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Alterations in Renal Functions of Market Gardeners Occupationally Exposed to Pesticides in West Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Author MGT did the study design and wrote the protocol. Authors MGT and DNT did the literature searches, field work and statistical analysis while analyses of the study was done by author PBT. All authors read and approved the final manuscript.

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ABSTRACT

Despite the tremendous advantages of pesticides in sustainable agriculture, their excessive misuse causes environmental and health hazards.

Objective: Assessment of the effects of pesticides on renal functions of market gardeners in the western highlands of Cameroon.

Design and Methods: A total of 124 male market gardeners in Foumbot, Massangam and Bantoum were questioned on their health status, attitudes and behaviour with respect to agricultural practices. Thereafter, their blood samples were collected for assessment of renal function biomarkers including serum urea and creatinine. Urine samples were also analysed using 11-parameter urinalysis test strips. Sixty men with no history of pesticide exposure were recruited as control group.

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Results: Fifty six pesticides containing 25 active ingredients were currently used by market gardeners enrolled in our study, and most of their symptoms (headache, fatigue, skin rashes, eye irritation and nausea) were related to spread/use of these chemicals. Compared to the control subjects market gardeners' urea and creatinine serum levels were significantly increased (11.4 ± 1.59 $mgdL^{-1}$ *vs.* 12.61 ± 1.94 $mgdL^{-1}$; $P< 0.05$) and (12.5 ± 3.0 $mgdL^{-1;}$ *vs.* 29.41 ± 12.98 $mgdL^{-1}$ $P < 0.001$), respectively. Though some positive cases were identified, no significant difference was recorded in urine nitrites, ketones and protein levels.

Conclusion: Our results suggest that market gardeners in West Cameroon are exposed to pesticides, and that this exposure could impair their renal functions. Despite the infancy of biomonitoring of effects of pesticides in Cameroon, a crucial need arises to increase awareness of market gardeners on the adverse effects of pesticides on kidney functions and increase government subventions on Personal Protective Equipments.

Keywords: Biomarkers; kidney; occupational exposure; pesticides.

ABBREVIATIONS

AChE : Acetyl Cholinesterase
ALT : Alanine aminotransferase
AST : Aspartate aminotransferase
FAO : Food and Agriculture Organisation of the United Nations
IU : International units
PPE : Personal Protective Equipment
UV : Ultraviolet
UNEP : United Nations Environment Programme
WHO : World Health Organization

1. INTRODUCTION

Pesticide use in agriculture is the fastest and most economical approach to control pests and disease, though they are considered major contaminants of our environment, and of varying toxicity to our health [1]. The World Health Organization [2] and the United Nations Environment Programme [3] have estimated one to five million cases of pesticide poisoning among agricultural workers each year with about 20,000 fatalities, mostly reported from developing countries. Exposure to pesticides is one of the most important occupational risks among farmers in developing countries [4]. Occupational exposures to these pesticides occur from skin absorption, inhalation and ingestion and their toxicity may be attributed to a number of reasons, including farmers' poor knowledge about pesticides and pesticide use, less protection against exposures, minimal understanding of the health risks and, most importantly, inadequate safety warnings on the packages provided by the manufacturers [5].

Evidence continues to accumulate that chronic pesticide exposure is associated with impaired health, including carcinogenesis, neurotoxicity, reproductive, developmental, and immunological effects [6]. Studies have shown that xenobiotics can result in glomerular dysfunction and tubular dysfunction, glomerular proteinuria, tubular proteinuria, reduction of glomerular filtration rate, and decrease in concentration function of the kidney. Misuse of highly toxic pesticides, coupled with weak extension measures and a partial legislative framework, is one of the major reasons for the high incidence of pesticide poisoning in developing countries [7]. In general, knowledge of the main determinants of pesticide exposure in developing countries is often poor [4].

To the best of our knowledge, there has been no attempt to study the adverse health effects of prolonged low-dose pesticide exposure, on market gardeners in the Western Region of Cameroon. This study to monitor the effects of prolonged low-dose exposure to pesticides on renal functions of market gardeners was imperative. The specific objectives were to evaluate market gardeners' attitudes and behaviour regarding the use of crop protection products and to assess kidney function by evaluating serum urea and creatinine levels.

2. MATERIALS AND METHODS

This study was a cross-sectional and transversal design of exposure and outcome, carried out from the month of February to June, 2014 in the Western Highlands of Cameroon. The geographical location of the four sites were; Foumbot (5°30' 28''N, 10°37' 57''E), Massangam (5° 25' 0''N, 10° 58 0''E) in Noun Division, Bantoum (4°55' 0''N, 10°46' 0''E) in Nde Division and Dschang (5°27' 0''N, 10°4' 0''E) in Menoua Division.

2.1 Study Population

A cohort of market gardeners in the West Region was recruited based on their potential exposure to pesticides. Adult males with no history of pesticide exposure were retained as control group in the study. Individuals presenting diabetes, neurological disorders, liver dysfunction, cancer or any other chronic condition were excluded from the population studied in order to avoid any interference with the biochemical parameters measured. Recruited individuals were interviewed and then examined by a general practitioner on a scheduled day. All individuals were offered health examinations consisting of a medical history, a physical examination and laboratory test.

Information on demographic characteristics (age of the subject, gender, height and weight), lifestyle (smoking habit and alcohol consumption) and occupational features (lifetime exposure to pesticides, use of personal protective equipment and type of pesticide used) were collected by means of a questionnaire administered by the health personnel. Participants were aged above 21, resident in small villages for at least 5 years with the majority working in farms.

2.2 Sample Collection

After an overnight fasting period, venous blood samples (4 -5 ml) were collected in tubes with clot activator (sodium citrate) and preserved cooled less than 2 hours until they reached the laboratory. Serum was separated by high-speed centrifugation (3000 g for 5 minutes), aliquoted and placed in labelled PCR tubes (eppendorf). These samples were then stored at 4-8℃ until analysed within 2-3 days. A second set of all serum samples was stored at -20℃ as reserve until analyses were completed (within the first month).

2.3 Exposure Assessment

An estimate of exposure was performed by calculating the cumulative pesticide exposure (CPE) for each farmer. It was calculated by multiplying the average number of working days per week by the average number of weeks per year and by the average number of years of pesticide use [8].

2.4 Biochemical Analysis

2.4.1 Serum creatinine (Cr)

Serum Creatinine (Cr) concentration was determined by modified Jaffe's method (1886) without deproteination. Liquick Cor-Creatinine diagnostic kit (CORMAY- Poland) was used.

$$\text{Creatinine concentration}(mg/L) = \Delta A(T) \times \Delta A(S) \times standard\ concentration$$

2.4.2 Creatinine clearance (Crcl)

Creatinine clearance (Crcl) was estimated using the Cockroft-Gault method, estimated as;

$$\text{Ccr (mL/min)} = \left(\frac{(140 - \text{age}) \times \text{body mass}}{\text{plasma creat } \times 72}\right) \times \text{ f}$$

(rounded to the nearest whole number. Multiply by 0.85 for females.)

2.4.3 Serum urea

Serum urea concentration was determined by Kassirer kinetic method (1971). Liquick Cor-Creatinine diagnostic kit (CORMAY- Poland) was used.

$$\text{Urea concentration} = \Delta A(T) \times \Delta A(S) \times standard\ concentration\ (mg/dL)$$

(1 mg of urea corresponds to 0.467 mg of urea nitrogen)

Key			
A	Absorbance	**Ccr**	Creatinine clearance
T	Test	**f**	constant
S	Standard		

2.5 Statistical Analysis

Data input and histograms were done in Microsoft Excel, 2013 computer program. Results were presented as mean ± S.D. Significant differences between control and exposed groups were analyzed using the Student-Newman-Keuls Multiple Comparisons Test of the computer program Graph Pad Instat 3.0. Results were considered statistically significant when $p < 0.05$.

3. RESULTS

3.1 Types of Pesticides Used

The overall size of farms ranged from 2 to 20 ha. A total of 26 fungicides, 15 insecticides, 2 insecticides-nematicides, 1 nematicide and 12 herbicides were used. Taken all together, the most used active ingredients were maneb, followed by cypermethrin, glyphosate and paraquat.

3.2 Attitudes and Behaviour Regarding Pesticide Use

The market gardeners were aged between 22 and 49, with a mean age of 36.47 years. Body Mass Index (BMI) recorded was on average 25.04 kg/m^2. Market gardeners had a mean Systolic Blood Pressure/ Diastolic Blood Pressure (SBP/DBP) of 140.09/71.29 mmHg. Few market gardeners had a slightly raised Blood Pressure (BP). Of those who responded to the survey, 57% were the farm owners, the remainder being relative, employees or contracted workers.

3.2.1 Education

59% of market gardeners had some education, although for the majority this only included attendance at a primary school (Fig. 1).

Table 1. List of commonly used pesticides in study areas

Active ingredient	Use	WHO Cat	Pesticides	% r.t.P	% r.t.MG
Maneb	F	II	Manesan, Trimagnol 80 WP, Plantineb 80 WP	11.3	84.5
Metatoxyl	F	II	Ridomil Plus 72 WP, Callomil Plus, Metalm 72 WP, Fongistar	8.6	72.4
Copper	F	II	Ridomil plus 72 WP, Metalm 72 WP, Callomil plus,	9.1	74.3
Mancozeb	F	II	Fongistar, Agrizeb 80 EC, Ivory 80 WP, Penncozeb 80 W,	6.8	53.2
Chlorothalonil	F	III	Balear 750 SC, Chloroplant 720 SC, Dacobre, Talonyl, Daconil	5.8	44.7
Carbendazim	F	III	Banko plus	4.4	31.9
Benalaxyl	F	II	Galben plus	0.6	2.5
Benomyl	F	II	Benlate	0.3	2.4
Glyphosate	H	U	Roundup 120, Glyphader, Kalach 120 SL, Sikosto 360 SL	9.1	70.3
Paraquat	H	III	Calloxone super SL, Gramoxone super	6.8	52.1
Diuron	H	III	Action 80 DF, Diuron 80 W	5.3	36.9
Linuron	I	III	Tromissil 50 WP	0.6	4.8
Trimesium	H	II	Touchdown	0.3	2.7
Cypermethrine	I	II	Nurelle D20/200 EC, Cypercal 100 EC, Cycogne 200 EC	11.4	80.5
Dimethoate	I	II	Cyperdim 220 EC, Callidim 400 EC. Dimezyl 400EC, Dimex 400 EC	9.8	81.2
Chlorpyrifos	I	II	Nurelle D20/200 EC, Dursban 4 EC	5.1	35.6
Chlorpyriphos-ethyl	I	II	Pyriforce	0.3	2.2
Lambda-cyhalothrine	I	II	Pilori 15 EC	1.9	10.4
Deltamethrine	I	II	Decis 60 EC	1.1	8.4
Malathion	I	III	Malathane	0.6	2.1
Profenos	I	II	Calfos 720 EC	0.3	2.6
Thiamethoxam	I	III	Actara 25WG	0.2	1.6
Ethoprophos	I/N	II	Mocap 10G	0.4	1.6
Oxamyl	I/N	II	Vydate 240 EC	0.2	3.4
Carbofuran	N	II	Sesame 5 G	100	-

II: moderately hazardous, III: slightly hazardous, U: unlikely to cause acute hazard
F: Fungicide, I: Insecticide, N: Nematicide, H: Herbicide, I/N: Insecticide-Nematicide.
% r.t.P: percentage relative to pesticides, % r.t.MG: percentage relative to market gardeners
WHO Cat. World Health Organisation category of pesticides

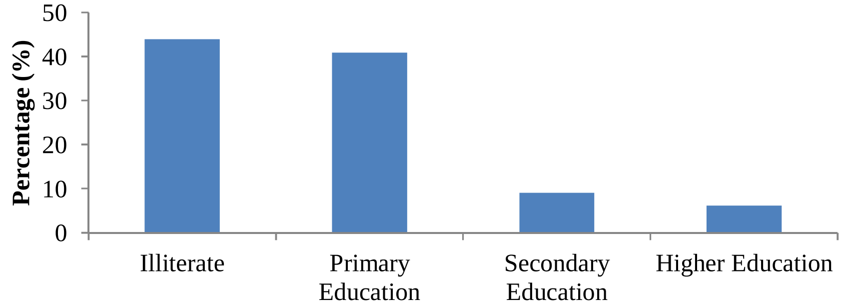

Fig. 1. Educational level of market gardeners

3.2.2 Types of sprayer, intensity of use and maintenance

A lever-operated knapsack sprayer was used on average by 93% of market gardeners. Pesticide application was done between 90 and 120 h a month. The average number of years of pesticide use was 10.26, and the average spraying frequency was 2.23 times/week. The mean CPE was 129.77 ± 46.1.

Of the market gardeners, 47% reported sprayers were usually either damaged or over-filled such that liquid obviously leaked on them. Most leakages occurred with intensive use and were not repaired due to lack of funds or negligence. When a nozzle was blocked, 32% used a probe (stick, wire or grass stem) to remove the blockage, although this can damage the nozzle and can increase its subsequent output resulting in overdosing. Only 46% of market gardeners washed their sprayer after each application. Lack of washing out the spray liquid after application resulted in damage to a different crop, especially where different types of pesticides were used through the same sprayer. Only 8% of the sprayer wash water was stored for reuse. The majority was disposed of in water courses situations where water courses could become contaminated.

3.2.3 Transport

Most (78%) transported the pesticides separately from food. Only 22% reported always carrying pesticides at the same time as food and these were primarily in Massangam.

3.2.4 Storage

62% of market gardeners reported storing pesticides, 58% of these in a locked cupboard, while 11% left the pesticides in the open. Storage in the house or in a bedroom was considered safe from theft.

Table 2. Intensity of use and maintenance of sprayers

Parameters	Actions	Percentage
Use of sprayers	Leaked with obvious spillage	47
	Never leaked	15
	Repaired leak immediately	38
Measures of unblocking a nozzle	Stick, wire or stem	32
	Fingers	16
	Water	42
	Mouth	8
	Others	2
Maintenance of sprayer	Wash after use	41
	Less often	39
	Others	12
	Stored sprayer wash water	8

Table 3. Transport and storage of pesticides by market gardeners in studied areas

Parameters	Actions	Percentage
Transport	Separately from food	78
	By car or Public transport	47
	By motorcycles or on foot	53
Storage	Locked location	58
	In house or bedroom	15

3.2.5 Consumption of stimulants

43.9% of market gardeners said they smoked occasionally i.e. at least weekly. On the other hand, 78.8% of market gardeners consumed alcohol at least weekly.

Table 4. Rate of consumption of stimulants by market gardeners in studied areas

Attitudes and behaviour		Market gardeners freq. (%)
Tobacco smoking	None	56.1
	Weekly	43.9
Frequency of alcohol intake	None	21.2
	At least weekly	78.8
Coffee/ tea intake	None	69.9
	Weekly	19.6
	Daily	10.9

3.2.6 Preparation of sprays

Most pesticides were mixed in a separate container and stirred with a stick (48%). Of the market gardeners 28% used their bare hands in mixing while 72% used gloves. Disposal of empty pesticide containers was rather haphazard, approximately 80% of farmers discarded pesticide waste cans in pits and water bodies, and some burned or buried the cans while about 6.1% of market gardeners brought the empty container back to the farmer's houses.

3.2.7 Personal protective equipment

Most farmers wore gloves and covered shoes during mixing and loading, 15% of market gardeners, irrespective of the age, wore the recommended five key items, including long trousers, and long-sleeved shirts (or overalls), gloves, boots and face-shield. Farmers reported a lack of clean water in the field and the small amount available was reserved for drinking.

3.2.8 Information on safe use

32% of respondents said that they had received information on safe use of pesticides within the previous 12 months, although many had learnt about safe use previously. The needs to wash after spraying, store pesticides safely and read the label were the key messages that many said they had learnt over the last 3 years. The main source of information was the local distributor/supplier of the agrochemicals.

3.2.9 Benefits vs. health concerns

Over 90% of market gardeners agreed or somewhat agreed that there was a benefit using pesticides with half of the respondents agreeing somewhat strongly that they should be concerned about their personal safety. 65% of respondents agreed that the public should be concerned about the use of pesticides, but 34% reported they would rather use alternative means to control weeds, pests and diseases.

Table 5. Attitudes and behaviour in the spraying process

Process	Habits and attitudes	Percentage
Spray preparation	Use stick	48
	Mixed in sprayer tank	46
	Used hand (72 % - gloved; 28 % - bare)	5
During mixing	All 5 items of PPE	15
	Wash off spill immediately	34
	Wash off spill at the end of the day	66
Measurement of pesticide	Use of graduated lid	60
	Personal graduated device	28
	Un-graduated container	12
	Felt mixing practices are acceptable	81
During spraying	All 5 PPE	5
	Boots or foot covering	31.8
	Overalls	21.2
	Long trousers and long-sleeved shirts	42
	Face shield	12
	No protective tool	22.7
	Avoided spraying during heat of the day	63
	Considered direction of the wind	30
After spraying	Had a shower immediately after spraying	18.7
	Washed spraying clothes after each spraying	12.4

3.3 Incidents

Participants had the same background cultures and were exposed to about the same amount of sunshine. 10% of health incidents recorded was associated with the transport of pesticides to the farm, especially spillage on back from broken lids of containers. The level of minor incidents was impressive and respondents confirmed spillage occurred often on hands. Incidents were mainly (73%) of a minor nature requiring no more than self-medication. The main complains were body cramps (86.4%), headaches and skin irritations (Table 2). Skin irritation (79.7%) was frequent when applying lambda-cyhalothrin (69%) and cypermethrin (63%). Most hospital cases were related to organophosphate insecticides, including cypermethrin and deltamethrin.

Table 6. Health incidents reported by market gardeners

Complaints	Number / 66	Frequency (%)
Body cramps	57	86.4
Headache	52	84.8
Body itches/ skin rash	46	79.7
Cough/ breathing difficulty	56	78.8
Gastritis	55	76.3
Tiredness	50	75.8
Eye irritation	48	72.7
Dizziness	53	70.3
Nausea	36	54.5
Nerve pain	34	51.5
Diarrhoea	15	22.7

3.4 Kidney Function in Studied Groups

3.4.1 Variation of serum markers of kidney function

Table 7 showed that serum levels of creatinine and urea were significantly higher among market gardeners as compared to the controls. Estimated creatinine clearance levels of market gardeners were lower compared to the control group.

3.5 Variation of Biochemical Parameters with Respect to Attitudes and Behaviour

3.5.1 Distribution of kidney function markers with respect to intake of alcohol

High levels of serum creatinine (1.5 – 1.8 mg/dL) occurred in 12% and 19% of the alcoholic and non-alcoholic subgroups of market gardeners respectively. Similar trends of higher than normal serum urea levels (>50 mg/dL) were recorded in 10% and 8% of the alcoholic and non-alcoholic subgroups of market gardeners respectively as shown in Fig. 1. Interestingly, 85% of the non-alcoholic subgroup contrary to 43% of the alcoholic subgroup had serum urea levels higher than the control maximum (20-50 mg/dL) even though these values were within reference limits.

Fig. 2 revealed the distribution of creatinine clearance with respect to alcohol intake. We observed lowest values (30 – 59 mL/min) indicating stage 3 Chronic Kidney Damage (CKD) with moderate Glomerular filtration Rate (GFR) in 10.5% and 15.4% of the alcoholic and non-alcoholic subgroups respectively. In the same light, stage 2 CKD with mild GFR (60 – 89 mL/min) occurred in 63.2% and 42.3% of the alcoholic and non-alcoholic subgroups respectively. There was however no significant difference ($p = 0.34$) between the S.Ds of these populations. Creatinine clearance, a sensitive marker of kidney function was not influenced by alcohol intake.

4. DISCUSSION

The first part of our work was a knowledge, attitude and behaviour (KAB) survey. Limited use of appropriate PPE was due to negligence, ignorance, and discomfort of muffler and extreme heat of protective overall. Similar behavioural trends were observed in previous studies [9]. Our study revealed that fungicides (e.g. maneb) and herbicides were the most used pesticides, stipulating that fungal diseases were the main threats to crops in these localities. This may be related to the farmers' preference use for tomatoes culture, most susceptible to these pests and diseases. An increase in incidence of illnesses was partly because market gardeners were unaware of the side effects of pesticide use as they did not read the label, probably due to; the small size of the letters, the language or illiteracy.

Headaches and feeling weakness (fatigue) were the most common complaints, which could be also due, to long exposure to sun/UV radiation, especially if no head protection was worn. The odour of some pesticides or co-formulants also elicited a feeling of nausea even when the chemical was not highly toxic. The recurrent bouts of fever or headaches could be partly due to malaria as most did not use mosquito nets.

Chronic dermal exposure to low dose pesticides was associated with allergic reactions. Our results were in accordance with those of [10,11].

In our study, exposure was increased due to; the lack of personal hygiene as water was a rare commodity in farms, the non-respect of wind direction as they still had to walk along the rows of the crop, so it was not always possible to hold the lance downwind, the muddy nature of some soils where the use of boots was unsuitable, the use of an adsorbent material to cover their faces which instead accumulates pesticide vapours thus increasing amount of pesticide inhaled and leakages from sprayers, poor storage conditions and careless disposal of pesticide leftovers and containers. The cases of leakages from lever-operated knapsack sprayers were either due to farmers using the less expensive and thus less robust equipment or lack of availability of spare parts. This observation was in line with a previous survey [10,12] where it was reported that, the worst behaviour involving the use of cheap sprayers, inadequate personal protection and ready availability of toxic pesticides occurs mostly due to the less effective registration and control of pesticides, exacerbated by a lack of concern for the well-being of the poor rural populations.

Table 7. Serum markers of kidney function in studied groups

Serum markers	Control	Agropesticide users
Creatinine (mg/L)	11.4 ± 1.59	12.61 ± 1.94*
Urea (mg/dL)	12.5 ± 3.0	29.41 ± 12.98***
Creatinine clearance (ml/min)	94.94 ± 13.77	82.84 ± 22.38**

*Significant difference between the control and market gardeners group is indicated by *, ** and *** representing p<0.05, p<0.01 and p<0.001 respectively*

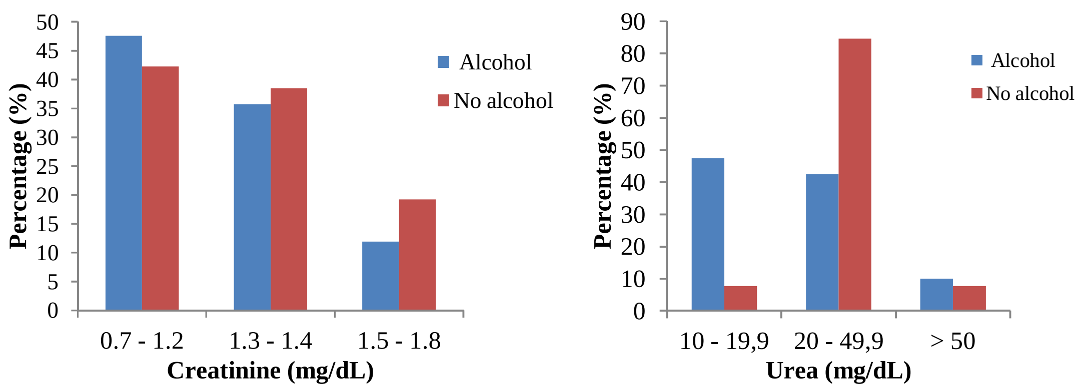

Fig. 2. Distribution of creatinine and urea with respect to alcohol consumption

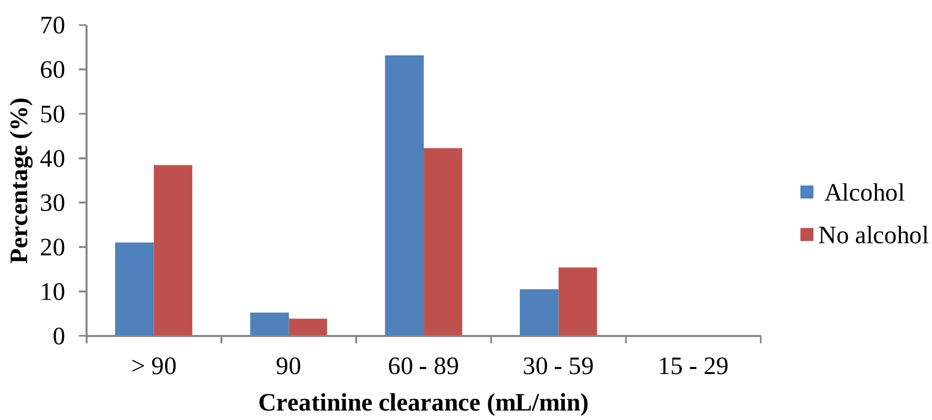

Fig. 3. Distribution of creatinine clearance with respect to alcohol consumption

Pesticide waste disposal was rather haphazard, mostly in pits and water bodies and such conditions may lead to exceeded recommended maximum residue limit (MRL) of pesticide residues in harvested crops, extending the threat to the consumers. In order to attenuate some of the side effects of pesticide application, market gardeners ate before pesticide manipulation, drank milk or water or applied the products early in the morning and in the afternoon (2-6 pm).

From our second aim which consisted of appreciating kidney function after chronic exposure to pesticides, we found significantly high urea and creatinine levels observed market gardeners which reflected early renal impairments or dysfunctions. Our results coincided with previous studies reporting minimal nephrotoxic changes with higher levels of serum creatinine and urea in workers of a chemical plant producing dust pesticides [13]. Our results also coincided with those of [14] and [8] who reported a subtle nephrotoxic change in workers occupationally exposed to pesticides, due to their higher levels of serum creatinine and/or blood urea.

Another interesting finding was a significant decrease in creatinine clearance levels in the pesticide-exposed group with a high prevalence of stage 2 and stage 3 chronic kidney damage (CKD) with mild and moderate glomerular filtration rate (GFR) respectively. Creatinine being a substance whose constant serum concentration is freely filtered through the glomerulus and undergoes no reabsorption or tubular secretion has a clearance that is equal to the Glomerular Filtration Rate (GFR) and is a good tubular indicator. Creatinine clearance is more sensitive than serum creatinine and any decrease in value reflects either organic kidney damage (a decrease in number of functional glomeruli or an alteration of the basal membrane) or functional kidney damage: [a decrease in renal blood flow (slow circulation or a decrease in efficient filtration pressure)]. This result coincided with that of [15]. Despite these significant alterations in liver and kidney function markers; there was no overt clinical disease with hospitalisation during the study period. This was probably because these key organs of detoxification and excretion have very high adaptability capacities. At the level of assessing risk factors, we found that, alcohol and level of education did not influence the alterations in biological parameters even though some differences occurred among population subgroups. Our results were in line with those of [8] who reported an insignificant difference in serum markers of kidney function of market gardeners who consume alcohol and tobacco those who do not.

5. CONCLUSION

Long-term low-dose exposure to pesticides can lead to impaired kidney functions. Thus, further research is needed to confirm these findings and to establish the possible mechanism of toxicity. Implementation of government regulations on pesticide use and sales must also be strengthened.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Cameroon Bioethics Initiative (CAMBIN), Reference number: CBI/240/ERCC/CAMBIN, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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