Epidemiological Study of Organophosphorus Compounds Insecticides Types Related To Acutely Intoxicated Patients Presented To Poison Control Center (PCC-ASU)–Egypt.

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Abstract

Background: Organophosphorus compounds are the most commonly used type of insecticides in Egypt. There are more than 40 different types known and used nowadays either individually or in mixed forms. This study aimed to detect the different types of organophosphorus compounds insecticides (OPC) used in the Egyptian environment detected by studying the acutely intoxicated patients presented to PCC-ASU and correlating it to the severity of their presentations and epidemiological backgrounds.

Materials and Methods: Seventy acutely intoxicated patients of both sexes were included in this study after being informed of the purpose of this study and consenting to their participation. Ethical committee approval was obtained and an informed written consent was signed by participants or their guardians to be included in this study. Socio-demographic data were collected including: age, sex, mode of intoxication, social class, residence and occupation. Biochemical tests included: Determination of serum potassium level, serum creatinine level, serum amylase activity, pseudo cholinesterase activity and organophosphorus insecticides type by gas chromatography. The obtained data and results were subjected to statistical analysis using SPSS windows version (18), and different statistical parameters were utilized in the analysis and interpreted accordingly.

Results: The study found 26% of intoxicated patients were from age group (10-20) 26% were from age group (30-40). 46% of the patients were exposed due to suicidal attempts, 40% were accidentally exposed and 14% were due to occupational hazard. 56% of the studied cases were from urban residents. 23% of total numbers of suicidal cases were unemployed, where 50% of them are from low socio-economic status. The study detected 7 different OPC (Malathion, Dimethoate, Chloropyrofois, Phenthoate, Prothiphenos, and Ethion). Serum Amylase and creatinine levels were significantly high in severe group. Pseudocholinesterase level was decreased significantly in deceased, severe and moderate group.

Conclusions: Although the basic mechanisms of acute toxicities are thought to be similar for all OPC but major differences in their clinical presentation course were noted.

Main conclusions: OPC's types differentiation according to their animal LD50 did not accord with their human toxicities. Although the basic mechanism of toxicity is thought to be the same for all OPs, this study found major differences in the clinical course of humans poisoned by particular OPC despite of identical treatment. The differences are fundamentally important for management. The study also shows that the relative human toxicity of OPC may not be related to animal toxicity. The widely used approach of differentiating according to their animal LD 50 did not accord with human toxicity and is probably of limited value in risk assessment or management of acute human intoxication.

Keywords: Organophosphorus Insecticides; Occupational; Egyptian; Environmental; PCC-ASU; Acutely Intoxication.

I. Introduction

Organophosphorus compounds insecticides (OPC) are the most widely used group of insecticides worldwide because of their rapid breakdown into environmentally safe products. [1] Environmental uncontrolled exposure in various Egyptian areas to various types of organophosphorus insecticides which has subsequently affected the clinical presentations of patients presented to the main poison control center in Ain Sham University thus necessitated further studies of the compounds used and illegaly mixed in different occupational exposures. However, they have far more acute toxicity than DDT. [2] In spite of the increasing numbers of OPC used in agriculture, there are serious concerns regarding their potential risk of exposure. [3]
Epidemiological Study of Organophosphorus insecticides Types Related To Acutely Intoxicated Patients

World Health Organization estimated the yearly death by 200,000 people world wide due to pesticides intoxication mainly in developing countries.[4] This can be attributed to their easy availability and wide occupational exposure altogether with inadequate protective procedures. [5,6] OPC are used in agriculture, domestics, gardens, and veterinary practices. They all act through inhibiting acetylcholinestrase enzyme (ACHE) and cause intoxication symptoms in the form of excessive sweating, salivation, lacrimation, nausea, vomiting, diarrhea, constricted pupil, poor concentration and tremors. In serious cases respiratory failure and death may occur. [7,8] This study aimed to detect the different types of organophosphorus insecticides used in different environmental areas in Egypt. The study also included some sociodemographic data concerning and monitoring of different types of OPC and its relation to severity of their clinical presentation.

II. Patients and Methods

1-Design and setting:
This study was conducted on 70 Egyptian patients of both sexes with organophosphorus insecticides (OPC) acute toxicity who were admitted to PCC-ASUand ten healthy adults individuals serving as control groups. Ethical committee approval was obtained and an informed written consent was signed by participants to be included in this study.

2-Patients' selection criteria:
The selected patients were of both sexes with acute moderate OPC intoxication presentations. The diagnosis of OPC intoxication was based on the following criteria as guided by (Karki et al., 2004):[9]
1. History of exposure to an OPC agent.
2. Clinical manifestations of OPC intoxication.
3. Low serum pseudo-cholinesterase activity.
All these criteria were required to be present in each patient to be included in the study.
All of the following patients are excluded from the study:
1. Patients with history of cardiac, pulmonary disease or renal impairment.
2. Patients with salivary gland lesions, intra-abdominal diseases and diabetic ketoacidosis that could elevate serum amylase level.
3. Patients with any of the following which could reduce the pseudo-cholinesterase activity as parenchymal liver disease, acute infection, metastatic carcinoma, malnutrition, iron deficiency anemia.

Patients were further grouped based on the severity of their clinical presentations into moderate, severe, deceased, chronic and control. Sociodemographic data were collected including: age, sex, mode of intoxication, social class, residence and occupation.

Biochemical parameters included:
Determination of serum potassium level after Burtis CA and Ashwood ER,( 1999).[10]
Determination of serum creatinine level by the kinetic method according to Houot, (1985).[11]
Determination of serum α-amylase activity after Murthy et al.,(1992).[12]
Determination of pseudocholinesterase activity( PChE level) was measured on admission to confirm OPC intoxication and was done daily during the hospital stay to evaluate relapse and the onset of recovery. It was determined using a kinetic colorimetric method according to Kalow and Genset K.,(1997).[13] Organophosphorus Insecticide residues analysis of the collected samples were carried out in three steps which are extraction of organophosphorous residues from serum samples; clean up of extracted samples and determination of organophosphorus insecticides concentration (ppm) by gas chromatography after Wolf M et al.,(2005). [14]

Statistical Analysis: The obtained data and results of investigations were subjected to statistical analysis using SPSS windows version (18), and different statistical parameters were utilized in the analysis.[15]

III. Results

In this study the mean age was 22.7±12.8 years old. Table 1 showed that the age group <10 years old showed highest incidence 50% in the accidental mode of toxicity. As for the occupational toxicity (30-40) age group showed highest incidence of toxicity by 50 %. Table 2 showed that males were more exposed to OPC intoxication than females. While females represented higher incidence of accidental exposure to OPC intoxications than males 55% and 45% respectively. While no cases were recorded in occupational toxicity for the female group. Table 3. showed that acute OPC intoxication showed highest incidence in unemployed patients 23% followed by farm workers19% then students and children equally17%, housewives,13% factory workers 7%, and lastly others include civil employers and manual Workers 4%.
As for the mode of toxicity: It was found that the highest incidence of suicidal cases were found in unemployed patients represented by 31% from total number of suicidal intoxications cases, while the highest incidence of accidentally intoxicated patients were children represented by 29%, from total number of patient accidentally exposed to OPC, while 50% of occupationally intoxicated were from farm workers and the other 50% were from factory workers.

Table 4. showed the social class distribution of patients included in the study where 53% of patients intoxicated accidentally were from middle social class followed by 26% from low social class while only 2% from high social class. It also showed that 28% from occupational toxicity was from low social class. While 50% of suicidal patients were of low social class followed by 47% of patients of middle social class and only 3% of high social class. Table 5. showed that majority of patients 56% were residents of urban areas while 44% were rural areas residents.

Table 6. showed highly significant relationship between types of OPC and severity of cases. Chloropyrifos represented the highest percentage of occurrence among morbidity group 55.7%. Dimethoate had the highest percentage of occurrence among the deceased group 40%. 74%. while the ethion showed the highest percentage of occurrence among chronic group 75%.

Table 7. showed that serum Potassium level showed significant decrease in deceased and severe group when compared with control group with no significant difference between chronic and control group. Highly significant increase in Serum creatinine in deceased and severe group with no significant difference between moderate, chronic and control groups. Mean serum level of amylase significantly increased in deceased and severe group when compared with (chronic, moderate group and control group) with no significant difference between those three groups. Regarding to pseudocholinesterase (PchE) level there was highly significant decrease in PchE levels in deceased and severe groups when compared with control moderate and chronic group.

IV Discussion

In the current study the most commonly affected age groups were ranging between (10-20) and (30-40) years old equally (26%) per group. This finding was similar to Yurumez et al. (2007) who found that 220 of the patients who were admitted to the emergency department of Kocatepe University in Turkey between January 1995 and December 2004. They found that the most affected age group was in between 15-34 years 40.5%, of both sexes. [16]

In the present study the highest percentage of patients who were accidentally poisoned were in the age group under 10 years old represents 92% from total number of this age group. This is attributed to that most accidental intoxications occurred in younger children who are too young to acknowledge the danger of swallowing something that may be harmful. [17]

This result is in agreement with Riegart and Roberts. (1999) who found that children under 10 years old have most commonly affected group on accidental intoxication. [18]

The highest percentage of suicidal patients were in the age group (10-20) years old represents 77% from the total number of this age group which is in agreement with Linakis and Frederick., (1994) who stated that amongst 2904 intoxicated patient 60.5% of them were suicidal cases and the percentage was higher in (15-19) age group. [19]

As for the occupational exposure the highest percentage of cases were in the age group (30-40) years old representing 28% of total number of cases. This is in agreement with Rastogi et al., (2008) who did their study on 50 male sprayers in the mango belt in India found that 34 from 50 insecticides applicators were in age group (25-40) years old. [20]

The current study showed that males were more exposed to OPC 55.7% than females 44.3%. This result may be attributed to the fact that in developing countries as Egypt the percentage of male agricultural workers is greater than female which in turn cause that male is more exposed to females. This finding is in agreement with Murali et al. (2009) who revealed that the mean age was 27.8 years and that 66% were males in a retrospective study which covered 15 years (1990 - 2004) and recorded 2884 cases admitted with acute intoxication during these years.[21]

In the current study the majority of cases were urban areas residents 56% these results are in accordance with Thomas et al., (2002), who found that 62% of cases on his study were from urban area. [22] Other studies in developing countries showed that OPC are common suicidal agents among adolescents especially in urban areas. [23]. This may be due to the availability of OPC without any restriction in Egypt, easy accessibility and feasibility.

In the current study 50% of suicidal patients were of low social class with unemployment and poverty problems these results are in agreement with Hassan et al. (2009) who stated that unemployment, poverty and low literacy are directly related to attempting suicide as they lead to the Psychiatric disorders and stressful situations specially in urban area. [24]
In the present study 23% of cases were unemployed followed by farm workers 19%. Both students and children were in their orders 17%, then housewives 13%, factory workers 7%, and lastly others including civil employers and manual workers. This in agreement with Saadeh et al., (1996) who found that unemployment constituted 43% of patients with OPC intoxication. As he did his study on 120 patients admitted with OPC toxicity. [25]

Stressful environment in urban areas with increasing percentage of unemployment issue could explain the highest incidence of intoxication in urban areas among unemployed males this explanation is consistent with Clegg and VanGemert, (1999). [26]

Chloropyrofis showed the highest percentage of occurrence among studied cases 55.7% this result is in consistence with Eddleston et al. (2008), who stated that in Sri Lankan hospitals Two-thirds of OPC poisoned patients 67.2% reported ingestion of one or two of OPC types, one of them is Chloropyrofois 36.8%. [3, 27]

The study found that malathion occurrence was 50% from total no of studied cases in mixed or single forms. This was in agreement with reports done by Godhwani et al., (2004) who suggested that it could be attributed to the fact that malathion is widely available and are used as insecticides in agriculture and in the household and many OPC in the market are neither registered nor licensed. [28]

In the current study 74% of the morbidity group patients 18% of the chronic group and only 8% of the mortality group were intoxicated with Chloropyrofis this is in agreement with Eddleston et al. (2005), who found that although chlorpyrifos is generally the most toxic in rats, it is least toxic in people. [29]while in dimethoate 60% of patients in morbidity group while 40% in mortality group with no patient recorded in chronic group this result is in agreement with Rahimi and Abdollahi (2007), who stated that Dimethoate intoxication was more severe than Chloropyrofis intoxication: the case of fatality for Chloropyrofis was 8.0% while dimethoate 23.1%. [19] also with Eddleston et al., (2005), who stated that Dimethoate poisoned patients dies sooner than those ingesting other pesticides and often from hypotensive shock. [30, 29]

Generally in this study the mortality rate of the poisoned patients was 24.3% which agreed with Katz et al., (2006) who stated that worldwide mortality studies reported mortality rates ranging from 13-25%. The compounds most frequently involved included Malathion, dimethoate, and fenthion/malathion. [31] In the present study significant decrease in the mean serum potassium level was noticed in deceased and severe group of patients. This result confirmed what was reported by Osmundson., (1998) who stated that hypokalemia is one of the most commonly described biochemical abnormalities that occur with OPC intoxications. [32] Hyperamylasemia was reported in this study in severe and deceased group of patients. Thus this increase in mean serum amylase level was coinciding with severity; the same result was reported by Alawadi, (2000). [33] As patients with Hyperamylasemia had normal mean serum lipase level, thus Hyperamylasemia was contributed to be of salivary origin and not of pancreatic origin, this finding is in agreement with Clark,( 2002). [34]

Mean serum creatinine level was elevated in all groups which is agreed with Hala et al (2006) who found that creatinine levels are elevated in OPC consuming patients. [35] In the present study the mean pseudocholinesterase level was non- significantly different amongst patients of the acute and deceased groups while a highly significant difference was present in severe and deceased groups in compared with control group.

This result suggested that grading system to identify high risk patients that are based on this measurement is unreliable, similar results were reported by Verhulst et al., (2002). [36] In this study chronically exposed farm workers who have normal pseudocholinesterase level and this is report by Patel., et al (2008) who stated that workers chronically exposed to OPC have (pseudo cholinesterase) activities within normal limits. [37]

IV. Conclusions

Although the basic mechanism of toxicity is thought to be the same for all OPCs, this study found many differences in the clinical course of humans poisoned by particular OPC despite of their identical treatment protocol. The differences are fundamentally important for management. The study also showed that the relative human toxicity of OPC may not be necessarily related to their animal toxicities patterns. The widely used approach of differentiating OPCs according to their animal LD 50 did not accord with human toxicity and is probably of limited value in risk assessment or management of acute human intoxication.

V. References

[4]. World Health Organization. The impact of pesticides on health, Pesticide exposure and suicidal ideation in rural communities in Zhejiang province, China (October 2009).
Epidemiological Study of Organophosphorus insecticides Types Related To Acutely Intoxicated Patients


DOI: 10.9790/2402-1006037278 www.iosrjournals.org 76 | Page
Table 1. Age distribution in relation to mode of OPCs intoxications in studied patients

<table>
<thead>
<tr>
<th>Mode Of Intoxication</th>
<th>Accidental</th>
<th>Suicidal</th>
<th>Occupational</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td>M  F</td>
<td>M  F</td>
<td>M  F</td>
</tr>
<tr>
<td>&lt;10</td>
<td>10</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>10-20</td>
<td>5</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>20-30</td>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>30-40</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>40-50</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>50-60</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Sex distribution in relation to mode of OPCs intoxications in studied patients

<table>
<thead>
<tr>
<th>Mode of exposure</th>
<th>Male n=39</th>
<th>Female n=31</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accidental</td>
<td>14 36</td>
<td>14 45</td>
<td>9.33</td>
<td>0.009 (S)</td>
</tr>
<tr>
<td>Occupational</td>
<td>10 26</td>
<td>0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicidal</td>
<td>15 38</td>
<td>17 55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X²: Chi square statistical analysis
P: > 0.05 insignificant difference
P: < 0.05 significant difference
P: < 0.0001 high significance

Table 3. Occupational distribution in relation to mode of OPCs intoxications in studied patients.

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Mode</th>
<th>Accidental n=28</th>
<th>Occupational n=10</th>
<th>Suicidal n=32</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Housewives</td>
<td>5 18</td>
<td>0 0</td>
<td>1 12</td>
<td>9 13</td>
<td></td>
</tr>
<tr>
<td>Farm worker</td>
<td>2 7</td>
<td>5 50</td>
<td>6 19</td>
<td>13 19</td>
<td></td>
</tr>
<tr>
<td>Student</td>
<td>6 21</td>
<td>0 0</td>
<td>4 13</td>
<td>10 17</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>6 21</td>
<td>0 0</td>
<td>10 31</td>
<td>16 23</td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>8 29</td>
<td>0 0</td>
<td>6 19</td>
<td>14 17</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 4</td>
<td>0 0</td>
<td>2 6</td>
<td>3 4</td>
<td></td>
</tr>
<tr>
<td>Factory worker</td>
<td>0 0</td>
<td>5 50</td>
<td>0 0</td>
<td>5 7</td>
<td></td>
</tr>
</tbody>
</table>

X² = 49.5
P = 0.0001 (H.S)

X²: Chi square statistical analysis
P: > 0.05 insignificant difference
P: < 0.05 significant difference
P: < 0.0001 high significance
H.S: Highly significant

Table 4. Distribution of modes of exposure in relation to social class of the studied cases

<table>
<thead>
<tr>
<th>Social class</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of exposure</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Accidental</td>
<td>9 26</td>
<td>17 53</td>
<td>2 19</td>
</tr>
<tr>
<td>Occupational</td>
<td>10 28</td>
<td>0 0</td>
<td>1 0</td>
</tr>
<tr>
<td>Suicidal</td>
<td>16 50</td>
<td>15 47</td>
<td>1 3</td>
</tr>
<tr>
<td>Grand Total</td>
<td>35 50</td>
<td>32 45.7</td>
<td>3 4.3</td>
</tr>
</tbody>
</table>

X²-value = 13.8
P-value = 0.008
Significance = Significant

X²: Chi square statistical analysis
P: > 0.05 insignificant difference
P: < 0.05 significant difference
P: < 0.0001 high significance
Table 5. Organophosphorous insecticides compounds types in relation to residence in studied patients

<table>
<thead>
<tr>
<th>Residence Type</th>
<th>Urban n=39</th>
<th>Rural n=31</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC</td>
<td>No %</td>
<td>No %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethoate</td>
<td>1 50</td>
<td>1 50</td>
<td>11.2</td>
<td>0.132 (N.S)</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>1 67</td>
<td>4 33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenthoate</td>
<td>18 25</td>
<td>3 75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothiophos</td>
<td>1 100</td>
<td>0 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>5 70</td>
<td>3 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profenfos</td>
<td>7 50</td>
<td>1 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethion</td>
<td>10 0</td>
<td>3 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>16 50</td>
<td>16 50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X²: Chi square statistical analysis  P: > 0.05 insignificant difference
P: < 0.0001 high significance  P: < 0.05 significant difference
N.S: Non significant

Table 6. OPCs types in relation to the degree of clinical presentation severity in studied patients

<table>
<thead>
<tr>
<th>Degree of Severity</th>
<th>Deceased gp</th>
<th>Morbidity gp</th>
<th>Chronic gp</th>
<th>Total no</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of OPC</td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
<td>No %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethoate</td>
<td>4 40</td>
<td>6 60</td>
<td>0 0</td>
<td>10 14.2</td>
<td>38.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>3 8</td>
<td>29 74</td>
<td>7 18</td>
<td>39 55.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenthoate</td>
<td>1 8</td>
<td>7 54</td>
<td>5 38</td>
<td>13 18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothiophos</td>
<td>1 6</td>
<td>10 55</td>
<td>7 39</td>
<td>18 25.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>7 20</td>
<td>25 71</td>
<td>3 9</td>
<td>35 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0 0</td>
<td>7 54</td>
<td>6 46</td>
<td>13 18.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethion</td>
<td>0 0</td>
<td>2 25</td>
<td>6 75</td>
<td>8 11.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Profenfos</td>
<td>1 11</td>
<td>3 33</td>
<td>5 56</td>
<td>9 12.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X²: Chi square statistical analysis P: > 0.5 insignificant difference
P: < 0.05 significant difference P: < 0.0001 high significance

Table 7. Mean biochemical parameters measurements in the studied patients

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Severe Mean ±SD</th>
<th>Moderate Mean±SD</th>
<th>Deceased Mean±SD</th>
<th>Chronic Mean±SD</th>
<th>CONTROL Mean±SD</th>
<th>Fc</th>
<th>CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (mEq/L)</td>
<td>3.2±0.5</td>
<td>3.5±0.7</td>
<td>3±0.6</td>
<td>4±0.4</td>
<td>4±0.3</td>
<td>18.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.6±0.35</td>
<td>0.9±0.3</td>
<td>2.1±0.5</td>
<td>1.1±0.2</td>
<td>0.8±0.2</td>
<td>46</td>
<td>0.25</td>
</tr>
<tr>
<td>Amylase (U/L)</td>
<td>49±22</td>
<td>53±50</td>
<td>108±69</td>
<td>65±27</td>
<td>47±13</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Pseudocholinesterase (U/L)</td>
<td>339±159</td>
<td>402±324</td>
<td>301±146</td>
<td>2076±973</td>
<td>2960±867</td>
<td>68.7</td>
<td>548</td>
</tr>
</tbody>
</table>

SD = Standard Deviation
Ft = tabulated variance ratio at P 0.05=2.55
Fc = variance ratio calculated by ANOVA one-way statistical analysis.
Fc > Ft =significant, Fc < Ft = non-significant.
CL = confidence limit, where difference between two groups > CL significant difference and if this difference < CL = insignificant difference.
P: > 0.05 insignificant difference
P: < 0.05 significant difference
P: < 0.0001 highly significant