Effect of Cannabis sativa on Hematological Indices in Rats and Men

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Abstract: This study was designed to assess the effects of injection of different doses of petroleum ether extract of Cannabis sativa on important hematological indices in rats. Similar effects in adult men who had been smoking C. sativa for different periods were also assessed. Blood hemoglobin concentration was not affected in both addicted humans and treated rats, whereas blood erythrocyte count (RBC) and total leukocytes count (TWBC) revealed significant (p<0.05) decreases in the group of rats treated with 0.2 mg/g of the extract and 0.6 mg/g respectively but no changes in addict men groups were observed. Neutrophils and lymphocytes percentages decreased and eosinophils increased significantly (p<0.05) in all treated groups of rats compared to the control group. In the addicted men eosinophils and monocytes showed significantly (p<0.05) lower levels compared to the control group, but lymphocytes percentages were elevated in all addicted men compared to the control group with a significant (p<0.05) difference in the heavy addicted group. These findings showed that injection of C. sativa petroleum extract to the rats resulted in different effects in some parameters compared to those in addicted men smoked C. sativa for long periods. This was clear for the lymphocytes percentage which decreased significantly in the treated rats and showed significantly higher percentage in the addicted men when compared to their controls.

Key words: Cannabis sativa, rats, addicted men

INTRODUCTION

Cannabis sativa use is reported at lower levels in developing countries, although data from many developing countries are limited there is long tradition of cannabis use for culinary, medicinal and ceremonial purposes (UNDCP, 1997). Various intake route of tetrahydrocannabinol THC (intravenous, smoke, inhalation and oral), cause plasma levels which are related to onset, degree and duration of clinical effects. The degree of response and plasma cannabinoids levels attained, vary in a dose-related manner depending upon potency of smoked marihuana. The peak pharmacological effects correlating with peak plasma carboxy-THC (inactive metabolite level) (Hollister et al., 1981; Perez-Reyes et al., 1982). Chronic cannabinoid smoke exposure impairs lung function (Tashkin et al., 1972) which has been suggested to result in the intrathoracic airway obstruction or pulmonary insufficiency leads to ventilation/perfusion imbalance that results in functional hypoxia or hypoxaemia increasing the demand on bone marrow for RBC production observed as increased Hb concentration (Rubin and Comitas, 1975). Brady et al. (2009) Reported that Cannabinoid receptors are divided into CB1 and CB2. CB1 receptors which are mainly found in the brain, but they can also be found in the kidneys, lungs and liver. CB2 receptors are found in the immune system and hematopoietic cells. CB1 and CB2 are G-protein receptors and when activated by cannabinoids, affect many intracellular structures such as calcium channels and protein kinase A. Benefits of cannabinoids include the suppression of inflammation and various types of cell-mediated immunity. Murkinati et al. (2010) reported that the activation of CB2 cannabinoid 2 receptor reduced ischemic injury and resulted in a reduction of the neutrophils number in the ischemic brain.

Danial and Watson (1987) found that the peripheral blood lymphocytes from chronic marihuana smokers, contained reduced number of T lymphocytes that from resette with Sheep Red Blood Cells (SRBC).

The objective of this study was to investigate and compare the effects of different doses of cannabis extract via intramuscular injection (0.2, 0.4 and 0.6 mg/gm BW) in rats to evaluate the effects of smoked cannabis on addict men using haematological parameters including haemoglobin, total white blood cells, total red blood cells and differential white blood counts.

MATERIALS AND METHODS

Twenty healthy albino rats 6-9 months old weighing between 70-200 gm, were divided randomly (male and female) into four groups, control and three treatment groups five animals in each group. The treated groups were injected with four doses of cannabis petroleum extract intramuscularly for 10 days, two days between one dose and another (0.2 gm per gm body weight low

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313
dose), (0.4 mg per gm body weight medium dose), (0.6 gm per gm body weight) respectively. The dose was prepared by weighing cannabis extract and calculated according to the animal body weight (Mahfouz et al., 1975; Rosenkrantz and Esber, 1980). Blood samples were collected (2.5 to 3 ml from the ocular vein of rats twice after the second obese and the last dose at 5 and 11 respectively. Human blood samples were collected from twenty men users and addicts and six healthy non-users (control) 18-60 years old. Addicts were divided into four groups according to the duration of usage (3-8 years—mild users), (11-17 years—medium users), (18-40 years—heavy users). Used to determine changes in haematological parameters. Haemoglobin concentration (Hb) was measured by (acid haematin method). Improved neubaur haemocytometer was used for counting erythrocytes (RBCs), total leucocytes count (WBCs) were counted in an improved haemocytometer using tracks solution as a dilution fluid (glacial acetic acid 1 ml, 1% aqueous gentian violet 1 ml, distilled water up to 200 ml). The percentage of lymphocytes, neutrophils, monocytes and eosinophils were determined microscopically from account of 100 leukocytes in thin Giemsa-stained blood smear.

Completely randomized design was used in this study. The data were tabulated and subjected to Analysis of Variance (on way ANOVA) using the Microsoft Computer Program as described by Steal and Torrie (1960).

RESULTS

Tables 1 and 2 give the result of the haemoglobin concentration and erythrocyte counts (RBCs) in rats and men groups. It is seen that the injection of cannabis extract resulted in significant (p<0.05) decrease in erythrocyte values when rats treated with 0.2 mg and 0.6 mg. However no significant changes were noticed in the addicted men groups as compared with control. Haemoglobin concentration showed no significant difference among treated groups of rats and addict men.

Tables 3 and 4 present the number of total leucocyte counts and differential leucocyte count in rats and men groups. Total leucocyte (WBCs) number decreased in the rats treated groups and reached a significant level (p<0.05) for the group which was treated with high dose (0.8 gm/g). On the other hand the addicted men groups showed no significant differences compared with control. Neutrophils values were decreased in all treated rats and addict men groups, with no significant difference in men groups. The increase in eosinophils value followed the increase in the dose until reached its maximum in group received the high dose (0.8 gm/g), however eosinophils decreased in all addict men groups and resulted the significant decrease in groups which duration of addiction was (3-8 years and 18-40 years). The number of monocytes presented a fluctuating pattern in rats, the level decreased for the group treated with low dose and increased for the group treated with high dose. Men groups presented significantly lower levels for all addiction groups compared to control group. Lymphocytes percentages decreased significantly (p<0.05) in all treated rat groups. Whereas, addicted men groups showed high levels compared to control with significantly (p<0.05) high level in the group of heavy users (18-40 years duration).

Table 1: The effect of C. sativa extract on Haemoglobin (Hb) Concentration and RBC counts in rats (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Haemoglobin conc. g/dl</th>
<th>RBC count/L x 10^12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.76±0.07</td>
<td>4.57±0.05</td>
</tr>
<tr>
<td>Low dose 0.2 mg/g</td>
<td>9.18±0.05</td>
<td>3.24±0.32</td>
</tr>
<tr>
<td>Medium dose 0.4 mg/g</td>
<td>9.63±0.20</td>
<td>3.79±0.17</td>
</tr>
<tr>
<td>High dose 0.8 mg/g</td>
<td>9.63±0.18</td>
<td>3.37±0.37</td>
</tr>
</tbody>
</table>

Table 2: The effect of C. sativa on Haemoglobin (Hb) Concentration and RBC counts in men (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Haemoglobin conc. g/dl</th>
<th>RBC count/L x 10^12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.57±0.52</td>
<td>4.81±0.40</td>
</tr>
<tr>
<td>Mild users</td>
<td>11.29±0.55</td>
<td>4.67±0.56</td>
</tr>
<tr>
<td>Medium users</td>
<td>11.54±0.47</td>
<td>4.16±0.73</td>
</tr>
<tr>
<td>Heavy users</td>
<td>11.49±0.38</td>
<td>4.91±0.30</td>
</tr>
</tbody>
</table>

Table 3: The effect of C. sativa extract in WBCs count and differential leucocyte counts in rats (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC count/L x 10^9</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.02±0.32</td>
<td>63.2±0.66</td>
<td>4.6±0.245</td>
<td>4.3±0.37</td>
<td>27.4±0.44</td>
</tr>
<tr>
<td>Low dose 0.2 mg/g</td>
<td>6.02±0.12</td>
<td>59.8±0.92</td>
<td>5.4±0.24</td>
<td>3.6±0.37</td>
<td>3.1±0.94</td>
</tr>
<tr>
<td>Medium dose 0.4 mg/g</td>
<td>6.23±0.92</td>
<td>59.9±0.24</td>
<td>6.9±0.31</td>
<td>4.8±0.20</td>
<td>4.8±0.24</td>
</tr>
<tr>
<td>High dose 0.8 mg/g</td>
<td>5.16±2.64</td>
<td>51.6±1.86</td>
<td>6.4±0.24</td>
<td>5.0±0.44</td>
<td>5.0±0.80</td>
</tr>
</tbody>
</table>

Table 4: The effect of C. sativa extract in WBC and differential leucocyte counts in men (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBC count/L x 10^9</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Monocytes</th>
<th>Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.42±0.57</td>
<td>53.5±0.78</td>
<td>6.3±0.89</td>
<td>10.3±0.92</td>
<td>29.5±2.17</td>
</tr>
<tr>
<td>Mild users</td>
<td>5.26±0.33</td>
<td>49.4±1.90</td>
<td>4.0±0.49</td>
<td>5.3±0.61</td>
<td>38.5±4.36</td>
</tr>
<tr>
<td>Medium users</td>
<td>5.27±4.14</td>
<td>42.3±3.26</td>
<td>4.8±0.83</td>
<td>4.8±0.83</td>
<td>38.14±4.46</td>
</tr>
<tr>
<td>Heavy users</td>
<td>4.6±3.44</td>
<td>45.6±2.92</td>
<td>3.7±0.47</td>
<td>3.7±0.47</td>
<td>42.8±3.68</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different (p<0.05)
DISCUSSION
Many authors discussed the effects of the cannabinoids on the hematological indices and immunity but controversial findings were reported. William et al. (1991) reported that comparison of blood chemistry and hematology values before, during and after exposure to Marijuana (MJ) smoke indicated no differences for most parameters. This was concluded after performing a large multidisciplinary study designed to determine whether chronic Marijuana (MJ) smoke exposure results in residual behavioral and/or neuropathological alterations in the rhesus monkey, their data indicated that long-term, experimental exposure to MJ smoke is feasible and does not compromise the general health of the rhesus monkey. Obonga et al. (2005) reported that after chronic administration of Crude Cannabis Resin (CCR), (20 mg/kg and 40 mg/kg) to Spraque-Dawley albino rats for 21 days, the changes in various hematological indices such as Packed Cell Volume (PCV), total leukocyte count, differential leukocyte count, Red Blood Cell (RBC) count, absolute lymphocyte count, monocyte count, neutrophils and eosinophil counts were evaluated. The results showed that the hematological indices such as erythrocytic and leukocytic counts were not significantly (p<0.05) affected by the treatment with the crude cannabinoid resin treated animal groups for the first two weeks of treatment. However in the third week, results showed significant increases (p<0.05) in the above mentioned indices while eosinophils disappeared from the blood of treated groups and concluded from his study that chronic administration of CCR at high doses (above 20 mg/kg) to rats has slight hematoxic potentials.

Curickshank (1976) reported elevated, but not significant hemoglobin values for human smoked C. sativa, this was suggested to be due to the lower arterial PO2 values and raised carbon monoxyhemoglobin values and was the most reasonable explanation for the elevated hemoglobin values. These findings are in line with the results obtained in rats in the present work. The groups received C. sativa as Low dose 0.2 mg/g BW and as High dose 0.6 mg/g BW presented significantly lower RBC count and numerically increased hemoglobin levels compared to control group. The slightly lower hemoglobin values obtained by addicted men groups compared to control group in the present work could be due to poor nutrition of the smokers. The decrease of total White Blood Cells (WBCs) in the treated rats groups resulted from the decreased lymphocytes and neutrophils counts. The reduction of the number of the neutrophils due to the application of the cannabinoids was reported in previous studies. Murikinati et al. (2010) suggested that the activation of CB2 cannabinoid 2 receptor reduced ischemic injury this action involved the reduction of the number of neutrophils in the ischemic brain of experimental mice. Also phagocytosis impaired in animals exposed to acute marihuana smoke was due to a water soluble cytotoxin in the gas phase of fresh smoke possibly impairing glycolysis necessary for some of the cell energy for phagocytosis (Huber et al., 1980). Danial and Watson (1987) reported that phagocytosis activity inhibited due to the inhibition of leukocyte migration in C. sativa smoker. These results agree with the present result also may be due to the effect of cannabis on bone marrow synthesis and maturation of neutrophil cells and on the proliferation of lymphocytes.

The elevation of eosinophil values in rat groups is possible to be due to the histaminic effect of cannabis extract injection. Archer (1963) demonstrated that in the horse there was a very close relationship between the plasma histamine level and the blood eosinophil count elevation. The reduction obtained in addict men groups was in agreement with the idea proposed by Bilenkisop and Blenkinsopp (1977), who demonstrated that dexamethasone caused migration of eosinophils into the reticuloendothelial system in rats, which accounted for the immediate decline in number and Long-term administration caused suppression of production. Monocyte values decreased for addict men group and rat group which injected with low dose this finding is similar to the result obtained by Mann et al. (1971) and Danial and Watson (1987), when macrophage bacterial activity was decreased and its spreading was inhibited by exposure of animals to acute marihuana smoke. Lymphocytes count decreased significantly in all rat groups treated with C. sativa in the present study. This may be due to decreased proliferation of the cells. In an earlier study, Peterson et al. (1976) reported that marihuana use appears to affect T-cells function transiently by decreasing rosette formation for 24-72 h after smoking, affecting T-memory cells in some individuals which observed as decreased phytohaemagglutinin stimulation response for 24-48 h and becoming normal by 8th day after smoking. However, Oklahoma state university reported about the Marihuana effect on immune system, an information adapted from the University of Notre Dame Office of Alcohol and Drug Education (2006) That marihuana use can weaken the immune system and interrupt maturation of white blood cells. Therefore, marijuana users may be more vulnerable to illness.

Conclusion: The study concluded that exposure to C. sativa using different routes can result in different effects. The total white cells count decreased significantly in all treated rats and that was due to the significant reduction of the neutrophils and lymphocytes percentages. The reduction of the neutrophils in the addicted smokers was not significant but with significant increase in the lymphocytes count.
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