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Evaluation of Cholinesterase Activity of Liver and Kidney Parameters in Market Gardeners Exposed to Pesticides in the Municipality of Bingerville

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Abstract **Original Research Article**

Pesticides are chemical or biological agents capable of destroying pests or controlling their growth and reproduction. Long-term exposure could have adverse effects on biochemical parameters. To this end, several parameters were analyzed, namely the subjects' knowledge and attitudes about pesticides, the determination of cholinesterase and transaminase activity, and the measurement of urea and creatinine levels using a Cobas C 111 automated analyzer and a spectrophotometer. Blood samples were taken. All subjects surveyed had a good knowledge of pesticides, and there was a correlation between their knowledge and their practices. The study population consisted of 30 market gardeners and 50 controls. The average age was 59.83 ± 7.751 and 33.56 ± 14.888 for market gardeners and controls, respectively. These analyses showed an average urea concentration of 0.24 ± 0.055 g/l and 0.24 ± 0.0261 g/l, respectively, and creatinine concentrations of 8.76 ± 1.912 mg/l and 8.67 ± 1.470 mg/l respectively in the control group and market gardeners ($p>0.05$); the mean values of transaminases (TGO) in the control group and market gardeners were 29.12 ± 12.25 IU/l and 16.93 ± 7.900 IU/l respectively ($p<0.05$); the mean cholinesterase values in the control group were 9584.42±1686.26 U/L and those in the market gardeners were 7590.63± 1606.27 U/L with ($p<0.05$); the cholinesterase activity of market gardeners was inhibited by 20%. This inhibition does not require any risk mitigation measures, as it is below 40%, which is the biological limit recommended by the WHO.

Keywords: Pesticides, Cholinesterase, Market Gardeners, Biochemical Parameters, Organophosphates, WHO.

INTRODUCTION

Cash crop farming has encouraged farmers to adopt new methods such as the use of machinery, fertilizers, and, above all, pesticides to improve the quality and quantity of agricultural products (Chuisseu *et al.*, 2020). Pesticides are substances that are mainly used in agriculture to protect plants from pests, weeds, or diseases (Nicolopoulou S. *et al.*, 2016). Although pesticides have proven benefits, numerous studies have revealed their harmful effects on the health of both consumers of agricultural products and farmers (Chuisseu *et al.*, 2020). According to the World Health Organization (WHO), there are one million cases of serious pesticide poisoning worldwide each year, causing approximately 220,000 deaths annually (Cherin P. *et al.*, 2012). Organophosphates (OPs) are organic compounds widely used as pesticides, plasticizers, flame retardants, and potentially as chemical weapons (Jacques P *et al.*, 2012). They are the most widely used class of pesticides worldwide, accounting for 30% of the global market and causing three million cases of poisoning and 100,000 deaths each year, as well as inducing debilitating neurological disorders, and therefore represent a serious public health and environmental toxicity problem. Organophosphates (OPs) primarily target cholinesterases in the body, whose enzymatic activity is used as a biological marker and main indicator of poisoning, with clinical symptoms reflecting the effects of OPs (Jalady M.A *et al*, 2013). Plasma and intraerythrocytic cholinesterase activities are the indicators used to monitor workers exposed to OPs, reflecting the effects of cumulative exposure over previous weeks or acute overexposure (INRS, 2012). Prevention consists of medical monitoring of phytosanitary operators through regulatory medical examinations. This monitoring is carried out on two levels. First, a clinical examination of exposed individuals is performed to look for possible signs of poisoning, followed by a blood test to measure serum cholinesterase levels. The WHO recommends the following measures to prevent poisoning: launch an investigation if plasma cholinesterase activity falls by more than 20% from the baseline value, in order to determine the cause of exposure and apply appropriate measures; remove the individual from their workplace if plasma cholinesterase activity falls below 40%. The WHO also recommends that people exposed to pesticides undergo biological testing every six months, including cholinesterase, urea, creatinine, and transaminases. A decrease in cholinesterase activity may be linked to liver damage. Increased activity is observed in cases of nephrotic syndrome (Biommis, 2012). In developing countries where pesticide use is widespread in agricultural practice, pesticide poisoning accounts for 99% of fatal poisonings due to a lack of regulation of the monitoring system and insufficient access to information systems. Côte d'Ivoire, a predominantly agricultural country, uses large quantities of pesticides to increase vegetable production, which contributes to the pollution of water resources and the contamination of the population (Akpo *et al*., 2016). Vegetables and protein crops occupy a prominent place in the diet in Côte d'Ivoire, where urban population growth is driving an increase in demand. Vegetable production has increased by 30% in ten years, reaching 700,000 tons in 2001 (CNRA, 2004). The desire to meet the food needs of large urban areas in Côte d'Ivoire is pushing market gardeners to use more plant protection products (MINAGRA, 1993). Unfortunately, this use is not without harmful effects. Previous studies have shown biochemical dysfunctions that could reflect hepatic cytotoxicity (Araoud M *et al*., 2012) (Hu R *et al*., 2015) and renal cytotoxicity (Payán-Rentería R *et al.,* 2012 ; (Abdul A *et al*, 2019) in agricultural workers exposed to pesticides. In CI, much work has been done on pesticides, but it has mainly focused on the attitudes and knowledge of market gardeners towards pesticides (Kpan *et al*., 2019), and work on market garden products (Angbo *et al*., 2024). However, this exposure to pesticides can be assessed either by measuring the pesticide and its metabolites in urine, blood, and other samples, or by measuring a biological effect of a pesticide such as cholinesterase inhibition (Kapka-Skrzypczak L *et al*., 2011). Research on the action of pesticides using biomarkers is rare. As the municipality of Bingerville is considered one of the centers of market gardening that uses pesticides for protection, we hypothesized that frequent and long-term exposure to pesticides would alter these biochemical parameters in exposed market gardeners. To this end, we sought to evaluate the effect of pesticides on the biochemical parameters of these market gardeners versus those of the control group.

MATERIAL AND METHODS

Study area and sample collection

This was a cross-sectional analytical study conducted between September 1 and October 10, 2024, in the municipalities of Bingerville and Cocody. The sample consisted of market gardeners working in market gardening and organized into a cooperative called SCOOPS-YERELON, as well as unexposed individuals. The study included people of all races, both sexes, all socioeconomic conditions, and all ages, whether or not they used pesticides. Individuals who did not give their consent to participate in the study were not included. Subjects with liver and kidney disease were excluded.

The methodology consisted of three main phases: the pre-survey, the survey, and the measurements.

Preliminary survey

This was the preparatory phase for the actual survey and took place at the selected site. This phase enabled contact to be made with the administrative managers of the vegetable production site. It also enabled data to be collected on worker participation and their acceptance of biological sampling.

Survey

In order to characterize the market gardeners, an interview was conducted at the study site. To this end, a questionnaire guide was submitted to each of the respondents. It addressed the socio-demographic status of the respondent, professional activities, seniority, exposure time, determination of TGO and TGP liver enzymes, determination of ChE, and measurement of urea and creatinine. Blood was then collected by superficial venipuncture in the morning on an empty stomach (at least twelve hours) in a dry tube. The samples were immediately transported in a cooler containing dry ice to the laboratory and centrifuged at 3000 rpm for 3 minutes, after which the plasma was stored in a refrigerator at +4°C until analysis (ISO 15189, 2022).

Assay

The urea assay was performed using the enzymatic method described by Tietz (1995). In the presence of urease, urea is hydrolyzed into ammonia (NH_3) and carbon dioxide (CO_2), then in a second reaction, the ammonia formed reacts with α-ketoglutarate through the action of glutamate dehydrogenase (GLDH) with parallel oxidation of NADH to NAD+. The rate of decrease in NADH concentration is directly proportional to the urea concentration in the test sample (Kaplan, 1984) and is measured by photometry. The reagents are prepared according to the manufacturer's instructions. The contents of one enzyme capsule (R2) (urease, glutamate dehydrogenase (GLDH, NADH)) were dissolved in a buffer vial (R1) consisting of tris (pH 7.8) and α-ketoglutarate. The vial was then closed and mixed to obtain the working reagent (WR). The resulting solution is stable for 6 weeks at 2-8°C or 7 days at room temperature (15-25°C). Ten (10) µL of serum were placed in a cuvette to which 1 mL of the working reagent was added.

The assay was performed using the Jaffé method (1886). Creatinine measurement is based on the reaction of creatinine with sodium picrate using the kinetic method: creatinine at 37°C in the presence of yellow alkaline picrate produces an orange coloration that can be read at 510 nm. The intensity of the 1443orme di directly proportional to the creatinine concentration in the test sample (Murray, 1984).

Creatinine measurement is based on the reaction of creatinine with sodium picrate using the kinetic method. The reagents are prepared according to the manufacturer's instructions. To do this, the working reagent (WR) was obtained by mixing equal volumes of picric reagent (R1) and alkalizing reagent sodium hydroxide (R2). This mixture was stable for 15 days in the refrigerator (2-8°C) or 7 days at room temperature (15-25°C). In a cuvette, 10 µL of plasma was mixed with 1 mL of working reagent and then incubated for 10 minutes at 37°C. The optical density (OD) was read at 505 nm in comparison with the reagent blank.

Alanine aminotransferase (ALT) activity was determined using the method recommended by the International Federation of Clinical Chemistry (IFCC) (Bergmeyer and Horder, 1985). In a tube containing 1 mL of working reagent R1, 100 µL of the sample to be 1443orme dis added. After shaking for 1 min, the optical density of the homogenate is read at 340 nm using a spectrophotometer to determine the enzymatic activity of ALAT. This activity is calculated based on the wavelength of 340 nm and the SGPT factor (F1747) using the formula below: ALAT enzyme activity (U/L) = Optical density (sample) x 1745.

Aspartate aminotransferase (ASAT) activity was determined using the Karmen method and in accordance with the recommendations of the International Federation of Clinical Chemistry (IFCC). In a tube containing 1 mL of working reagent R1, 100 µL of the sample to be tested was added. After shaking for 1 min, the optical densities were read at 505 nm using a spectrophotometer to determine the enzymatic activity of ASAT (ECCLS, 1985). Enzyme activity is calculated from the wavelength of 505 nm and the SGOT factor (F1745) using the following formula: ASAT enzyme activity (U/L) = Optical density (sample) x 1745.

Plasma cholinesterase activity was determined using the kinetic method (Artiss, 1982) based on the following principle: cholinesterase hydrolyzes butyrylthiocholine into butyrate and thiocholine. Thiocholine reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to form nitro-2-mercapto-5-benzoate. The nitro-2-mercapto-5-benzoate 1443orme dis proportional to the enzymatic activity of cholinesterase in the sample (King, 1984; Whittaker, 1983). The phosphate buffer, which is working reagent R1, was obtained by dissolving the contents of vial R1 in 50 ml of distilled water, and working reagent R2 by dissolving the contents of vial R2 in 3 ml of distilled water. This dissolved solution is stable for 8 weeks at 2-8°C (King, 1984). It must be protected from direct light and sunlight. 1.5 ml of reagent R1, 50 µl of reagent R2, and 10 µl of serum diluted 1: 2 with 9 g/l NaCl were placed in a test tube. After homogenizing the contents of the tube, the optical density was measured with a spectrophotometer at 405 nm.

Ethical considerations

Consent

All participants in this study were informed of its purpose. In addition, informed consent was obtained from each individual before the study began.

Confidentiality

Respondents were assured that the information collected would remain confidential.

Statistical analysis

All test results were first entered into an Excel spreadsheet. They were then statistically processed using Graph Pad Prism software (version 10). The analysis of means was performed on all results obtained using Student's t-test combined with Welch's correction for robustness of the analysis in order to determine the existence of statistically significant differences between the calculated means. Statistically significant differences were highlighted at a significance level of 5%.

Results

Sociodemographic characteristics

The results revealed that market gardening is an activity carried out by men, who account for 71% of participants, with women accounting for 29%; in contrast, 82% of the control group were male and 18% were female.

The distribution of the population by age group shows that the average age of the controls was 33.56 ± 14.888 and that of the market gardeners was 59.83 ± 7.751, with a significant difference ($p < 0.05$) (Figure 1).

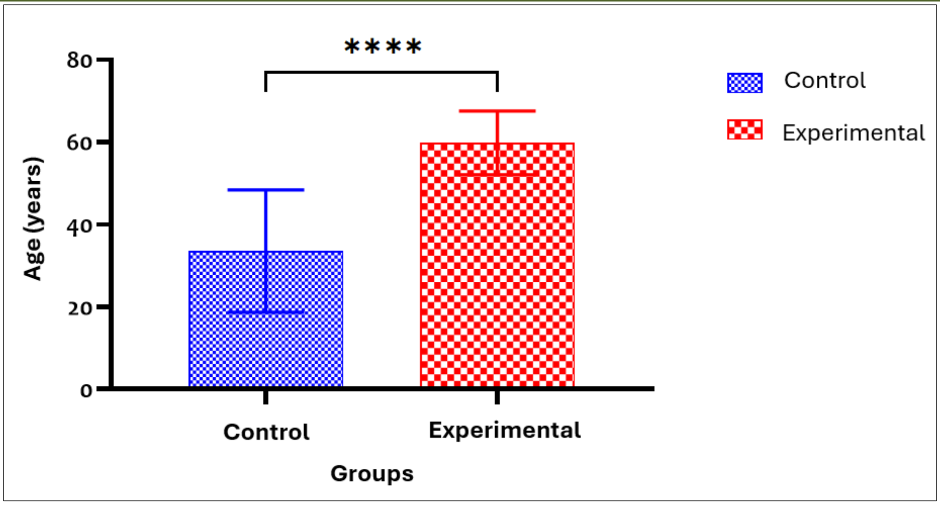

Figure 1: Distribution of the study population by age group

Professional information

Data on professional experience indicate that market gardeners have an average of 36.50±13.836 years of experience.

In terms of professional training, 100% of market gardeners acknowledged having received awareness training on the dangers of pesticides.

The majority of market gardeners stated that they were aware of the usefulness of pesticides.

With regard to safety at work, market gardeners have received training on safety measures related to the use of pesticides.

With regard to the availability of personal protective equipment (PPE), information gathered from producers at the various sites visited revealed that the most commonly used protective measures among market gardeners are gloves, face masks, and boots.

Biological aspects

Serum cholinesterase activity in the general populationThe mean values for serum cholinesterase activity in market gardeners were 7590.63± 1606.27 U/L and in controls 9584.42 ± 1686.263 U/L. The mean values for market gardeners showed a statistically significant difference compared with those for controls ($P < 0.05$) (Figure 2).

Enzyme activity inhibition is calculated relative to the cholinesterase activity of the controls according to the following reaction :

Average cholinesterase values Controls $\xrightarrow{100\%}$

Average cholinesterase values Users $\xrightarrow{\times\%}$

×% correspond à l'inhibition recherchée.

Cholinesterase activity was inhibited by 20% in market gardeners (Table 1).

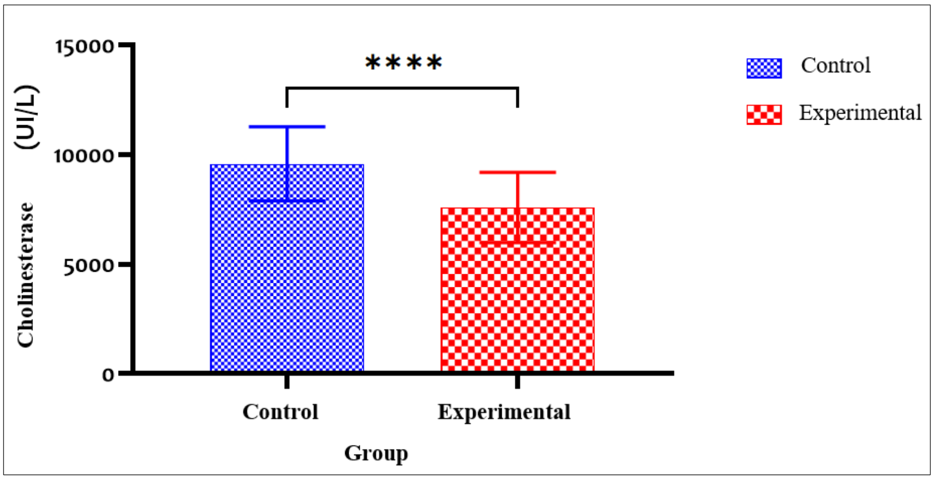

Figure 2: Distribution of average cholinesterase values among market gardeners and controls

Table 1: Variation in the inhibition of enzyme activity in market gardeners

	Average cholinesterase values (U/L)	% cholinesterase activity (%)	% inhibition of cholinesterase (%)
Market gardeners	7590,63 ± 1606,27	79,19	20,80

Urea

There is no significant difference between the average urea values of market gardeners and those of the control group (Figure 3).

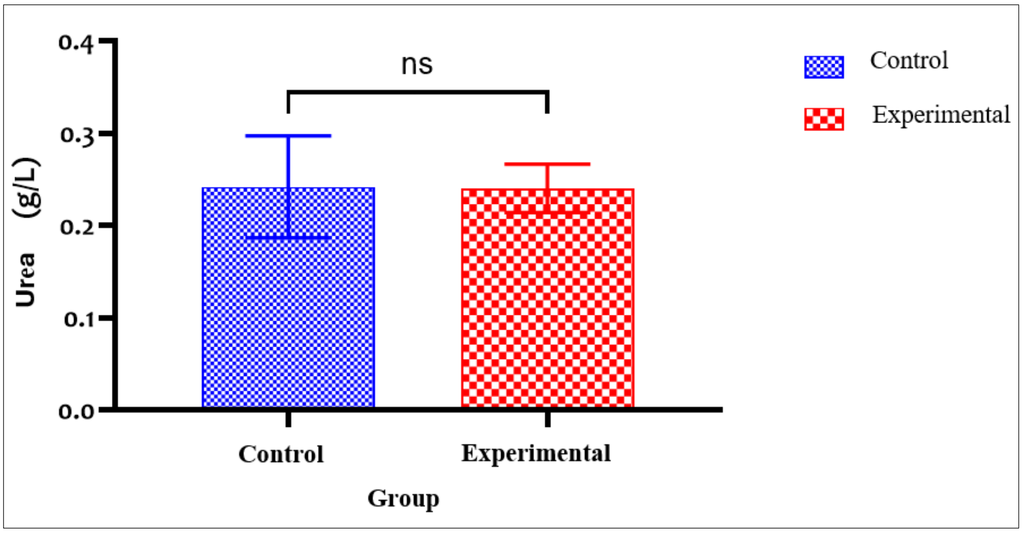

Figure 3: Distribution of average urea values for market gardeners and controls

Creatinine

There is no significant diffrende between the mean creatine values of market gardeners and those of the control group (Figure 4).

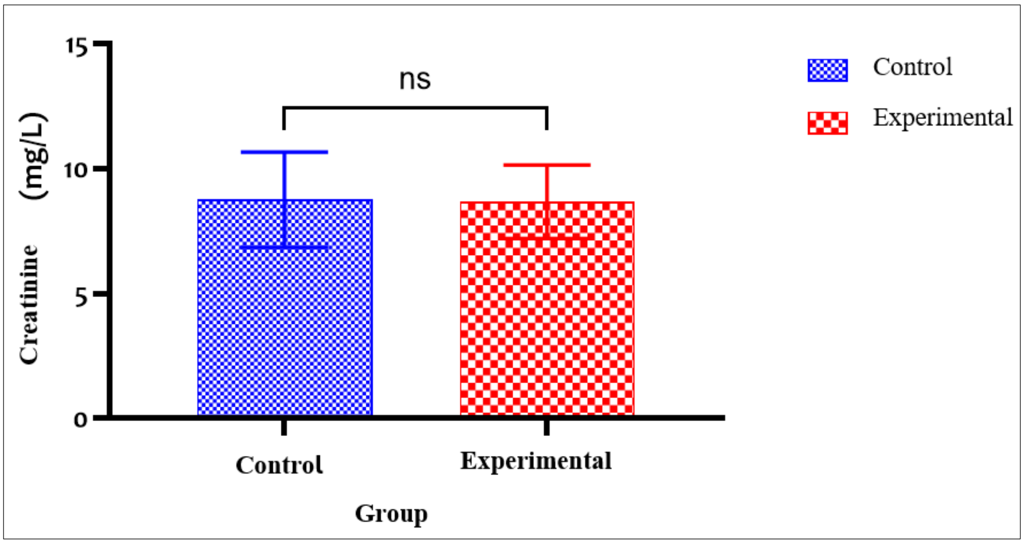

Figure 4: Distribution of mean creatinine values among market gardeners and controls

TGO

There is a significant difference between the average TGO values of market gardeners and those of the control group (Figure 5).

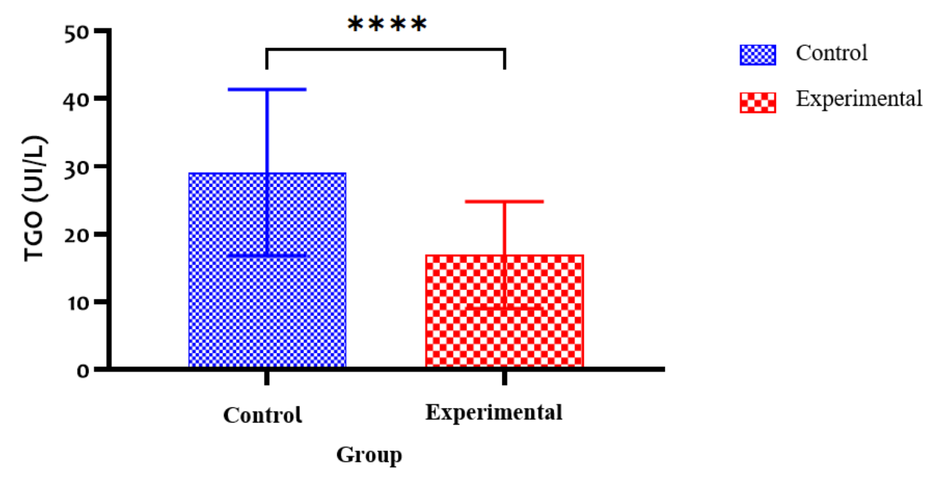

Figure 5: Distribution of average TGO values for market gardeners and controls

TGP

There is a significant difference between the average TGP values of market gardeners and those of the control group (Figure 6).

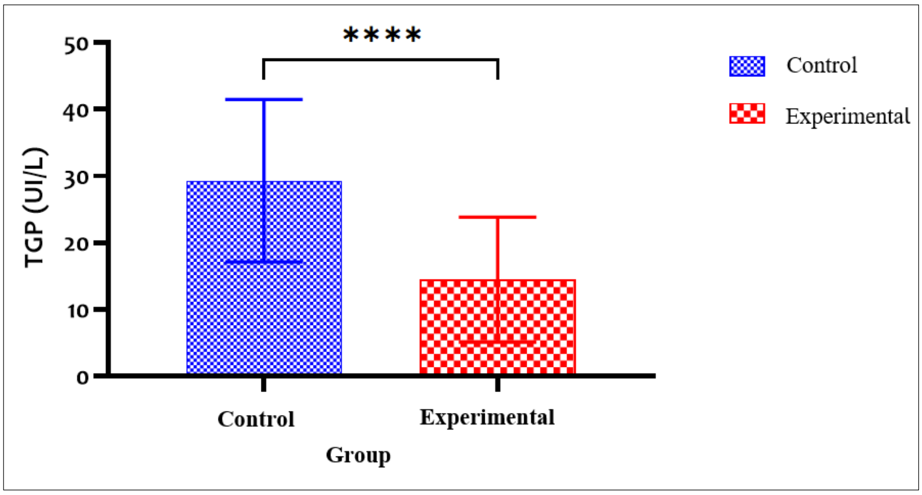

Figure 6: Distribution of average TGP values for market gardeners and controls

DISCUSSION

The aim of this study was to assess the effect of pesticides on the biochemical parameters of market gardeners belonging to a cooperative in the municipality of Bingerville, compared with unexposed controls in the municipality of Cocody. This study was conducted among 30 market gardeners in the municipality of Bingerville and 50 controls. Among the market gardeners, men accounted for 71% of the workforce, while among the controls, they accounted for 82%. This gender imbalance is similar to that described in a study of farmers in northwestern Tanzania (Philbert *et al*., 2019) and in Cameroon (Chuisseu *et al*., (2020). This could be explained by the fact that in these agricultural areas, manual farming practices are used in conjunction with agrochemicals, without the use of machinery, requiring a great deal of physical effort. There is a significant difference between the average age of market gardeners and controls. The average age of market gardeners was 59.83±7.751.

The average serum cholinesterase (BChE) activity among market gardeners was 7590.63± 1606.27 U/L (workers versus 9584.42±1686.26 U/L for controls. The average BChE activity of market gardeners (n = 30) was significantly lower than that of controls (n = 50). Comparison of these averages with those of the controls showed a significant difference ($p < 0.05$). Numerous studies have highlighted the importance of cholinesterase in monitoring poisoning. A study conducted by Chanese A. *et al*., (2021) showed a significant reduction in mean plasma cholinesterase in chronically exposed subjects compared to the control group. In another study conducted by Chanese A. *et al*., (2021), average plasma cholinesterase values were found to be lower in those exposed to insecticides. This has been confirmed by authors such as Arum N. *et al*., (2020) and Surat H. *et al*., (2018). A similar study conducted by Tanandra B. *et* *al*., (2020) and Sine H *et al*., (2019) also showed that plasma cholinesterase in two exposed subgroups (pesticide applicators and other agricultural workers) was significantly lower than in controls.

The cholinesterase inhibition rate among market gardeners was 20%. These cholinesterase inhibition rates were below 40%. Our results are similar to those of Abdou *et al*., (2008), who found inhibition rates ranging from 31% to 36%. As can be seen, these values do not require any risk mitigation measures, as they are below 40%, which is the biological limit recommended by the WHO (1982, 1986). According to the International Labor Organization (ILO), a decrease in serum cholinesterase of more than 40% results in the removal of the subject and their transfer to another position until the level returns to its initial values (ILO, 2010).

Biochemical parameters of pesticide exposure include urea, creatinine, and transaminases. There were no significant differences between the mean values of exposed individuals and controls in terms of urea and creatinine. The mean values were within the normal reference range. These results are consistent with those of Soraya *et al*., (2015) regarding the effect of pesticides on renal function (creatinine). However, in terms of liver function (ASAT, ALAT), a significant decrease in transaminase activity was observed in market gardeners compared to controls. However, some authors have found that liver function was affected by exposure to organophosphate pesticides, resulting in a significant increase in transaminase activity (Cestonaro L.C. *et al*., 2020 ; Tanandra B. *et al*., 2021). In contrast, Raafat and Mandour (2013) found that the average transaminase activity (ASAT, ALAT) of exposed patients was significantly higher than that of the control group. With regard to the degree of poisoning, the average AST level increased with the severity of the poisoning, and the average ALT level was close to that of the control group in cases of mild and moderate poisoning (whereas it was significantly higher in cases of severe poisoning). These results would indicate that in our study, the poisoning was very mild.

Measuring serum cholinesterase activity is an excellent indicator of exposure to organophosphates Measuring plasma butyrylcholinesterase (BuChE or plasma cholinesterase or pseudocholinesterase) is often preferred because BuChE activity varies in parallel with AChE activity and plasma samples are easier to store for this assay (Biotox, 2013). Decreased cholinesterase activity may be linked to liver damage (viral hepatitis, cirrhosis, liver failure), anemia, and organophosphate pesticide poisoning. Increased activity is observed in cases of nephrotic syndrome and diabetes (Biommis, 2012). The present study does not reveal any liver damage, as transaminase levels are normal. There is also no nephrotic syndrome, as urea and creatinine concentrations are normal.

The results within normal limits for all parameters in this study could be explained by the fact that market gardeners in this municipality follow the advice of their supervisors. The market gardeners are organized into a cooperative and are supervised by the National Agency for Rural Development Support (ANADER). Their secretary told us during interviews that they scrupulously follow the recommendations they receive from their supervisors. The supervisors who participated in our study indicated that they had received training in the application of pesticides.

Conclusion

The objective of this study was to investigate the exposure of market gardeners in the municipality of Bingerville to organophosphate pesticides. To this end, several parameters were analyzed, namely the subjects' knowledge of pesticides, the determination of cholinesterase activity, and the measurement of biochemical parameters. With regard to knowledge of pesticides, the results showed that all the subjects surveyed had a good knowledge of pesticides, and that there was a correlation between their knowledge and their practices.

In addition, during this study, biochemical parameters were measured to assess the impact of exposure to organophosphate pesticides among market gardeners. No significant difference in creatinine or urea levels was observed between market gardeners and controls, but there was a significant decrease in transaminase activity in market gardeners compared to controls. There was also a significant difference in cholinesterase activity in market gardeners compared to controls. However, the average values for all these parameters were within normal limits. A 20% inhibition of butyrylcholinesterase activity was also noted in these market gardeners. This inhibition, as can be seen, does not require any risk mitigation measures, since it is less than 40%, which is the biological limit recommended by the WHO. The market gardeners in our study were not affected by exposure to organophosphates.

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