A potential indicator for the evaluation of oxidative stress in birds. J. Comp. Physiol. B, 176: 653-661.

36. Chauvigné, F., S. Plummer, L. Lesné, J.P. Cravedi, N. Dejucq-Rainsford, A. Fostier and B. Jégou, 2011. Mono-(2-ethylhexyl) phthalate directly alters the expression of leydig cell genes and CYP17 lyase activity in cultured rat fetal testis. PLoS ONE, Vol. 6. 10.1371/journal.pone.0027172.
37. Kong, D., J. Zhang, X. Hou, S. Zhang and J. Tan *et al*., 2017. Acetamiprid inhibits testosterone synthesis by affecting the mitochondrial function and cytoplasmic adenosine triphosphate production in rat leydig cells. Biol. Reprod., 96: 254-265.
38. Agarwal, A., A. Aponte-Mellado, B.J. Premkumar, A. Shaman and S. Gupta, 2012. The effects of oxidative stress on female reproduction: A review. Reprod. Biol. Endocrinol., Vol. 10. 10.1186/1477-7827-10-49.