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ABSTRACT:

Serum Cholinesterase (SChE) or Pseudocholinesterase can be used for assessing the sensitivity to Succinylcholine (Scoline) or as indicator for insecticide and pesticide poisoning. This cross-sectional study assessed the SChE and Dibucaine number (DN) among students in the University of Papua New Guinea. The single proportion formula was used to estimate the sample size. A total of 276 apparently healthy students were selected by simple random sampling. The "Linear Chemicals" reagent kit for assay of SChE was used to determine the SChE activity. The DN was estimated by assay of the SChE with and without the inhibitor Dibucaine. Signed informed consent was obtained from 217 students, which consisted of 99 (45.6%) males and 118 (54.4%) females. The mean ages for the male and female students were 26.0 ± 6.5years and 23.8 ± 5.3 years respectively.

The median SChE activity was 3.74KU/L for the males and 3.51KU/L for the females. The Interquartile Ranges (IQR) of the SChE activities for the male and female students was 2.80 – 4.48KU/L and 3.02 – 4.13KU/L respectively. Although there was no statistically significant difference between the SChE activity of the male and female students, considerable variation was observed in the SChE activity in both the male and female students.

The median DN for the male students was 85.43 % and the IQR was 81.6% – 88.2%. For the female students the median was 86.1% and the IQR was 83.5% – 88.0%. No statistically significant difference was obtained between the DN for the male and female students.

The data strongly supports the need for screening of students and other patients before administering Scoline which is the muscle relaxant commonly used in the Port Moresby General Hospital and other hospitals in National Capital District in Papua New Guinea.

Key words: Serum, Cholinesterase, Pseudocholinesterase, Dibucaine, Scoline, Students,
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INTRODUCTION:

The most physiologically important Cholinesterase (ChE) is Acetylcholinesterase (AChE) located in the presynaptic knobs of cholinergic neurones, red blood cells, lungs and spleen [1, 2]. The functions of AChE are well defined [1, 2, 3]. The other ChE is Serum Cholinesterase (SChE), which is mainly located in the plasma and serum [1, 2, 3]. The alternative names for SChE are Pseudocholinesterase, Acetylcholine Acylhydrolase, Benzoyl Cholinesterase and Cholinesterase II [1, 2]. Although the major biological function of SChE is not well defined, several researchers have suggested that it catalyzes the hydrolysis of acetylcholine and other esters of Choline in the blood, such as, Succinylcholine (Suxamethonium) and Mivacurium used as muscle relaxants during surgical procedures [2 – 5]. Some ester type compounds like Procaine, Chloroprocaine, Tetracaine, Cocaine and Heroin are hydrolysed by SChE [6, 7].

Both AChE and SChE activities are inhibited by organophosphorus compounds in pesticides and insecticides [1, 2]. During exposure to these toxicants the SChE activity usually falls more rapidly compared to AChE, thus clinical signs or symptoms are not detected under limited exposures [1, 2]. The change in SChE activity is used as a sensitive biochemical parameter for assessing the exposure of an individual to organophosphorus compounds and sensitivity to Succinylcholine [2 – 5].

Two major genetic variants ("atypical" and "normal") of SChE are known [2,3,6]. This gave rise to three forms of SChE. Individuals with the "atypical variant" form of SChE are homozygous for the "atypical" gene; those with the "normal variant" form are homozygous for the normal gene [2, 3, 6]. The third variant are individuals that are heterozygous for SChE [2,3,6].

The SChE activity is usually low in an individual with the atypical variant compared to the high SChE activity in an individual with the normal variant [2,3,6]. For the heterozygous individual the SChE activity is

between these two extremes [2,3,6]. These three variant forms of SChE can be identified by reaction with Dibucaine, which is an inhibitor of SChE [3, 7 – 9]. Dibucaine (Nupercaine) is a local anaesthetic [7]. Irrespective of the level of SChE in blood the activity of the normal variant is greatly inhibited by Dibucaine compared to the activity of the atypical variant [7, 8, 9]. Dibucaine inhibits the activity of the normal variant form of SChE by 80%, compared to a 20% inhibition of the atypical variant form of SChE [7, 8, 10]. Dibucaine inhibits the heterozygous variant form of SChE by between 40 to 60% [7, 8, 10].

The Dibucaine number (DN) is the percentage inhibition of SChE by Dibucaine under specified conditions [7 – 10]. The DN is an important diagnostic characteristic of an individual because it is independent of the amount of SChE in the blood [7]. Thus the adequacy of SChE in an individual can be assessed both quantitatively and qualitatively.

The SChE activity is used as the parameter for the quantitative assessment and the DN is used as the parameter for the qualitative assessment [7].

The low SChE activity in some individuals may be an inherited enzyme abnormality that results in abnormally slow degradation of the acetylcholine like esters. These individuals may be Suxamethonium-sensitive and may have qualitatively low SChE level, which is a genetically determined trait. The clinical consequence of this may include prolonged paralysis of the respiratory and other skeletal muscles after administration of a standard dose of Succinylcholine [4 – 7, 10]. It is therefore important to assess the SChE activity and DN prior to the administration of Suxamethonium, to minimise the risk of prolonged muscular relaxation and apnoea in susceptible individuals [4 – 7, 9].

In most developed countries the assay of SChE is part of the routine laboratory tests carried out either for assessing the sensitivity to Succinylcholine or for assessing liver function or as an indicator for insecticide and pesticide poisoning [5]. The lack of such routine laboratory tests in most developing countries with high incidence of malaria is worrisome. This is because the predominant types of insecticides and pesticides used to reduce the spread of the malaria parasites are organophosphates and carbamates that are highly toxic to both humans and other organisms [9].

A review of the literature indicates that published data on the prevailing SChE and the SChE variants (DN) in the Papua New Guinea (PNG) population is scanty [11]. The

use of a variety of insecticides in the form of sprays and coils for protection against mosquito bites is a common practice among students in the University of PNG (UPNG). Chronic exposure to these insecticides can results in depression of SChE activity without affecting the DN. This study was prompted by the apparent lack of data to characterize SChE among students in the UPNG.

The aim of this study was to assess the SChE activity and DN among students in the University of Papua New Guinea. Knowledge of the SChE activity and variants that are prevalent among the students can be used by appropriate authorities to make informed recommendations for the screening of students and other patients before using Scoline as muscle relaxant in the Port Moresby General Hospital (PMGH), which serve as the major general, specialist and reference hospital in NCD and PNG.

SUBJECTS AND METHODS

This was a prospective cross-sectional study carried out in the NCD, which is the incorporated area around Port Moresby the capital city of PNG. The sampling sites included both campuses (Waigani and Taurama) of the University of Papua New Guinea (UPNG). This study was conducted among students because of their easy accessibility and willingness to donate blood samples for research purpose. The single proportion formula was used to estimate the sample size, which was calculated using a design effect of one, a relative precision of 7.0% and a confidence level of 95%. An assumed prevalence rate of 25% was used because of the non availability of data on the characteristics of SChE among student in the UPNG. The sample size of about 270 apparently healthy students was considered adequate for a study, with a predicted non-response rate of 20%.

A total of 276 apparently healthy students (subjects) were selected by simple random sampling. The purpose of the study was appropriately explained to each of them before requesting their signed informed consent. All students had been living in the UPNG for at least twelve weeks before sample collection, which is less than the fifteen weeks academic session in the UPNG. Blood sample was collected only once from a consented student. About 0.5ml of blood was collected into a plain sterile microtainer after finger-stick using contacted activated single-use lancet. Each clotted blood was centrifuged to obtain the serum which was then decanted and stored frozen at – 70°C, until required for analysis.

The “Linear Chemicals” reagent kit for assay of SChE (total and inhibited) was used [10]. All additional reagents used were

of analytical grade. The assay of SChE and determination of the DN were carried out as indicated in the manufacturer's protocol [10]. The SChE catalyzes hydrolysis of the substrate Butyryl-Thio-Choline, forming Butyrate and Thiocholine. The Thiocholine interacts with 5,5`-Mercaptobis-2-Nitrobenzoic acid (DMNB) to form a yellow coloured compound {5-Mercapto-2-Nitrobenzoate (5-MNBA)}. The progress of the reaction (at 30^{o} Celsius) was monitored at 405nm and the change in Absorbance (ΔA) recorded ever 30 seconds [10].

The SChE activity was calculated thus: SChE Activity (KU/L) = (ΔA/30sec) x (23111/1000) [10].

The DN was estimated by assay of the SChE with and without the inhibitor Dibucaine. The percent inhibition of the SChE was calculated as follows: One minus the ratio of inhibited to un-inhibited SChE expressed as percent. The percent inhibition is the DN of SChE in the sample assayed [10]. Internal bench quality control (QC) was carried out using the normal and abnormal QC samples supplied by the manufacturer [10].

Data analysis was by the Microsoft Excel Data Pack and Statistical Package for Social Sciences (SPSS) softwares. Kolmogorov-Smirnov test for normality was used to assess the distribution of the data. Chi-square test, Mann-Whitney test and Wilcoxon U tests were used as appropriate.

Ethical clearance and approval for this project were obtained from the Research and Ethics Committee in the School of Medicine and Health Sciences UPNG and the Chief Executive Officer PMGH.

RESULTS:

Blood samples were collected from 217 of the 276 apparently health students randomly selected for participation in this study. This gave a consent rate of 78.6%. The 217 students consisted of 99 (45.6%) males and 118 (54.4%) females. Table 1 shows the summary statistics of the age for the male and female students. The mean age for the male students was 26.0 ± 6.5 years (Mean ± STD), the median age was 23.0 years and the Interquartile Range (IQR) was 22.0 – 30.0 years. For the female students the mean age was 23.8 ± 5.3 years, the median was 22.0 years and the IQR was 21.0 – 24.0 years.

The Kruskal-Wallis and Chi-square tests indicated that the mean age of the male students was significantly (p = 0.001) higher than that of the female students. The Mann-Whitney and Wilcoxon U tests also indicated statistically significant (p = 0.001) differences between the mean ages of the male and female students.

The Kolmogorov-Smirnov test for normality of distribution indicates that the SChE activities (KU/L) obtained for the male and female students were not normally distributed. This was confirmed by the outliers shown in the Box-plots of the SChE activities (KU/L) for the males and females presented in Fig. 1. Thus, non-parametric statistics were used for analyzing the data.
Table 2 shows the summary statistics of SChE activities (KU/L) for the male and female students.

The median SChE activity was 3.74KU/L for the males and 3.51KU/L for the females. The Interquartile Ranges (IQR) of the SChE activities for the male and female students was 2.80 – 4.48KU/L and 3.02 – 4.13KU/L respectively. Mann-Whitney and Wilcoxon U tests ($p = 0.432$), Kruskal-Wallis test ($p = 0.432$) and Chi-Square test ($p = 0.379$) indicated that the SChE activity (KU/L) of the male students was not statistically different from that of the female students.

Analysis of Variance (ANOVA) after Post-Hoc analysis (Scheffe) also indicated that there was no statistically significant ($p > 0.05$) difference between the SChE activity of the male and female students.

Table 1: Summary statistics of the age (years) for the male and female students

Parameters	Male students (n = 99)	Females students (n = 118)
Mean (years)	26.0	23.8
Median (years)	23.0	22.0
95% Confidence Interval (95% CI)	24.6 – 27.3	22.8 – 24.8
Standard Deviation (STD)	6.5	5.3
Range (years)	15.0 – 56.0	14.0 – 51.0
Interquartile Range (IQR) (years)	22.0 – 30.0	21.0 – 24.0

Fig. 1: Box-plots of Serum Cholinesterase activity (KU.L)

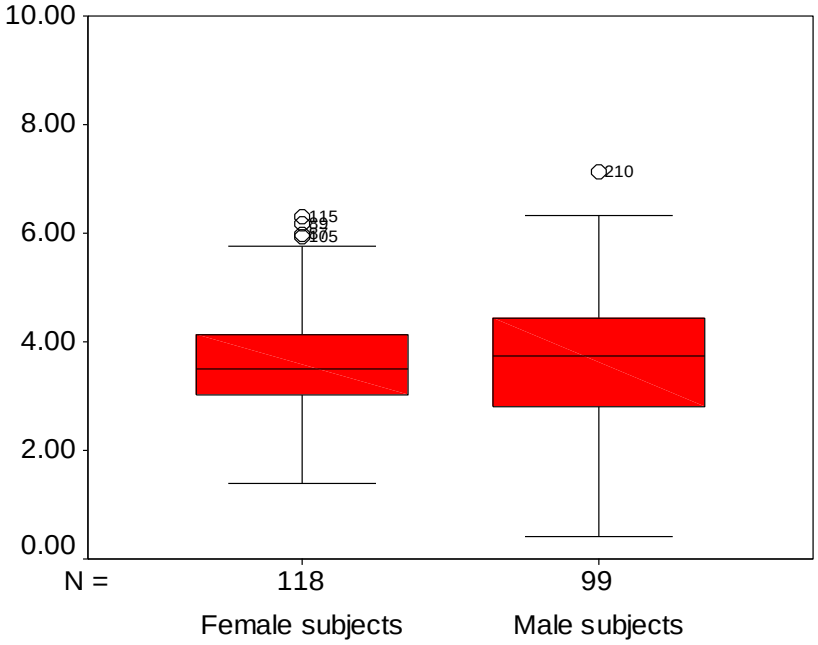

Table 2:
Summary statistics of the Serum Cholinesterase activity (KU/L) for male and female students

SChE activity (KU/L)	Male students (n = 99)	Female students (n = 118)
Median	3.74	3.51
IQR	2.80 – 4.48	3.02 – 4.13
Mean	3.64	3.36
95% CI	3.37 – 3.90	3.44 – 3.81
STD	1.32	1.02
Range	0.51 – 7.13	1.40 – 8.76
Calculated Reference Ranges	0.67 – 6.43	0.85 – 6.13

Table 3:
Percentile cut-offs for Serum Cholinesterase activity (KU/L) for male and female students

Percentiles	Male students (n = 99)	Female students (n = 118)
5th	1.16	2.11
10th	1.91	2.62
25th	2.8	3.02
50th	3.74	3.51
75th	4.48	4.13
90th	5.17	4.49
95th	6.02	5.76

Table 4:
Distribution of male and female students according to their Serum Cholinesterase Activity (KU/L)

SChE activity (KU/L) [10]	Male students (n = 99)	Female students (n = 118)
< 2.4	14 (14.1%)	7 (5.9%)
2.4 – 8.5	85 (85.9%)	110 (93.2%)
> 8.5	0	1 (0.9%)

*Reference 10

The percentile cut-offs for the SChE activities for the male and female students are presented in Table 3. The 5th to 25th Percentile cut-offs for the males were lower than that of the females. This trend changed for the 50th to 95th Percentile cut-offs were the values for the males were higher than the values for the females.

The distribution of the male and female subjects according to their SChE activity is presented in Table 4. Normal SChE activity was prevalent in 85.9% of males and 93.2% of females. The SChE activity was below

the lower limit of normal in 14.1% of males and 5.9% of females. When asked about the use of mosquito coils and sprays positive responses were obtained from 4.0% of males and 7.6% of females. Most of the male and female students had no knowledge about the content of the mosquito coils and sprays that they are using against malaria.

The Spearman's rho (r = - 0.03, p = 0.76) indicated weak non-statistically significant inverse relationship between the SChE activity and age among the male students. A similar relationship was indicated by the Pearson coefficient of correlation (r = - 0.085, p = 0.4). For the female students, Spearman's rho (r = 0.06, p = 0.516) showed weak linear non-statistically significant relationship between the SChE activity and age. The Pearson coefficient of correlation also indicated weak (r = 0.06) linear non-significant (p = 5.1) relationship between the SChE activity and age.

The reference range (Mean ± 2 STD) of the SChE activity (KU/L) was calculated after log-transforming the SChE data. The calculated Reference Range of the SChE activity for the males was 0.67 – 6.43KU/L and for the females was 0.85 – 6.13KU/L.

Test for normality of distribution of the Dibucaine Numbers (DN) for the male and female students indicates that the DN was not normally distributed. The Box-plots of the DN data for the male and female students are shown in Fig 2. Outliers are indicated in the Box-plot for only the female students.

The summary statistics of the DN for both male and female students are presented in Table 5. The median DN for the males was 85.43 % and the IQR was 81.6% – 88.2%. For the females the median was 86.1% and the IQR was 83.5% – 88.0%.

No significant difference was obtained between the DN for the male and female students, when tested statistically using the Mann-Whitney and Wilcoxon U tests (p = 0.225), the Kruskal-Wallis test (p = 0.225) and Chi-Square test (p = 0.304). Similar trend was observed (p = 0.34) using the analysis of variance (ANOVA) after Post-Hoc analysis (Scheffe).

The percentile cut-offs obtained for the DN for the male and female students are presented in Table 6. The 5^{th} to 50^{th} Percentile cut-offs for the males were lower than that of the females.

Fig. 2: Dibucaine Numbers (%) for male and female subjects

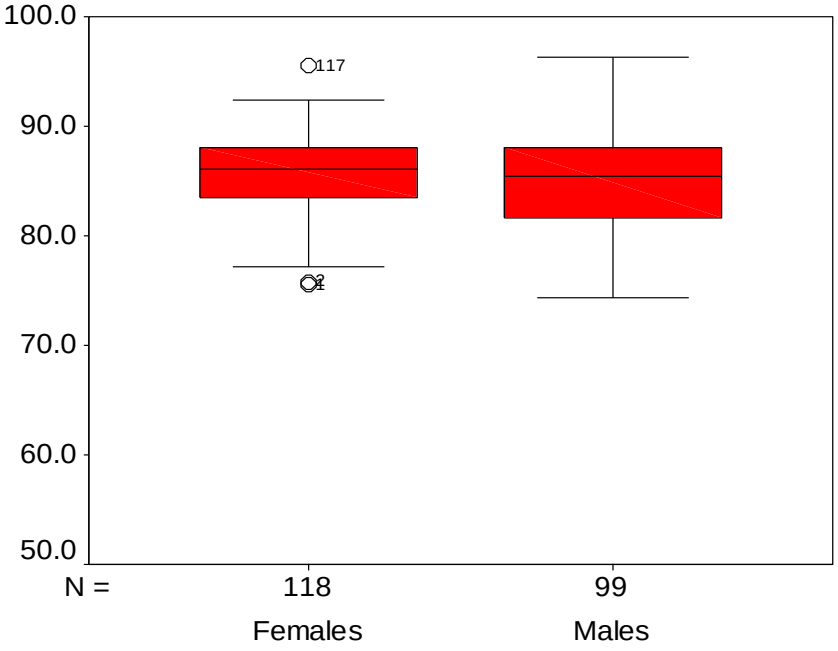

Table 5: Summary statistics of the Dibucaine Number (%) for male and female students

Dibucaine Number (%)	Male students (n = 99)	Female students (n = 118)
Median	85.4	86.1
IQR	81.6 – 88.2	83.5 – 88.0
Mean	84.4	85.6
95% CI	83.7 – 85.8	85.0 – 86.3
Std Dev	5.1	3.6
Range	61.9 – 96.3	75.5 – 95.5

Table 6: Percentile cut-offs for Dibucaine Numbers (DN) for the male and female students

Percentiles	Male students (n = 99)	Female students (n = 118)
5th	77.0	77.6
10th	77.8	80.8
25th	81.6	83.5
50th	85.4	86.1
75th	88.2	88.0
90th	90.4	89.6
95th	92.5	90.8

Table 7: Distribution of the male and female students according to their Dibucaine Number (variant form) of Serum Cholinesterase

*Dibucaine Number (DN %) [10]	Male students (%)	Female students (%)
0 – 20 (Atypical variant)	0	0
20 – 6 (Heterozygous)	1.0 (1.0%)	0
70 – 90 (Normal variant)	86.0 (86.9%)	109.0 (92.4%)
> 90	12.0 (12.1%)	9.0 (7.6%)

*Reference 10

The distribution of the male and female students according to their DN is presented in Table 7. The atypical variant SChE (DN between 0 – 20%) was not detected in both the male and female students. Normal variant SChE (DN between 70 – 90%) was prevalent in 86 (86.9%) of males and 109 (92.4%) of females. The DN was greater than 90% in 12 (12.1%) males and 9 (7.6%) females.

The Bivariate (2-tailed) correlation tests between the SChE activities and DN indicated strong linear statistically significant relationship for the males students (Spearman's rho = 0.453, p = 0.01; and Pearson coefficient of correlation r = 0.454, p = 0.01). Similar trends were obtained for the female students, Spearman's rho = 0.359, p = 0.01; and Pearson coefficient of correlation r = 0.382, p = 0.01.

The Spearman's coefficient of correlation (rho = 0.083) indicated weak linear non-statistically significant (p = 0.414) relationship between the DN and age of the males students. A weak (rho = - 0.081) inverse non-statistically significant (p = 0.38) relationship between the DN and age of the female subjects was indicated by the Spearman's coefficient of correlation.

DISCUSSION:

The unit for expressing SChE activity depends on the procedure and the method used to assay the enzyme [7, 8, 10 – 13]. Thus, there is no universally acceptable normal reference range that can be used for the comparison of the SChE activity obtained from laboratories using different assay techniques [7, 8, 10 – 13]. However, most commercial kits for assay of SChE activity tend to use the International Unit (U) under specified conditions [8, 10, 13].

In the present study the results were interpreted using the reference range for SChE activity and the cut-off points for the DN recommended by the manufacturer ("Linear Chemicals") of the analytical kits [10]. The total non-response rate of 21.4% (59 students) was slightly higher that the predicted non-response rate of 20.0% used in the calculation of the sample size for this study.

The mean SChE activity for the male (3.64KU/L) and female (3.63KU/L) students were within the normal range (2.4 – 8.5KU/L) recommended by the manufacturer of the SChE analytic kits [10] used in the present study. Both values were also within the normal range (2.2 – 5.2KU/L) reported by other authors that have used similar analytical procedure [14]. Although there was no statistically significant difference between the SChE activity of the male and female students in the present study, the SChE activity of 14.1% of the males was below the lower limit of the normal range compared to 5.9% of the females. Our data supports the findings reported by Curtain et al [11] that no individuals were found with extremely low or zero SChE activity and that considerable variation was observed in the SChE activity between the male and female students in Papua New Guinea [11]. The 10th percentile

SChE activity (1.91KU/L) for the male students was significantly lower (p = 0.01) than the corresponding value (2.61KU/L) for the female students and was also below the lower limit of the normal range (2.4KU/L).

The 5th and 95th Percentile SChE activities for the male and female students (Table 3) were within the calculated reference ranges obtained for these groups in the present study (Table2). Several researchers have suggested that when reference ranges are established locally, SChE activity becomes a sensitive indicator and of pesticide poisoning, loss of hepatic biosynthetic capacity and unusual sensitivity to Succinylcholine [12 – 14]. The calculated reference ranges obtained for the male and female students in the present study can server as guidelines for the quantitative assessment of SChE activity among the population in NCD. The lower SChE reference range can serve as the exposure index for assessing low-level and chronic residual exposures among students that regularly use mosquito coils and sprays. There is the need to advocate for improve knowledge and awareness among students about the negative impact on health of the long term use of insecticides that contain organophosphates and carbamates.

The DN below 20% which is characteristic of the atypical variant of SChE was not present in the male and female students. This result supports the earlier report by Curtain et al [11] that homozygote for the atypical variant of SChE was not found in peoples in Papua New Guinea. The non-detection (zero percent) of individuals with the atypical variant of SChE in our present study and that of Curtain et al may be due to the low number of subjects (217 students in our study and 2144 individuals in that of Curtain et al) studied. Pestel et al [15] in a study of 24,830 Europeans reported a prevalence of 0.07% atypical homozygous variant which gave an incidence rate of 1:1400. Pestel et al also reported 1.23% prevalence of the heterozygous variant amount Europeans. This is higher than the 0.46% prevalence of the heterozygous variant among the 217 subjects in our present study. The 12.1% and 7.6% of male and female students respectively with DN greater than 90% which is above the upper limit of the cut-off points for the normal variant (DN 70 – 90%) requires further investigation. Several authors [7, 16] have indicated that appearance of additional variant forms of SChE may be caused by a variety of mutation on the gene locus encoding for the enzyme.

CONCLUSION:

Although there was no statistically significant difference between the SChE activity of the male and female students,

considerable variation was observed in the SChE activity in both the male and female students. However, none of these students were found with extremely low or zero SChE activity. No statistically significant difference was obtained between the DN of the male and female students.

Our data strongly supports the need for screening of students and other patients before administering Scoline which is the muscle relaxant commonly used in the PMGH and other hospitals in NCD. The SChE activity is the parameter for the quantitative assessment and the DN is the parameter for the qualitative assessment.

There is the need to advocate for improve knowledge and awareness among students about the negative impact on health of the long term use of insecticides that contain organophosphates and carbamates.

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