Anticancer activity of ylang-ylang essential oil in Ehrlich Ascites Carcinoma cell-treated mice

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ABSTRACT

Essential oils are secondary metabolites contains a complex mixture of terpenes with a diverse array of chemical structures, play a crucial role in the management of complex diseases like cancer via synergistic and antagonism effect. Ylang Ylang essential oil (YYEO) extracted from the flowers of Cananga odorata is renowned for its fragrance, contained more than 150 essential components within it, and utilized in various diseases and cosmetics. Traditionally YYEO is being used as an aphrodisiac, anxiolytics, antihypertensive, antiseptic, in food and beverages as a fragrance agent. Due to the presence of a complex mixture of essential components in YYEO, we aimed the current study to assess the anticancer potential against Ehrlich Ascites Carcinoma (EAC) bearing mice. In vitro antioxidant, tumour growth, body weight, biochemical, haematological, and serum estimation was evaluated with subsequent histopathology of the liver. 5-Fluorouracil (5-FU) was used as a standard drug. YYEO showed potent antioxidant activity by DPPH assay. YYEO significantly reversed the Hb, lymphocytes, WBC, and RBC numbers in the treated group compared with the disease control group. YYEO administration has restored the imbalanced levels of antioxidant biomarkers such as MDA, GSH, and SOD activity. YYEO reversed the histopathology of the liver altered by the EAC in mice. In conclusion, a complex mixture of terpenes contained in YYEO could be the potent anticancer therapy in the future. Further studies are needed to identify the active principles and the mechanism involved in this anti-tumour activity.
use of single and / or co-administered chemotherapy, radiation, and surgery (Islam et al., 2014). Although these options are evolving with higher survival rates, patients are undergoing a lot of strain with long term side effects. This has necessitated to deploy novel cost-effective treatments involving minimal human suffering (American Cancer Society, 2007). Herbal medicines including essential oils have received considerable attention in recent years as there is increasing realization that these remedies can impact the progression of carcinoma and its treatment can aid in reestablishing balanced body systems (Rajapoor et al., 2007; Takeoka and Dao, 2003). Also these medications are easily available and cost-effective. Essential oils contain a multi-component system mainly terpenes. These essential oils being secondary metabolites of the plant have antimutagenic, antiproliferative, antioxidant, and detoxifying properties (Blowman et al., 2018).

Ylang Ylang (Cananga odorata), which belongs to the Annonaceae family, is a traditional plant and is cultivated in Asian regions such as the Philippines, Indonesia, Malaysia and Madagascar. Ylang Ylang essential oil (YYEO), derived from the flowers of Cananga odorata, recently introduced in countries such as China, Africa, India, America, etc., is well known for its aroma (Mazari, 2020). There are about 161 bioactive phytoconstituents reported in Ylang Ylang oil including Linalool, Geranyl acetate, Germacrene-D, β-caryophyllene, Benzyl acetate, Geraniol, Meethyl benzoate, Germenyl acetate, Farinasene & Benzyl benzoate and so on. It also contains monoterpine hydrogen, which contains oxygen, monoterpenes, sesquiterpenes, benzonaid and phenol. monoterpene hydrocarbons containing oxygen, monoterpenes, sesquiterpenes, benzonaid and phenols (Brokl et al., 2013). Traditionally YYEO is employed as aphrodisiac (Kodithala and Murali, 2018), anxiolytics (Zhang et al., 2016), antihypertensive (Jung et al., 2013), antiseptic (Caacbay and Jacinto, 2009), in food and beverages as a fragrance agent (Burdock and Carabin, 2008). Cananga odorata extract was proven to have a cytotoxic effect against hepatocellular carcinoma cancer cell lines, HepG2, and Hep2.2.15 Tan et al. (2015) and anti-inflammatory effect (Maniyar and CH, 2015). The cytotoxic o xoaporphine alkaloid liriodenine, isolated from Cananga odorata, was found to be a potent inhibitor of topoisomerase II both in vitro and in vivo (Woo et al., 1997). However, there are no scientific reports on the anti-cancer activity of Ylang Ylang essential oil on EAC tumor-bearing mice. Therefore, the current study was conducted to investigate the anticancer potential of Ylang Ylang essential oil in EAC tumor-bearing mice.

**MATERIALS AND METHODS**

**Procurement of YYEO**

Ylang Ylang essential oil was procured from Allin Exporters, B-75, Sector- 6, Noida, Uttar Pradesh-201301, India.

**In vitro antioxidant activity of YYEO**

The in vitro antioxidant assay was performed by the DPPH radical scavenging assay method (Noreen et al., 2017; Caacbay and Jacinto, 2009). Briefly, 3.6 ml of a methanolic solution of DPPH (0.004% w/v) was mixed with 0.4ml solution of different concentration (50-800 g/ml) of YYEO. After incubating at 37ºC for 40-45min absorbance was read at 517nm using a spectrophotometer. Ascorbic acid was used as standard. The inhibition curve was plotted and IC50 calculated.

**Experimental animals**

Balb/c female mice (20–30g) were procured from In Vivo Bio Sciences, Bengaluru. All mice were kept in clean cages for acclimatization under standard husbandry conditions (22-28°C) and relative humidity was maintained at 65±10% for a 12hr light-dark cycle with food and water ad libitum. The experimental protocol was reviewed and experimental animals were approved by IAEC, KLE College of Pharmacy, Belagavi (IAEC Reg No. 221/ Po/Re/S/2000/ CPCSEA) and the experiment was carried out in accordance with the CPCSEA guidelines.
Figure 3: Effect of YYEO on vital organ weight

Figure 4: Effect of YYEO on tumour volume and weight
Figure 5: Effect of YYEO on serum biomarkers

Figure 6: Effect of YYEO on haematological parameter
Figure 7: Effect of YYEO on antioxidant biomarkers

Figure 8: Effect of YYEO on liver
Acute toxicity studies
Ylang Ylang Essential oil was found to be safe and showed no mortality amongst the treated animals at a dose of 2000mg/kg for 14 days (Naik and Rasal, 2019).

Dose selection
Based on the LD₅₀ obtained from the acute toxicity study, doses of 200, 400, 800 mg/kg were chosen to evaluate the therapeutic effect. Furthermore, YYEO is an oil, the dose of YYEO (mg) has been converted to ml in accordance with the specific gravity of YYEO. As the quantity was not measurable, the oil was diluted through emulsion with Tween 20 as an emulsifier in the ratio 2: 2: 1, Ylang Ylang oil: Water: Tween 20.

Ehrlich ascites carcinoma
The mice with Ascitic Carcinoma (Donor) were taken after 15 days of tumor inoculation. The ascitic fluid was withdrawn using a 24-gauge needle into a sterilized syringe and tested for microbial contamination. The ascetic fluid was appropriately diluted in normal saline to obtain a concentration of 10⁶ cell/ml of tumor suspension and was administered intraperitoneally (0.1ml x 10⁶ cells/ml) to induce a tumor (Gupta et al., 2004).

Experimental design
Animals were divided into six groups of six mice in each. Except for the Normal group, all other groups were inoculated with EAC. Further, treatment with YYEO was started after 24 hours of inoculation for 14 days.

Group I: Normal group
Group II: EAC cell line (0.1ml x 10⁶ / ml) (Disease Control (DC))
Group III: EAC cell line (0.1ml x 10⁶ / ml) + 5- Fluouracil (20 mg/kg i.p.)
Group IV: EAC cell line (0.1ml x 10⁶ / ml) + YYEO (200 mg/kg p.o.)
Group V: EAC cell line (0.1ml x 10⁶ / ml) + YYEO (400 mg/kg p.o.)
Group VI: EAC cell line (0.1ml x 10⁶ / ml) + YYEO (800 mg/kg p.o.)

Body weight and vital organ weight
The change in body weight (BW) was recorded once every 3 days. At the end of the study, all the animals were decapitated and the weight of the vital body organ was recorded.

Tumor volume and tumor weight
Tumor weight (TW) was determined by collecting ascetic fluid after sacrificing the animal. Tumor volume (TV) is quantified by collecting ascetic fluid from the peritoneal cavity.

Serum estimation
At the end of the study, blood was collected via retro-orbital route and centrifuged at 3000rpm for 10 min to separate serum for the estimation of antioxidant enzyme mechanism such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Lactate Dehydrogenase (LDH), Triglycerides, and Total Protein using standard ERBA and UNICHEM kit protocols.

Hematological parameters
Blood was collected from retro-orbital route to determine the level of WBC, RBC, Haemoglobin, lymphocytes.

Biochemical estimation
The liver tissue was collected from the animal and homogenated to determine the in vivo antioxidant levels of Lipid Peroxidation (LPO), Glutathione (GSH), and Superoxide Dismutase (SOD). All the estimations were carried out by the method (Chandraшекhar et al., 2013).

Histopathology
At the end of the study, animals were euthanized and the liver was collected and stored in 10% neutral buffered formalin for further evaluation. The liver tissues were stained with hematoxylin-eosin and tissue lesions were evaluated using an electronic microscope at ×40 magnification.

Statistical analysis
All values are expressed as Mean ± SEM. ANOVA followed by a Tukey’s multiple comparison test using GraphPad Prism 5.0. The degree of freedom of 5% with a confidence interval of 95% was applied for all the tests; *p<0.05, **p<0.01 and ***p<0.001 compared to Normal group; ^p<0.05, ^^p<0.01 and ^^^p<0.001 compared to Disease control, #p<0.05, ##p<0.01 and ###p<0.001 compared to 5- FU treated group, @p<0.05, @@p<0.01 and @@@p<0.001 compared to 200mg/kg, $p<0.05, $$p<0.01 and $$$p<0.001 compared to 400 mg/kg.

RESULTS

In vitro antioxidant activity
DPPH scavenging activity of the YYEO and ascorbic acid was found to be dose-dependent as shown in Figure 1. 800μg/ml concentration of YYEO showed the highest DPPH scavenging property i.e. 85.6% compared to other concentration of YYEO. Similarly, ascorbic acid has a percentage inhibition of 90.4%.
### Table 1: Effect of YYEO on body weight

<table>
<thead>
<tr>
<th>Days</th>
<th>Normal Body Weight (gm)</th>
<th>Disease Control Body Weight (gm)</th>
<th>EAC + 5- FU EAC+ 500 mg/kg</th>
<th>EAC+ 200 mg/kg</th>
<th>EAC+ 400 mg/kg</th>
<th>EAC+ 800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22.53 ± 0.82</td>
<td>23.68 ± 1.27</td>
<td>23.48 ± 0.70</td>
<td>23.23 ± 0.76</td>
<td>24.1 ± 0.93</td>
<td>23.86 ± 0.77</td>
</tr>
<tr>
<td>3</td>
<td>23.03 ± 0.81</td>
<td>24.83 ± 1.18</td>
<td>22.90 ± 0.75</td>
<td>25.46 ± 0.86</td>
<td>26.28 ± 0.90</td>
<td>25.23 ± 0.70</td>
</tr>
<tr>
<td>6</td>
<td>23.26 ± 0.59</td>
<td>29.18 ± 1.09</td>
<td>22.41 ± 0.40</td>
<td>27.41 ± 0.87**</td>
<td>26.95 ± 0.48**</td>
<td>25.81 ± 0.73</td>
</tr>
<tr>
<td>9</td>
<td>23.88 ± 0.96</td>
<td>32.33 ± 0.96</td>
<td>22.00 ± 0.49</td>
<td>29.26 ± 0.92**</td>
<td>27.25 ± 0.96**</td>
<td>26.45 ± 1.21</td>
</tr>
<tr>
<td>12</td>
<td>24.06 ± 0.70</td>
<td>35.16 ± 1.46</td>
<td>21.61 ± 0.49</td>
<td>32.10 ± 1.05***</td>
<td>28.03 ± 0.83***</td>
<td>27.03 ± 1.23</td>
</tr>
<tr>
<td>14</td>
<td>24.66 ± 0.67</td>
<td>37.45 ± 0.72</td>
<td>21.11 ± 0.24</td>
<td>33.81 ± 0.70***</td>
<td>29.01 ± 1.29***</td>
<td>26.68 ± 1.67</td>
</tr>
</tbody>
</table>

### Table 2: Effect of YYEO on organ weight

<table>
<thead>
<tr>
<th>Organ Weight (gm)</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1.18 ± 0.11</td>
<td>0.158 ± 0.004</td>
<td>0.28 ± 0.01</td>
<td>0.228 ± 0.006</td>
</tr>
<tr>
<td>Disease Control</td>
<td>1.77 ± 0.55</td>
<td>0.221 ± 0.006</td>
<td>0.53 ± 0.02</td>
<td>0.348 ± 0.009</td>
</tr>
<tr>
<td>EAC + 5- FU</td>
<td>1.123 ± 0.007</td>
<td>0.160 ± 0.003</td>
<td>0.31 ± 0.02</td>
<td>0.248 ± 0.003</td>
</tr>
<tr>
<td>EAC + 200 mg/kg</td>
<td>1.57 ± 0.01</td>
<td>0.208 ± 0.006</td>
<td>0.49 ± 0.02</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>EAC + 400 mg/kg</td>
<td>1.483 ± 0.009***</td>
<td>0.201 ± 0.006</td>
<td>0.43 ± 0.02</td>
<td>0.30 ± 0.007</td>
</tr>
<tr>
<td>EAC + 800 mg/kg</td>
<td>1.30 ± 0.02</td>
<td>0.191 ± 0.004</td>
<td>0.41 ± 0.01</td>
<td>0.28 ± 0.005</td>
</tr>
</tbody>
</table>

### Table 3: Effect of YYEO on tumor volume and weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor Volume (ml)</th>
<th>Tumor Weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Disease Control</td>
<td>5.56 ± 0.16</td>
<td>5.917 ± 0.12</td>
</tr>
<tr>
<td>EAC + 5- FU</td>
<td>1.92 ± 0.08</td>
<td>2.20 ± 0.09</td>
</tr>
<tr>
<td>EAC + 200 mg/kg</td>
<td>4.06 ± 0.19</td>
<td>4.30 ± 0.20</td>
</tr>
<tr>
<td>EAC + 400 mg/kg</td>
<td>3.26 ± 0.24</td>
<td>3.60 ± 0.23</td>
</tr>
<tr>
<td>EAC + 800 mg/kg</td>
<td>2.41 ± 0.16</td>
<td>2.75 ± 0.13</td>
</tr>
</tbody>
</table>
Table 4: Effect of YYEO on serum parameters

<table>
<thead>
<tr>
<th>Serum markers</th>
<th>Normal</th>
<th>Disease Control</th>
<th>EAC + 5-FU</th>
<th>EAC + 200 mg/kg</th>
<th>EAC + 400 mg/kg</th>
<th>EAC + 800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>52.67 ± 2.18</td>
<td>123.5 ± 4.11 ***</td>
<td>61.33 ± 2.04</td>
<td>98.17 ± 2.05 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>42.17 ± 1.86</td>
<td>88.83 ± 1.79 ***</td>
<td>49.83 ± 1.07</td>
<td>74.83 ± 1.75 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>495.7 ± 8.37</td>
<td>2205 ± 73.0 ***</td>
<td>614.2 ± 11.18</td>
<td>1611 ± 32.55 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>76.33 ± 2.17</td>
<td>146.8 ± 3.02 ***</td>
<td>90.83 ± 1.49</td>
<td>129.7 ± 3.85 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>7.40 ± 0.33</td>
<td>3.85 ± 0.29 ***</td>
<td>6.90 ± 0.22</td>
<td>5.03 ± 0.22 ^^</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Effect of YYEO on haematological parameters

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Normal</th>
<th>Disease Control</th>
<th>EAC + 5-FU</th>
<th>EAC + 200 mg/kg</th>
<th>EAC + 400 mg/kg</th>
<th>EAC + 800 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (cells/mm³)</td>
<td>7.42 ± 0.59</td>
<td>20.54 ± 0.57 ***</td>
<td>11.40 ± 0.64</td>
<td>17.03 ± 0.77 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC (cells/mm³)</td>
<td>9.89 ± 0.45</td>
<td>5.00 ± 0.38 ***</td>
<td>10.66 ± 0.45</td>
<td>6.92 ± 0.99 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g %)</td>
<td>14.73 ± 0.57</td>
<td>7.58 ± 0.41 ***</td>
<td>12.60 ± 0.48</td>
<td>8.98 ± 0.70 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>84.33 ± 2.88</td>
<td>32.50 ± 1.66 ***</td>
<td>78.50 ± 1.85</td>
<td>42.17 ± 2.49 ^^^</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Effect of YYEO on antioxidant parameters

<table>
<thead>
<tr>
<th>Antioxidant parameter</th>
<th>Normal</th>
<th>Disease Control</th>
<th>EAC+5-FU</th>
<th>EAC+200mg/kg</th>
<th>EAC+400mg/kg</th>
<th>EAC+800mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO</td>
<td>7.25 ± 0.48</td>
<td>27.78 ± 0.42 ***</td>
<td>11.45 ± 0.41</td>
<td>20.35 ± 0.28 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>20.58 ± 0.44</td>
<td>6.38 ± 0.39 ***</td>
<td>16.15 ± 0.43</td>
<td>9.33 ± 0.27 ^^^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>40.52 ± 1.00</td>
<td>18.77 ± 0.45 ***</td>
<td>33.62 ± 0.59</td>
<td>22.83 ± 0.50 ^^^</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The IC$_{50}$ value of ascorbic acid and YYEO was found to be 151.84 µg/ml and 223.90 µg/ml respectively. The scavenging effect of DPPH is plotted in a graph with Absorbance vs. Concentration of oil (Figure 1).

**Body weight**

Tumor development was observed from day 6 and at the end of the study about 52% increase in body weight was observed in Disease control (DC) (37.45 ± 0.72g) compared to Normal group (NG) (24.66 ± 0.67g). However, a significant (p<0.001) change in BW was observed in 800mg/kg YYEO treated group (26.68±1.67g) compared to DC group. Similarly, BW of 33.81±0.70 and 29.01±1.29g was observed in 200 and 400mg/kg in EAC bearing YYEO treated animals. We examined that, administration of YYEO from day 1 to day 14 showed significant change compared to DC group which indicates YYEO potency on inhibiting tumor growth. The change BW among the groups are shown in (Figure 2) and change in BW from day 1 to day 14 are shown in Table 1.

**Organ weight**

Increase in vital organ weight (Liver, Heart, Kidney, and Lungs) were observed in DC group. However, treatment with YYEO significantly reduced organ weight on day 14. The effect of 200, 400, and 800mg/kg dose of YYEO on EAC bearing mice organs were shown in Figure 3 and Table 2.

**Tumor volume and tumor weight**

Mice inoculated with EAC showed a significant increase in tumor volume (p <0.001) compared to the NG until end of the study. The TV and TW of DC group was found to be 5.56 ± 0.16ml and 5.91 ± 0.12g respectively whereas EAC bearing mice treated with YYEO significantly reduced to 4.06 ± 0.19ml and 4.30 ± 0.20g in 200mg / kg, 3.26 ± 0.24 ml and 3.60 ± 0.23g in 400 mg / kg, 2.41 ± 0.16 ml and 2.75 ± 0.13g in 800 mg / kg. Whereas in EAC bearing mice treated with 5- FU treated group was found to be 1.92 ± 0.08 ml and 2.20 ± 0.09 g. Compared to the 200 mg / kg group, the 400 mg / kg i.e. 3.26 ± 0.24 ml and 3.60 ± 0.23g and 800 mg / kg i.e. 2.41 ± 0.16 ml and 2.75 ± 0.13g groups showed a significant reduction in TV and TW. (Table 3) (Figure 4).

**Serum markers estimation**

The level of AST were significantly increased (p <0.001), by) in EAC bearing DC group to 123.5 ± 4.11 U/L when compared to the NG i.e. 52.67 ± 2.18 U/L. After administration of YYEO at different doses (200 mg/kg, 400 mg/kg, 800 mg/kg) to EAC bearing mice the levels of AST was significantly reduced (p <0.001) to 98.17 ± 2.05 U/L, 79.50 ± 2.12 U/L, 70.17 ± 1.55 U/L respectively as compared to disease control. Also 5-FU showed decrease in AST level when compared with DC. (Table 4) (Figure 5).

Inoculation of EAC drastically increased the level of ALT, LDH and Triglycerides in DC group as compared to NG (p <0.001). Administration of YYEO at different doses (200 mg/kg, 400 mg/kg, 800 mg/kg) significantly decreased (p <0.001) ALT, LDH and Triglycerides levels when compared to DC group (Table 4) (Figure 5).

The level of total protein in the DC group 3.85 ± 0.29 gm% significantly decreased (p <0.001) in comparison with NG i.e. 7.40 ± 0.33 gm%. The 5- FU group, 6.90 ± 0.22 gm% also showed significant increase (p <0.001) when compared to DC group. After administration of YYEO at different doses (400 mg/kg, 800 mg/kg) the level of total protein was significantly increased (p <0.001) to 5.70 ± 0.24 gm% and 6.23 ± 0.19 gm% respectively when compared to DC group (Table 4) (Figure 5).

**Haematological parameter**

It was found that all hematological parameters of mice with tumors on day 14 significantly altered from NG (Table 5) (Figure 6). In malignancy, there was a decrease in the level of Hb, RBC and Lymphocytes, which was accompanied by an increase in WBC. At the same time interval YYEO (400 and 800 mg/kg p.o.) treatment significantly changed these altered parameters (p <0.001) to almost normal in dose dependent manner. The 5-FU group also showed altered parameters (p <0.001) when compared to NG and DC.

**Biochemical parameter**

The levels of LPO in liver tissue was significantly (p <0.001) increased to 27.78 ± 0.42 in DC group as compared to NG i.e 7.25 ± 0.48. In 5- FU group, decreased level of LPO was observed when compared to DC group (p <0.001). After the administration of treatment doses (200 mg/kg, 400 mg/kg, 800 mg/kg) to EAC bearing mice the level of LPO was reduced by 20.35 ± 0.28, 17.28 ± 0.31, 14.42 ± 0.26 respectively as compared to DC group (p <0.001) (Table 6) (Figure 7).

EAC inoculation significantly decreased (p <0.001) the level of GSH in liver tissue of DC group by 6.38 ± 0.39 when compared to NG i.e. 20.58 ± 0.44. On comparison with DC, the administration of 5-FU group i.e.16.15 ± 0.43 and different doses of YYEO (200 mg/kg, 400 mg/kg, 800 mg/kg) showed significant increase (p <0.001) in GSH level by 9.33 ± 0.27, 11.78 ± 0.48, 13.08 ± 0.38 respectively (Table 6) (Figure 7).

The level of SOD in the liver of DC group decreased (p <0.001) in comparison with NG. After administra-
To date, chemotherapy is a serious issue as it inhibits the repair of hepatic injury caused by EAC. Treatment with YYEO showed a remarkable reversal of protein synthesis (Onifade and Tewe, 2010). Previous studies have shown that depletion in protein exhibits liver dysfunction and inhibition of protein synthesis (Onifade and Tewe, 2010). Treatment with YYEO showed a remarkable reversal of all biochemical variables towards normal signifying the repair of hepatic injury caused by EAC. To date, chemotherapy is a serious issue as it causes myelosuppression and anemia during malignant growth. Anemia occurs in mice with a tumor mainly due to a decrease in the Red Blood Cell or Hb level and may reoccur due to iron insufficiency or myelopathy (Ve and Re, 1958). Treatment with the YYEO roughly normalized WBC, Red Blood Cells, Hb, and Lymphocytes. This reflected the drug activity of the hematological variables. LPO is a process associated with free radicals in biological systems that can occur under enzymatic control (Fenninger and Mider, 1954). Malondialdehyde (MDA) being the end product of LPO, was found to be higher in the DC group than in treated groups. Due to the excessive oxidative stress, GSH levels were decreased in the DC group but in the treatment group, GSH levels were increased to normal levels, which may be due to decreased proliferation of the cells (Arrick and Nathan, 1984). Similarly, Tumor growth is recorded due to blockade of SOD (Sun et al., 1989). The treated group showcased an enhanced level of SOD reflecting the restoration of natural antioxidant enzymes.

**DISCUSSION**

The current study utilized the in vivo cancer models to assess the anticancer potential of YYEO against EAC bearing mice. Initially, we treated with YYEO at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg to the EAC bearing mice and 5-Fluorouracil was used as an internal standard. YYEO at doses of 200 mg/kg, 400 mg/kg and 800 mg/kg significantly reduced Tumor volume and Tumor weight at the final level of the study. The standard drug 5-Fluorouracil showed a significant result compared with YYEO treated groups and was more effective. The antioxidant potency of the YYEO was evaluated by DPPH, the effects were compared against the ascorbic acid. The result revealed that the effect of both YYEO and ascorbic acid was dose dependent i.e. as the concentration increases, the inhibitory potency also increased.

High serum enzyme levels such as AST and ALT are indicatory of cell proliferation and various diseases of the liver and bones and the drugs used for treatment should lower the level of these enzymes to a normal level (Benirschke et al., 1978). The increased level of LDH presence in the blood or body fluid can be directly attributed to the extent of injured body tissues (Ramalingam et al., 2019). Elevated triglycerides are indicative of damage to the liver and cell membranes (Patra et al., 2015). The liver is the main source of serum protein. Protein intake is directly determined by the level of total proteins. Previous studies have shown that depletion in protein exhibits liver dysfunction and inhibition of protein synthesis (Onifade and Tewe, 2010). Treatment with YYEO showed a remarkable reversal of all biochemical variables towards normal signifying the repair of hepatic injury caused by EAC.

**Histopathology**

It has been observed that hepatocellular architecture damaged with neoplastic lesions and modified hepatocytes have developed with more than one nucleus and of a hyperchromatic nature. Lymphocyte invasion and marked central vein enlargement were found in the DC group. Slight hepatocellular architecture damaged with neoplastic lesions was observed in groups of 200 mg/kg and 400 mg/kg. Inversion of this damage observed in the 5-Fluorouracil and 800 mg/kg group (Figure 8).

**CONCLUSIONS**

In conclusion, the treatment of YYEO was effective in inhibiting the tumor growth in EAC treated mice model. Further studies are needed to characterize the active principle and to elucidate mechanism of action involved in anti-tumor activity.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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