Protective and Curative Effect of Thymoquinone on Ehrlich Solid Carcinoma Inoculated Mice

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ABSTRACT

Background: Thymoquinone (TQ), the main constituents of the volatile oil from Nigella sativa seeds and it is reported to protect laboratory animals against chemical toxicity and induction of carcinogenesis. This study was undertaken to investigate the potential protective and curative effect of TQ on Ehrlich solid carcinoma cells (ESC) inoculated mice.-induced carcinogenesis.

Material and methods: (50) Swiss albino mice were divided into five groups. Control group, Thymoquonone group: animals of this group were orally treated with TQ (10 mg/kg/day) for 4 weeks, Ehrlich Carcinoma group : animals of this group were inoculated intramuscularly with 0.2mL Ehrlich Ascites Carcinoma (2.5×10^6 cells) in the right thigh of the lower limb, Thymoquinone and Ehrlich carcinoma group: animals of this group were pre-treated with TQ for 14 days then inoculated with (EAC) and Ehrlich Carcinoma and Thymoquinone group : in this group animals were inoculated with EAC then after 8 days received TQ orally for a month. Morphological, Molecular as well as histopathological and ultrastructural changes were examined.

Results: Our results revealed that TQ showed a significant anti-tumor activity in ESC bearing mice represented by a reduction in tumor weight and volume. Flow cytometric analysis illustrated that the level of apoptosis is significantly decreased in ESC inoculated group. Otherwise, TQ+ESC and ESC+TQ groups showed a highly significant increase in apoptosis G0/1 peak. The level of P53 protein expression showed a significant decrease in ESC inoculated group, and this decrease was ameliorated in TQ+ESC and ESC+TQ groups when compared to ESC inoculated group. Histopathological observations showed a reduction in tumor size after treatment with Thymoquinone and this tumor was found to be discontinuous and fragmented with slowly growing. Conclusion: our results revealed that TQ has potential benefits in the prevention of the onset and progression of solid tumor model in mice.

Key Words: Thymoquinone, Anti-tumor activity, Ehrlich ascites carcinoma, Ehrlich solid tumor

INTRODUCTION

Thymoquinone (TQ) is a naturally-occurring volatile oil extracted from (Nigella sativa) seeds. Previous studies on TQ showed in vitro and in vivo anticancer, anti-inflammatory and anti-oxidant activities. [1] The combination of TQ with other natural compounds like diosgenin exhibited antineoplastic activity against squamous cell carcinoma in vitro and sarcoma-180-induced tumors in vivo. [2] Effect of TQ as an immunomodulator was also reported. [3] Toxicity studies showed that TQ can reduce the hepatotoxicity induced by some compounds including carbon tetrachloride, [4] tert-butyl hydroperoxide, [5] and cyclophosphamide. [6] Furthermore, TQ had no effect on liver integrity and hepatic enzyme activity when tested on mice and rats. [7] Importantly, the intraperitoneal therapeutic dose of TQ is 10–15 times lower than its LD_{50} value in rats. [8] Thus, it is reasonable to assume that TQ is a safe compound and has the potential to work as a therapeutic and hepatoprotective agent. Many potential targets which TQ regulates for its anticancer activities have been identified including p53, p73. [9] In addition, the combination of TQ with conventional medicine can result in greater anticancer effect, for example in NCI-H460 non-small cell lung cancer cells multiple myeloma cells. [10] Solid Ehrlich tumor is an undifferentiated solid tumor that is frequently used in tumor studies. It is both used to develop a tumor model and in chemotherapy investigations. [11] Following subcutaneous
injection of Ehrlich tumor cells, a tumor of 1 cm in diameter is obtained approximately within 1 week. This highly virulent tumor causes death of almost 100% of the experimental animals in a short period. Large-scale virulence, quick development and infiltrative nature of the tumor reflect its high-grade malignancy. [12] Tumor growth can cause antioxidant disturbances in certain tissues of the tumor host. [13] One of the characteristics of tumor growth and invasion is the increased flux of oxy-radicals and loss of cellular redox homeostasis. Cancer cells can generate large amounts of hydrogen peroxide, which may contribute to their ability to mutate, damage normal tissues and invade other tissues. This suggests that there is a direct correlation between changes in the rate of cancer cell proliferation and changes in the antioxidant machinery. Furthermore, some anticancer agents can act as antioxidant. [14]

This study aimed to investigate the potential protective and curative effect of TQ on Ehrlich solid carcinoma cells (ESC) inoculated mice-induced carcinogenesis.

Material and Methods

Chemicals

Thymoquinone (TQ) was purchased from Sigma–Aldrich Company Ltd. All other reagents and chemicals used in this study were of analytical and molecular grade.

Animals

Male Swiss albino mice, 6-8 weeks old weighting 19±2 g were kept for 7 days with free access to water ad libitum and fed on balanced diet.

Mice were divided into five groups, 10 mice for each one. Group 1, (C) served as the normal control. Animals of this group did not receive any treatment for 4 weeks. Group 2, (TQ) was dissolved in bi distilled water and orally administrated to mice at a dose of 10 mg/kg/day for consecutive 4 weeks based on LD50. [15,16] Group 3, (ESC), mice were inoculated intramuscularly with 0.2mL Ehrlich Ascites Carcinoma (EAC) (2.5×10^6 cells) in the right thigh of the lower limb then left for extra 2 weeks. Group 4, (ESC+TQ), mice were inoculated intramuscularly with 0.2mL (EAC) (2.5×10^6 cells) in the right thigh of the lower limb then after 8 days they received TQ (10 mg/kg/day) orally for successive 4 weeks. At the end of the experiment, mice were sacrificed and the samples were collected.

Body weight Changes

Animals were examined for BW changes: (initial BW on day 0, last and net final BWs on day 30). Net final BW = (final BW – tumor weight). BW gain was determined as body weight gain (final BW-intial BW/initialBW) x100.

Tumor volume (TV) and tumor weight (TW) analysis.

Time interval measurements of TV using digital Vernier calipers were conducted from day 8 to day 30 ESC and ESC+TQ groups. Data collected were plugged into the following formula to obtain tumor volume: TV (mm3) = 0.52AB^2, where A is the minor axis and B is the major axis. On day 30, mice were euthanized and solid tumors were excised for TW determination, photographed for tumor regression and processed for the histopathological studies.

Apoptosis as determined by flow cytometry

The flow cytometric analysis was performed in the Mansoura University Hospital using FACS (Flow activated cell sorter) Calibur Flow Cytometer (Becton Dickinson, Sunnyvale, CA, USA) equipped with a compact air-cooled low power 15 m WArgon ion laser beam (488 nm). DNA histogram derived from flow cytometry was obtained with a computer program for Dean and Jett mathematical analysis. [18] This software calculated the percentage of cells in each phase (G0/G1, S and G2/M) of the DNA cell cycle for each sample. The analysis of apoptotic cell death was performed by measuring DNA content. [19] Flow cytometry P53 protein expression was performed using fluorescence threshold using FACS Calibur Flow Cytometer (Becton Dickinson, Sunnyvale, CA, USA). [20]

The histopathological examination

Liver and tumor tissues were collected from mice, washed carefully by cold normal saline 3 times, then fixed in formalin solution 10%,
processed and embedded in a paraffin wax. Sections of 4-μm thickness were cut. Slides were stained with haematoxylin and eosin for the histological examination.

**Electronic Microscopy.**
Liver and tumor tissue were fixed, for 2 hours, in 2.5% glutaraldehyde buffered in 0.1 M cacodylate buffer (pH 7.2) at 4°C and post fixed in 1% cold osmium tetroxide in 0.1M cacodylate at pH 7.2, for 3 hours. Ultrathin sections were obtained from specimens embedded in Lowicryl K4M resin after dehydration through graded ethanol series, substitution and polymerization at −200°C. Ultrathin sections were obtained using an Ultracut S microtome (Leica, Vienna, Austria). Sections were mounted on 400-mesh collodion-carbon-coated nickel grids and examined with a Joel Electron Microscope (Japan) operating at 60 kV.

**Statistical analysis**
All values are expressed as the mean ± SE. Data were evaluated by using SPSS windows. The one way ANOVA test was used to examine whether there are any significant differences between the treatment groups and the value of $P < 0.05$ was considered significant.

**RESULTS**
**Protective effect of TQ on body weights and tumor sizes of Ehrlich solid tumor in mice:**
Treatment of Ehrlich solid tumor in mice with TQ showed a significant (P<0.05) ability to reduce the tumor growth. Measuring the change in body weight showed a decrease in body weight for all treatments compared to the control TQ. The highest reduction in body weight was observed in Ehrlich solid tumor in mice (ESC) with a percentage change in body weight of -3.27% compared to +33.48% recorded for untreated mice (Table 1). A slight increase in body weight was observed in mice treated with TQ, while mice treated with ESC-TQ showed a reduction in body weight (Table 1).

A significant decrease in tumor weight was observed in ESC+TQ treated group, as compared to ESC only inoculated mice. This represents -69.47% ($P < 0.05$) decreases. In otherwise, there was a complete absence of the tumor in TQ+ESC group compared to ESC inoculated group (Table 1).

A significant decrease in tumor volume was observed in ESC+TQ treated group, as compared to ESC only inoculated mice. This represents -69.47% ($P < 0.05$) decreases in tumor volume. Otherwise, there was a complete absence of the tumor in TQ+ESC treated group compared to ESC inoculated group (Fig. 1).

**Flowcytometric analysis of Ehrlich solid tumor cell cycle phase distribution.**
The cell cycle analysis of control and different treated mice groups were shown in table (2) and figures (2). Results illustrated that the level of apoptosis (sub G1) is significantly decreased in ESC inoculated group (31.45±0.22). This decrease was overcome in TQ+ESC and ESC+TQ treated groups (73.85±0.7) and (45.08±0.04) respectively. Otherwise, TQ treated group showed non-significant change from the control group. The mean G0/1 peak showed a significant decline in ESC inoculated group (12.26±0.14). A decline was restored in TQ+ESC group which showed a highly significant increase in G0/1 peak (23.73 ±0.34) and relative increase in ESC+TQ group (15.85±0.01) (Fig.2). On the other hand, S phase % was not altered in the control and TQ group. While, there was a significant increase in ESC inoculated group(52.29±0.09) when compared to that of the control group, this percent declined in TQ+ESC and ESC+TQ groups when compared to ESC inoculated group; where they recorded 7.82±0.57 and 37.73±0.04 respectively.

The mean G2/M peak was not altered in both the control and TQ treated group. While, there was a significant increase (7.84±0.02) in ESC inoculated group when compared to that of control, this percent declined in TQ+ESC and ESC+TQ groups when compared to ESC inoculated group only group since they showed 1.87±0.19 and 5.72±0.01 respectively (Fig.2).

**Flow cytometric analysis of p53 protein expression.**
The expression of p53 protein in Ehrlich solid tumor in mice is shown in Figure 3. The present results illustrated that the level of P53 protein
expression (%) was not significantly altered in both control and TQ treated groups. While, there was a significant decrease (p<0.05) in ESC inoculated group when compared to that of the control group. This decrease was ameliorated in TQ+ESC and ESC+TQ groups when compared to ESC inoculated group (Figs. 3 A and B).

**Histopathology of Ehrlich solid tumor in mice:**

The inoculation of Ehrlich tumor cells into the control mice induced intramuscular tumors at the point of inoculation. These tumors were prominent and revealed fast growth with mixed inflammatory reaction predominantly lymphocytes, white conglomerated mass and sometimes infiltrated into the muscle fibres of the animals shown in figs.4 A&B, also, a number of mitotic figures and lymphocytic reaction indicating a continuous proliferation activity in the positive control group. These tumors showed sheets of small, higher chromatophilic tumor cells of variable shapes representing cell proliferation surrounding areas of necrosis and differentiated cells as shown in figs.4A&B. Necrosis was increased and marked reduction in the incidence of mitosis with extended necrosis, indicated slow growth. There was a marked reduction in tumor size after treatment with Thymoquinone, the tumor was found to be discontinuous and appeared growing slow and fragmented. This indicates a partial prevention of the effects of EAC cells by Thymoquinone.

Ultrastructurally, tumors isolated from Ehrlich tumor cells inoculated group revealed tumor cells without any apoptotic morphology. There were more or less rounded shape large nucleus quite euchromatic and quite evident nucleoli and a prominent porous nuclear envelope, besides electron-lucent nuclear vesicles dispersed in the nucleoplasm. There were also, threadlike processes projecting "microvilli" all-over the cells surface, some with bifurcation as shown in figs. 5A&B). In some instances, the nuclei were fragmented and quite heterochromatic showed no nucleoli. The cytoplasm showed a depletion of organelles which was characterized by of free ribosomes and small degenerated mitochondria and lipid vesicles and abundant vacuoles. Some tumor cells had high amounts of glycogen, while others had high amounts of lipid in the cytoplasm, with occasional tumor cells having crescent-shaped nuclei partially encircling the lipid as shown in figs. 5A&B.

Ultrastructurally, isolated tumors from post treated group with TQ showed changes in the shape of tumor cells. There were also some apoptotic cells which showed shrinkage rather than swelling, which was a morphologic feature of apoptosis. The cells were smaller in size with dense cytoplasm. The nuclear envelopes were deeply invaginated and sometimes broke up producing two or more fragments (karyorrhexis) as shown in figs. 5C&D. Some nuclei underwent karyorrhexis (the nuclear chromatin was aggregated into numerous masses and being released by rupture of the nuclear envelope; the dark masses especially in the periphery probably represent fragmented nuclear remnants) and the others were pyknotic or karyolytic. The cytoplasm contained deformed mitochondria, short profiles of endoplasmic reticulum and abundant vacuoles. The cell surface had numerous projections. Sometimes these apoptotic cells showed surface blebbing and then underwent fragmentation of the cytoplasm as shown in figs. 5C&D.

**DISCUSSION**

Thymoquinone (TQ), the main constituents of the volatile oil from *Nigella sativa* seeds is reported to protect laboratory animals against chemical toxicity and induction of carcinogenesis. In this regard, the present study was undertaken to evaluate the potential protective and curative effect of TQ on Ehrlich solid carcinoma cells inoculated animals-induced carcinogenesis. The mice inoculated with Ehrlich carcinoma showed a significant decrease in body weight gain with respect to the control ones. This result agrees with those obtained by Badr El-Din et al., [22] Viela et al., [23] and Metwally et al., [24] Reduction in body weight resulted in the stunted growth not only due to reduced food consumption but also due to the tumor burden with its massive growth rate. Our results revealed that (TQ) exhibited antitumor activity against ESC tumor in mice, presented by the decrease in tumor weight and tumor volume. The reduction in body weight gain and the decrease in tumor volume are the criteria for
judging on the volume of any antitumor drugs. [25]

On the other hand, flow cytometric data revealed that mice inoculated with Ehrlich carcinoma showed remarkable alterations in cell cycle analysis, represented by a significant elevation of cell accumulation at S % phase as well as on G2/M phase, in contrast, a significant decrease in apoptosis % was observed by many authors. [22, 26, 27] The Ehrlich tumor is a rapidly growing carcinoma so that cells increase their nuclear materials continuously on the expense of their normal apoptotic rate. [28] Accumulation of sub-G1 phase cells arrested in G1/G0 phase of the cell cycle as well, showed remarkable decrease in cells at S% and G2/M phase of the cell cycle. These findings establish the antiproliferative and apoptosis-inducing abilities of TQ.

The activation of a defective G1 checkpoint apparent causes the accumulation of cells within the phase which might be due to the presence of wild-type p53 protein. The tumor suppressor protein p53 is the primary regulator of the G1 checkpoint of cell cycle, where it co-ordinates the cellular ability of DNA repair with the binary decision between cell survival and death. [29] Hence, pre-treatment induced high fraction of cell death by apoptosis in EAC cells might be ascribed to the defective p53 checkpoint. [30-32] The p53 tumor suppressor was initially identified as the guardian of the genome based on its ability to mediate G1 arrest following DNA damage. [33-34] However, as indicated above, p53 is now known to act in many cellular processes, including cell-cycle checkpoints, DNA repair, senescence, angiogenesis, surveillance of genomic integrity and apoptosis. [35-36]

Anti-tumor properties of *Nigella sativa* were studied. [37-38] The active principle of *Nigella sativa* seeds was identified as a mixture of long-chain fatty acids. [37] These medicinal effects and others are due to thymoquinone (The major active constituents of *Nigella sativa*) which is a promising natural compound with important *in vitro* and *in vivo* anti-cancer activities against many different cancer cell lines, including cancers of the breast, colon, ovaries, lung and larynx, as well as myeloblastic leukemia, Ehrlich ascites carcinoma, Dalton's ascites lymphoma, and osteosarcoma. [39] In the present study, subcutaneous inoculation with Ehrlich tumor cells was found to display increased eosinophilic, dense and pleomorphic nuclei, cytoplasmic vacuolization, hydropic degeneration (Oedema), leucocytic infiltration and necrosis. [40-41, 42] Moreover, leucocytic inflammatory infiltration was observed in liver of Ehrlich inoculated animals in the present study. These leucocytic infiltrations were considered as a prominent response of the body tissue facing any injurious impacts. [43]

The ultrastructural investigation of ESC inoculated animals showed a destruction or loss of microvilli in bile canaliculi and space of Disse with destruction of the endothelial cells. Decreased number of microvilli in both bile canaliculi and space of Disse has been noted in mice inoculated with hepatitis and after administration of carbon tetrachloride. [44-45] In the present study, the anti-tumor activity of TQ may be due to the presence of fatty acids and may be also due to other components. Ando et al. [46] suggested the possible mechanism involved in the anti-tumor activity of fatty acids. Firstly, the fatty acids acted as a detergent on the tumor cell membrane and subsequently altered or destroyed the cell membrane. [47] Morgan et al. [48] reported that the length of fatty acid carbon chain had some influences on the anti-tumor activity *in vitro*. A similar study using *Nigella sativa* seeds was carried out by Salomi et al. [37] who concluded that *in vivo* EAC tumor development was completely inhibited by the active principle at the dose of 2mg/mouse per day for 10 days. From the previous studies, attempt was undertaken to test the *Nigella sativa* seeds powder against the chemical carcinogenesis in rat liver. The present observations reinforce the view that TQ has potential scavenging power against EAC induced intracellular oxidative stress. Results of the present work are in agreement with those of Verrill [49] who examined the potent anti-tumor activity of Zizyphus leaves extract against EAC cells, *Saliva aegyptiaca* and *Trigonella foenum graecum* which have a potential therapeutic effect on EAC bearing cells. [50]

Apoptosis is a pathway of cell death induced by a suicidal program in which cells destined to die by activate enzymes that degrade the cell nuclear DNA. [51] This is a normal
phenomenon that serves to eliminate cells that are no longer needed during development. It is also a pathologic event when diseased cells become damaged beyond repair and are eliminated without eliciting a host reaction, thus limiting collateral tissue damage. [53] Initiation of apoptosis occurs principally by signals from two distinct pathways: the intrinsic or mitochondrial pathway and the extrinsic or death receptor-initiated pathway. These pathways that are induced by distinct stimuli involve different sets of proteins. Both pathways converge to activate caspases, which are the actual mediators of cell death. [53]

However, besides significantly increasing the central area of necrosis, TQ treatment also promoted necrosis in the peripheral areas of the tumors, which are areas of undifferentiated and infiltrative proliferative neoplastic character. [54] TQ affects multiple targets, including suppression of anti-apoptotic genes expression and thus enhances apoptosis induction. [55] Moreover, TQ inhibited cell proliferation of many types of cancer cell lines, including breast adenocarcinoma, ovarian adenocarcinoma, [56] human pancreatic adenocarcinoma, colorectal cancer, [39,57] uterine sarcoma, [58] neoplastic keratinocytes [59] fibrosarcoma and lung carcinoma. [55]

Banerjee et al. [60] have shown that more potent synthetic analogues of TQ can be prepared, which can potentially be developed for future human use. For instance, TQ has been shown to suppress the proliferation of various tumor cells, including colorectal carcinoma, breast adenocarcinoma, osteosarcoma, ovarian carcinoma, myeloblastic leukemia and pancreatic carcinoma. [39, 61, 56, 62] While, it is minimally toxic to normal cells. [63] Furthermore, we observed that endothelial cells were more sensitive to thymoquinone-induced cell apoptosis, cell proliferation and migration inhibition. Thus our results suggest that thymoquinone inhibits tumor angiogenesis and tumor growth, and can be used as a potential drug candidate for cancer therapy.

In conclusions: this study demonstrated that TQ has potential benefits in the prevention of the onset and progression of solid tumor model.

REFERENCES

13- Wenger FA (2001): Influence of octreotide on liver metastasis and hepatic lipid peroxidation in


Fig. 1: Effect of TQ administration on tumor volume (mm$^3$) and morphology in mice-bearing Ehrlich solid carcinoma. (A) Histogram of tumor volume in ESC and ESC+TQ treated group. B- Morphological appearance of solid Ehrlich tumor in ESC and ESC+TQ treated group.
Fig. 2: Flow cytometric analysis of Ehrlich solid tumor cell cycle phase distribution. A- Control TQ group, B- ESC group, C- ESC+TQ group and D- TQ+ESC group.
Fig. 3: Expression of p53 protein in the different treated groups of mice. A- Flow cytometric analysis of p53. B- Histograms of flow cytometric analysis of p53 protein expression in the different treated groups.
Fig. 4: **Histopathology of solid Ehrlich carcinoma tumor (SEC) of mice.** (A and B) Longitudinal sections of Ehrlich tumor bearing mice; thigh muscle displaying muscle fibres (M) invaded by deeply stained tumor cells (arrow heads) and large area of necrosis (N). (C and D) Sections of Ehrlich tumor bearing mice with TQ showing a partial prevention of the effects of EAC cells by thymoquinone, and muscle fibres (M) invaded by deeply stained tumor cells (arrow heads).
Fig. 5: The ultrastructural estimation of solid Ehrlich carcinoma tumor (SEC) of mice. (A and B) Electron micrographs of Ehrlich solid tumor showing large nucleus (N) with condensed chromatin, many vacuoles (V), fat droplets (FD), microvilli (arrow), (R) ribosomes and mitochondria (M). (C and D) Electron micrographs of Ehrlich solid tumor treated with TQ showing many vacuoles (V), fat droplets (FD), karyolysis (KI), cell debris (CD), fragmented cells (*), karyolysis (KI), many vacuoles (V), fat droplets (FD), mitochondria (M), membrane blebs (MB), (arrow heads) and necrosis.
Table 1- Protective effect of TQ on % of body weight change and tumor sizes of the different treatments groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>C</th>
<th>TQ</th>
<th>ESC</th>
<th>TQ+ESC</th>
<th>ESC+TQ</th>
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<tbody>
<tr>
<td>Initial body weight (g) (at day 0)</td>
<td></td>
<td>20.1±0.1</td>
<td>20.63±0.12</td>
<td>20.78±0.24</td>
<td>20.6±0.17</td>
<td>20.6±0.01</td>
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<td>Final body weight (g) (at day 30)</td>
<td></td>
<td>26.73±0.14</td>
<td>27.2±0.3</td>
<td>26.5±0.7</td>
<td>27±0.05</td>
<td>26.6±0.3</td>
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<tr>
<td>Tumor weight (g)(at day 30)</td>
<td>--</td>
<td>--</td>
<td>6.31±0.04</td>
<td>--</td>
<td>2.85±0.24</td>
<td></td>
</tr>
<tr>
<td>Net final body weight (g)</td>
<td>26.73±0.14</td>
<td>27.2±0.3</td>
<td>20.1±0.7</td>
<td>27±0.05</td>
<td>23.73±0.31</td>
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<tr>
<td>Body weight gain (g)</td>
<td>+6.73±0.3</td>
<td>+6.56±0.3</td>
<td>-0.68±0.7</td>
<td>+6.4±0.24</td>
<td>+3.13±0.24</td>
<td></td>
</tr>
<tr>
<td>% of body wt. change</td>
<td>(+33.48%)</td>
<td>(+31.79%)</td>
<td>(-3.27%)</td>
<td>(+31.06%)</td>
<td>(+15.19%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean±SE, (a) significant compared to the control P<0.05 , (b) significant compared to ESC, P<0.05.

Table 2- Flow cytometric analysis of cell cycle distribution in the different treated groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ESC+TQ</th>
<th>TQ+ESC</th>
<th>ESC</th>
<th>TQ</th>
<th>C</th>
<th>Apoptosis (M1) %</th>
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<tbody>
<tr>
<td>G0/G1phase (M2) %</td>
<td>15.85</td>
<td>23.73</td>
<td>12.26</td>
<td>15.7</td>
<td>15.18</td>
<td></td>
</tr>
<tr>
<td>S phase (M3) %</td>
<td>37.73</td>
<td>7.82</td>
<td>52.29</td>
<td>22.6</td>
<td>23.78</td>
<td></td>
</tr>
<tr>
<td>G2/M phase (M4) %</td>
<td>5.72</td>
<td>1.87</td>
<td>7.84</td>
<td>2.41</td>
<td>2.53</td>
<td></td>
</tr>
</tbody>
</table>

a=significant as compared to the control group (P<0.05). b =significant as compared to ESC treated group (P<0.05)