Curative role of green tea and *Moringa oleifera* extracts on the changes of AFP and CD34 expression in mice with liver cirrhosis

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Abstract

The present study aims to investigate the effect of green tea and *Moringa oleifera* extracts on the expression of AFP and CD34 immunohistoochemistry (IHC) in the liver cirrhotic mice induced experimentally by CCl4. Eighty adult male albino mice weighing 25 ± 3 g were used and divided into 8 equal groups (10 mice/each); Group I: normal control mice group received no treatment. Group II: control mice treated with olive oil only at a dose (1ml/kg/bw) twice a week for 6 weeks, Group III: mice administered with green tea extract only at a dose (600mg/kg/b w/d) for one month, Group IV: mice administered with *Moringa oleifera* extract only at a dose (400mg/kg/bw/d) for one month, Group V: mice injected intraperitoneally (i.p.) by CCl4 at a dose (1 ml/kg/bw) added to olive oil (1:1ml) twice a week for 6 weeks to induce liver cirrhosis, Group VI: cirrhotic mice administered with green tea extract only at a dose (600 mg/kg/bw/d) for a month, Group VII: cirrhotic mice administered with moringa extract only at a dose (400 mg/kg/bw/d) for a month and Group VIII: cirrhotic mice administered with a mixture of green tea and moringa extracts at the same previous doses daily for a month. AFP was expressed in the liver sections of control mice group I as weak immunostain in the hepatocytic cytoplasm as well as in groups II - IV .The cirrhotic liver mice group V expressed intense AFP immunoreaction in most hepatocytes. The cirrhotic mice group administered with either green tea or moringa or a mixture of both together (groups VI - VIII) improved the hepatic tissues and expressed weak AFP immunostain in most hepatocytes. The CD34 in control liver of mice was expressed as a strong immunostain in the endothelia of blood sinusoids and many hepatocytes. The cirrhotic liver of mice expressed no CD34 immunostain in many lobules of the hepatic tissues, and few lobules expressed weak CD34 immunostain in the hepatic tissues. The administration of cirrhotic liver mice with green tea or moringa or a mixture of both improved the hepatic tissues and expressed strong CD34 immunostain in endothelia of blood sinusoids, and appearance of many intense oval cells in the hepatic tissues. In conclusion, green tea is more effective than moringa or co-administration of both together in improvement of AFP and CD34 expression in liver cirrhosis of mice

Keywords: liver, cirrhosis, green tea, *Moringa oleifera*, IHC, alfa fetoprotein (AFP), CD34, mice.

Introduction

Liver cirrhosis is defined as a condition in which the liver slowly deteriorates and is unable to function normally due to chronic or long lasting injury. Scar tissue replaces healthy liver tissue and partially blocks the flow of blood through the live, and a healthy liver is necessary for survive **(1).** There are many causes of cirrhosis are: chronic hepatitis C and B, alcohol-related liver disease, nonalcoholic fatty liver disease and nonalcoholic steato-hepatitis, autoimmune hepatitis, inherited diseases that affect the liver, rare viral infections, prolonged exposure parasitic infections, chronic heart failure with liver congestion and toxic chemicals **(2).** Liver cirrhosis is responsible for more than 1 million deaths annually and the majority of these deaths are preventable. There is marked geographical variation in rates of mortality due to cirrhosis, and this variation in liver disease burden exemplifies the links between population risks for liver disease and mortality **(3).** In Egypt, up to 85% of HCV infections persist for life, leading to chronic hepatitis. The major cause of death is primarily associated with cirrhosis **(4).**

Cirrhosis is divided into two categories (compensated and decompensate).

Compensated cirrhosis is known that the liver is heavily scarred but can still perform many important functions of the body. Many people with compensated cirrhosis have few or no symptoms and can live for many years without serious complications. Decompensated cirrhosis is known that the liver is extensively scarred and unable to function normally. People with decompensated cirrhosis eventually develop many symptoms and complications that can be life threatening **(5).**

Green tea (GT) is one of many herbal therapies effect on cirrhosis and liver disorders. The term tea (*Camellia sinensis*) is used for a family of mostly woody flowering plants belonging to the family Theaceae. Theaceae family contains about 520 species and contains subtropical areas, but most species occur in Eastern Asia and South America contains 28 genera. GT chemical composition contains; proteins(15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-N-ethyl-glutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium ,fluorine, and aluminum; and trace amounts of lipids (linoleic and a-linolenic acids), sterols (stigmasterol vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds (aldehydes, alcohols, esters, lactones hydrocarbons) **(6).**

GT has polyphenls which include flavanols, flavandiols, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the GT polyphenols are flavonols, commonly known as catechins. Products derived from GT are mainly varied in the proportion of polyphenols (45-90%) and caffeine content (0.4-10%). Four kinds of catechins are found in GT: epicatechin, epigallocatechin epicatechin-3-gallate, and (-)-epigallocatechin-3-gallate (EGCG) **(7).**

The most biologically active constituent in GT is EGCG has been known as a component that provides the beverage with potential benefits for human health **(8).**

Moringa oleifera (MO) is a multi-purpose vegetable tree with a variety of potential uses, of which the medicinal properties are initially considered the most interest and is an important tropical crop that is used as human food and in oil production. There are in total 13 species in the genus MO, belonging to the family Moringaceae and it is the most commonly cultivated species. In Pakistan and India, MO is locally known as Sohanjna and is grown and cultivated all over the country, and it is also known as Ben oil tree **(9&10).**

MO leaves (W/W) have the calcium equivalent of four times that of milk, the vitamin C content is seven times that of oranges, while its potassium is three times that of bananas, three times the iron of spinach, four times the amount of vitamin A in carrots and two times the protein in milk **(11).** MO is suggested as a viable supplement of dietary minerals. High amount of Ca, Mg, K, Mn, P, Zn, Na, Cu, and Fe are contained by MO pods and leaves **(12&13).** It is also a source of protein, β-carotene, vitamins B and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids and various phenolic compounds **(14).**

Medicinally, various parts of moringa are generally known for their multiple pharmacological effects including their antitumor, antihyperglycemic and anti-inflammatory effects **(15&16).** Furthermore, the extract of moringa has been shown to have potent antioxidant action *in vivo* studies **(17&18)** and hepatoprotective against CCl4 induced liver damage in male rats **(19).**

Alpha-fetoprotein (AFP) is a tumor-associated fetal glycoprotein containing 3-4% carbohydrate moieties and a molecular mass of 69,000 daltons. During development, AFP is expressed and synthesized

sequentially by cells of the yolk sac, and fetal liver and gastrointestinal tract. In adults, the AFP gene is silenced by methylation processes and AFP reappears only in instances of hepatic damage/regeneration and in tumors such as hepatomas and germ cell cancers AFP antibody initiate by binding the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric and finally abnormal hepatocytes and liver carcinoma under light microscope **(20&21).**

The CD34 protein is a member of a family of single-pass transmembrane sialomucin proteins CD34 is also an important adhesion molecule and is required for T cells to enter lymph nodes. It is expressed on lymph node endothelia, whereas the L-selectin to which it binds is on the T cell. Cells expressing CD34 (CD34+ cell) are normally found in the umbilical cord and bone marrow as hematopoietic cells, or in mesenchymal stem cells, endothelial progenitor cells, endothelial cells of blood vessels, mast cells and dendritic cells **(22&23).**

The present investigation is designed to study the possible role of green tea and *Moringa oleifera* extrats separately or together to improve the expression of AFP and CD34 by using IHC method in the liver tissues of mice induced- experimentally by CCl4.

Materials and Methods

1. Animal selection and care:

Eighty adult male albino mice weighing 25 ± 3 g were used in the present investigation and were obtained from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt. The animals were housed in plastic cages (10 per cage) for one week acclimatization under the same condition of temperature and natural dark- light cycle. Food and tap water were freely available to the animals throughout the experiment. All care and producers adopted for the present investigation were in accordance with the approval of the Institution Animal Ethics committee of National Research Center and in accordance with recommendation of the proper care and use of laboratory animals.

2. Induction of cirrhosis:

Carbon tetrachloride (CCl4) and olive oil were received from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt and used for induction of liver cirrhosis. CCl4 was added to olive oil (1:1ml) and was injected intraperitoneally (i.p.) to mice at a dose 1ml/kg/bw twice a week for 6 weeks according to **Sakaida *et al*. (24).**

3. Treatment:

a) Green tea (GT) extract was received from local pharmacy and administrated orally (by gastric tube) to mice at a dose 600 mg/kg/bw/d according to **Thomas and Thomas (25)** for one month, **b)** *Moringa oleifera* (MO) extract was received from Vacsera 51 Wezaret El Zeraa St. Agouza, Giza, Egypt and given orally to mice at a dose 400 mg/kg/bw/d for a month according to **Sharifudin *et al*. (26),** and **c)** mixture of GT and MO extracts (1:1ml) were administrated to mice at a dose 600 mg/kg/bw/d of GT and 400 mg/kg/bw/d of MO daily for one month.

4. Experimental design:

Eighty mice were divided into 8 equal groups (10 mice/each). **Group I:** normal control mice group received no treatment. **Group II:** mice injected i.p. with olive oil only at a dose 1 ml/kg/bw twice a week for 6 weeks. **Group III:** mice received orally GT extract only at a dose 600 mg/kg/bw/d for one month. **Group IV:** mice received orally MO extract only at a dose 400 mg/kg/bw/d for one month. **Group V:** mice injected i.p. by CCl4 at a dose 1 ml/kg/bw that added to olive oil (1:1ml) twice a week for 6 weeks to induce cirrhosis. **Group VI:** cirrhotic mice administered orally with GT extract only at a dose 600 mg/kg/bw/d for one month. **Group VII:** cirrhotic mice administered orally with MO extract only at a dose 400 mg/kg/bw/d for one month**. Group VIII:** cirrhotic mice

administered orally with a mixture of GT and MO extracts at the same previous doses of GT and MO daily for one month.

At the end of experimental period, mice were sacrificed after 5 hrs and the blood were collected from the reto-orbital plexus from all mice groups and the liver specimens were carefully removed and fixed in 10% neutral buffered formalin then processed for light microscope.

5. Immunohistochemical preperation:

The fixed liver pieces were processed and embedded in paraffin wax. Monoclonal antibody against AFP and polyclonal antibody against CD34 were used by applying avidin-biotin complex (ABC) technique **(27).** AFP was used as a marker against abnormal hepatocytes results from cirrhosis, and it was received from Biocare Polymer Detection Biosciences, San Diego, CA, USA **(28).** CD34 was used as a marker for vascular endothelial cells and expression of hematopoietic progenitor stem cells and it was received from Thermo Fisher Scientific Industries, Waltham, MA, USA **(23&29).** The ABC method, a biotinylated secondary antibody reacts with peroxidase conjugated streptavidin molecules. Endogenous peroxidase activity was inhibited by incubation with 3% H2O2 for 5 min. The sections were blocked with normal goat serum for 1h to prevent non-specific binding followed by incubation with the primary monoclonal antibody (anti-AFP) or polyclonal antibody (anti-CD34) for 1h at room temperature. The sections were incubated with the secondary antibody (anti-rabbit peroxidase) for 30 min. The staining was visualized by using diaminobenzidine (DAB) that gave a brown colour, then the slides were washed and counterstained with haematoxylin **(27).** AFP immunoreactivity was expressed as a brown colour in abnormal hepatocytes results from liver cirrhotic mice. CD34 immunoreactivity was expressed as a brown colour in the progenitor stem cells and endothelial cells.

Results

a) AFP immunostain expression in the liver:-

AFP marker is a polyoclonal antibody against hepatocellular injuries. The normal control liver of mice (group I) expressed weak AFP immunostain in the hepatocytic cytoplasm as well as that observed in the liver mice treated with olive oil or administered with either GT or MO extracts (group II-IV) **(Fig1).** The cirrhotic liver sections of mice induced by CCl4 (group V) expressed intense AFP immunoreactivity to the hepatocytes of most hepatic tissue sections as brown colour **(Fig.2).**

The administration of cirrhotic mice with GT extract (group VI) expressed an obvious improvement and a reduction of AFP immunostain in the hepatic cells and most of them appeared approximately similar to normal ones **(Fig.3).** The administration of cirrhotic mice with MO extract (group VII) expressed moderate decrement AFP immunoreactivity to the hepatic cells as well as the administration with a mixture of GT and MO extracts (group VIII) **(Figs.4&5).** It means that either GT or MO or both together improved the AFP of cirrhotic liver and they recovery most of the hepatocytes to normal structure but GT is more effective than MO or a mixture of both.

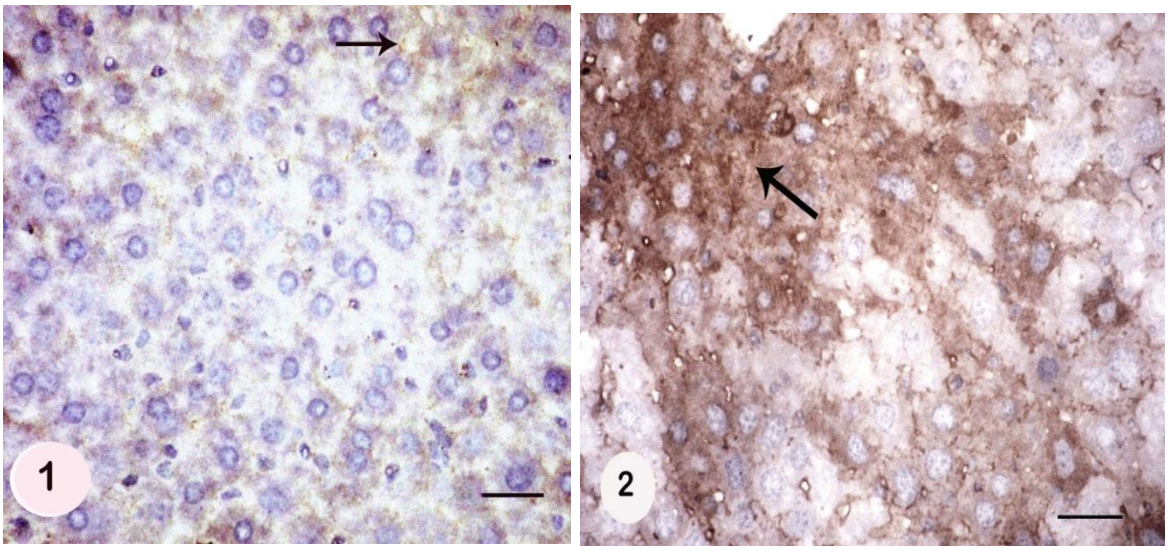


Fig. (1): Section of the liver of a normal control mouse expressing weak AFP immunoreaction in the hepatic cytoplasm (arrow). AFP immunostain, Bar = 6.25 µm.

Fig. (2): Section of the cirrhotic liver of a mouse injected with CCl4 at a dose 1ml/kg/bw twice a week for 6 weeks expressing intense AFP immunoreaction in the most of hepatocytes (arrow). AFP immunostain, Bar = 6.25 µm.

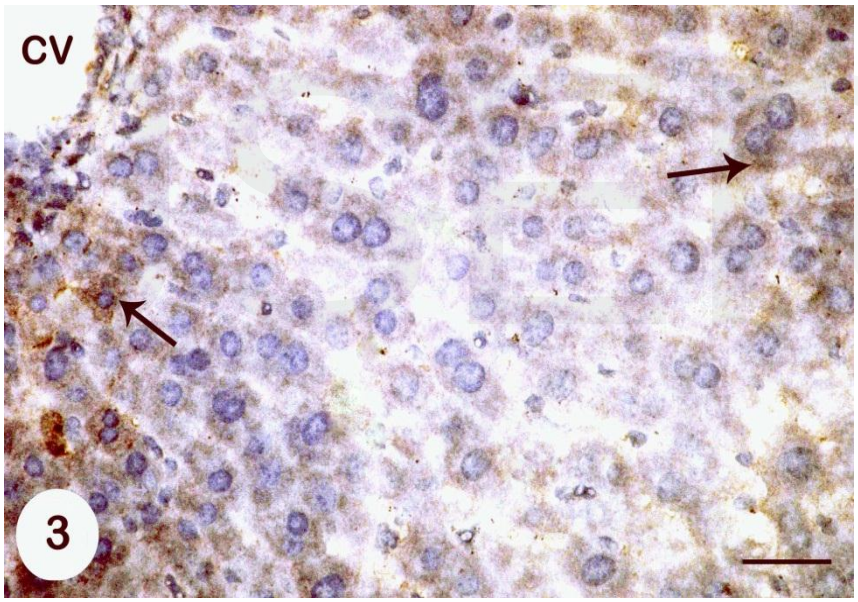


Fig.(3): Section of the cirrhotic liver of a mouse administered with GT extract at a dose 600mg/kg/bw/d for one month expressing an obvious improvement and a reduction of AFP immunostain in the hepatic cells (arrows). AFP immunostain, Bar = 6.25 µm..

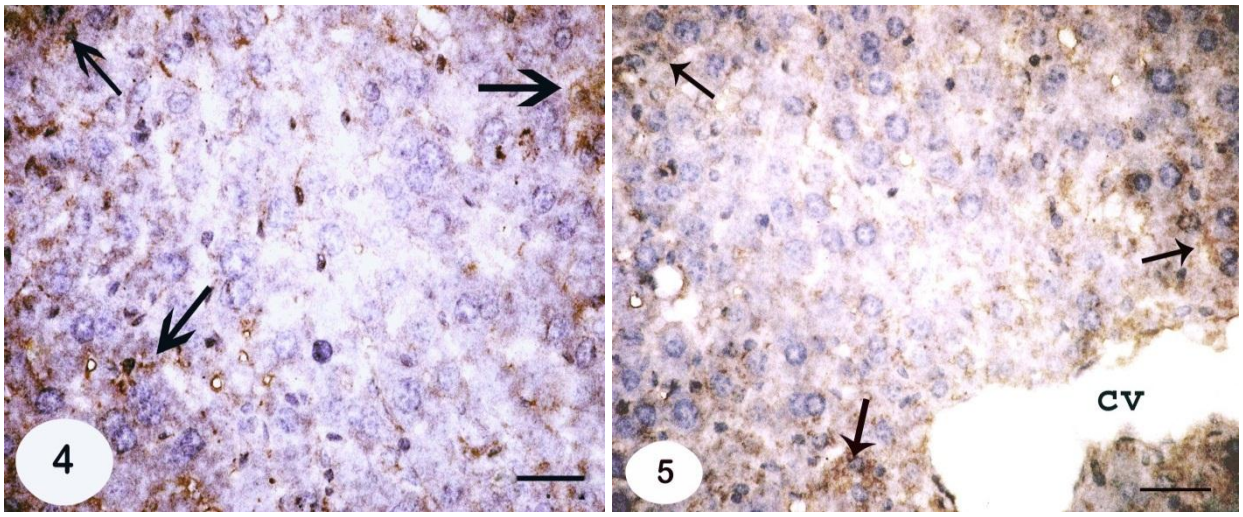


Fig.(4): Section of the cirrhotic liver of a mouse administered with MO extract at a dose 400mg/kg/bw/d for one month expressing moderate improvement and decrement of AFP immunostain to hepatocytes (arrows). AFP immunostain, Bar = 6.25 µm.

Fig.(5): Section of the cirrhotic liver of a mouse administered with a mixture of GT and MO extracts expressing AFP immunostain in a lot of hepatocytes (arrows). AFP immunostain, Bar = 6.25 µm.

b) <u>CD34 expression in the liver</u>:-

CD34 marker is a monoclonal antibody against CD34 protein that expressed in a vascular endothelial cells and progenitor stem cells. Sections of the liver of normal control mice (group I) expressed a strong positive CD34 immunostain in endothelial cells of the blood sinusoids and many hepatocytes as well as that observed in the control groups that given olive oil or either GT or MO (group II-IV) **(Fig.6).**

The cirrhotic liver sections of mice induced by CCl4 (group V) expressed no CD34 immunostain in many lobules of hepatic tissues and few hepatic lobules expressed weak CD34 immunostain in endothelia of blood sinusoids and hepatocytes **(Figs.7&8).** The administration of cirrhotic liver sections of mice with GT extract (group VI) or MO extract (group VII) or a mixture of both (group VIII) expressed strong CD34 immunostain in endothelial cells of blood sinusoids and appearance of intense brown oval cells **(Figs.9-13).**

In breif, GT extract is more effective to improve of both AFP and CD34 proteins of the cirrhotic liver of mice than MO or co-administration of both together.

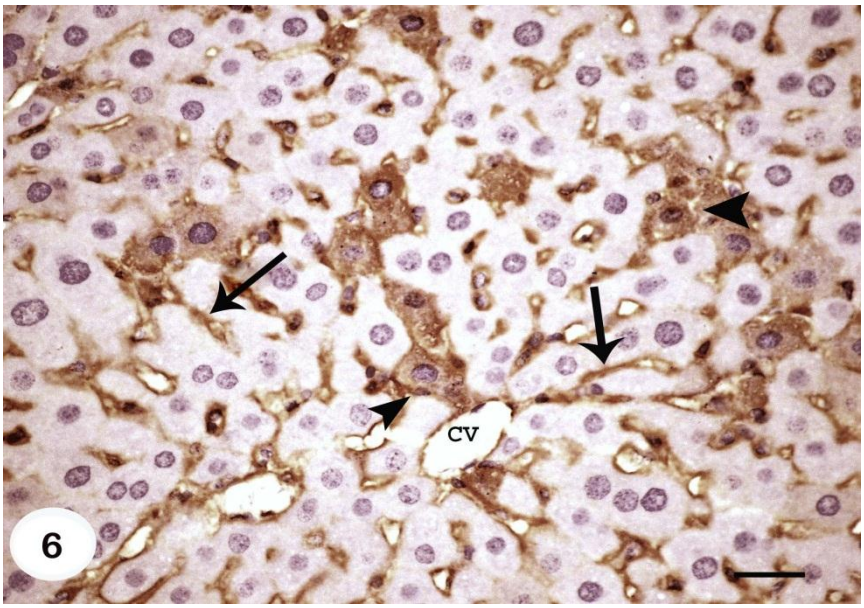
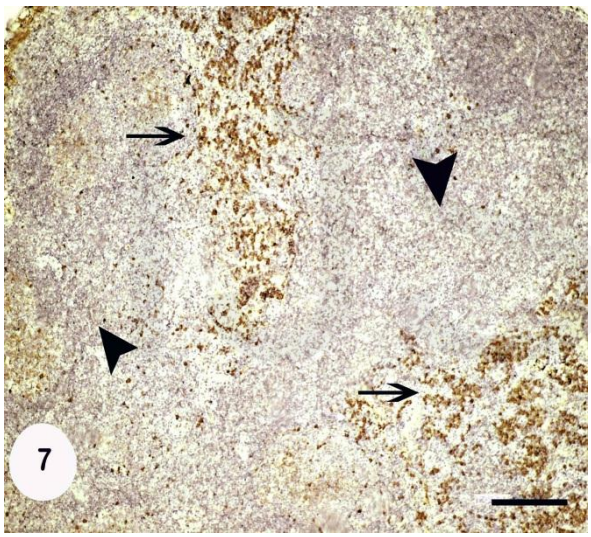
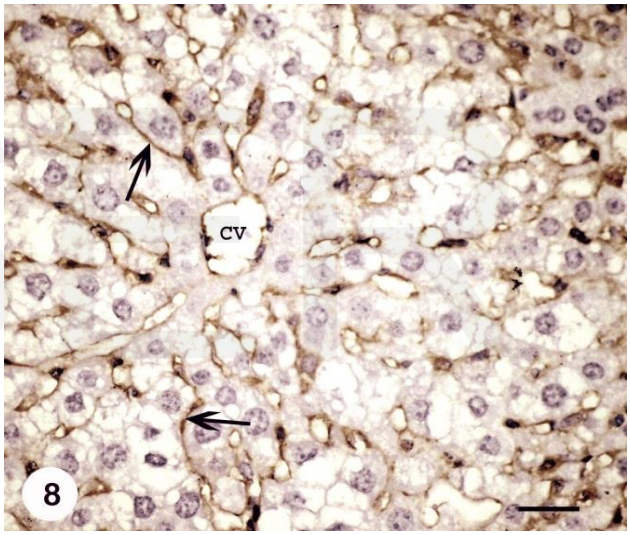
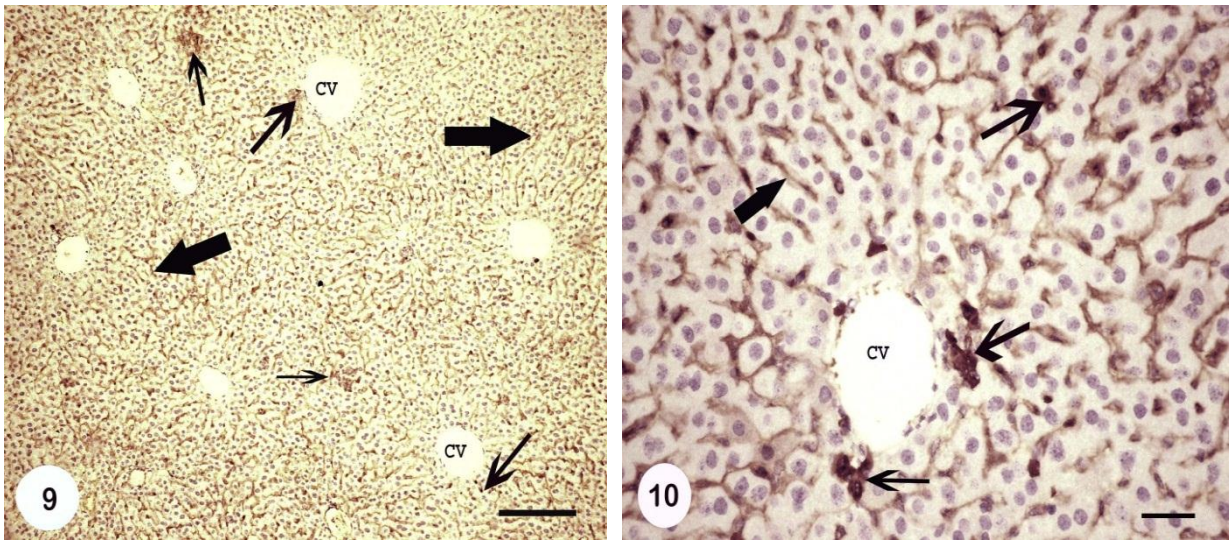


Fig.(6): Section of the liver of a normal control mouse demonstrating strong positive expression of CD34 immunostain in endothelial cells of the blood sinusoids (arrows) and many hepatocytes (arrow heads). CD34 immunostain, Bar=6.25µm.

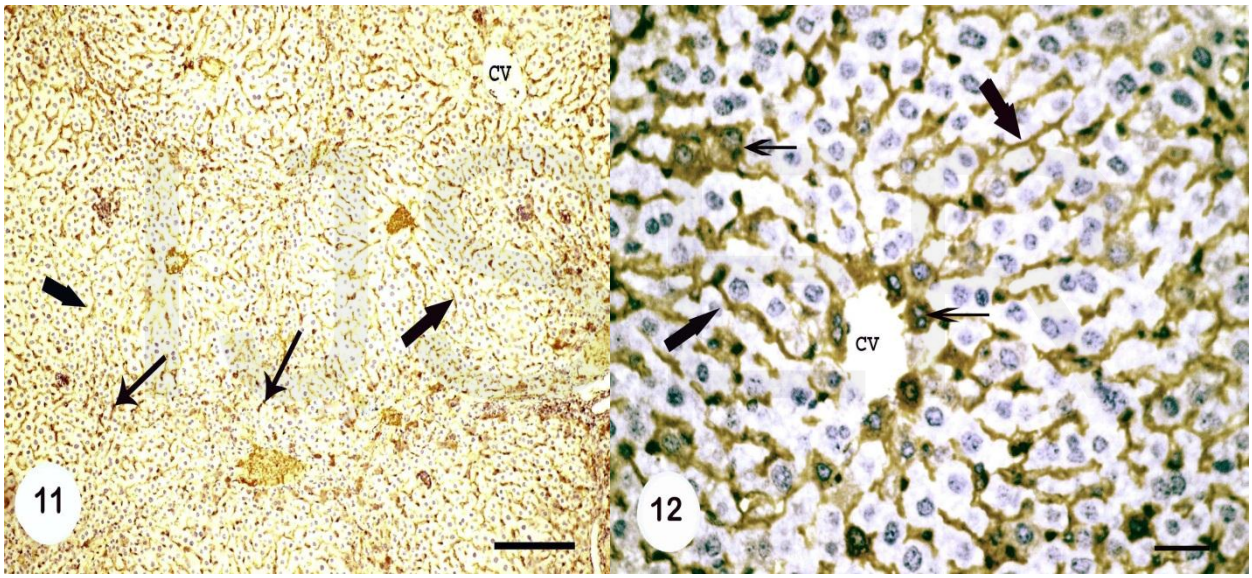




Figs.(7&8): Sections of the cirrhotic liver of mice injected with CCl4 at a dose 1ml/kg/bw twice a week for 6 weeks expressing; **Fig.7:** no CD34 immunostain in many lobules of the hepatic tissues (arrow heads), and few lobules have weak expression (arrows), Bar=25 µm. **Fig. 8:** a decrease of CD34 immunostain in endothelial cells of blood sinusoids (arrows) and no CD34 immunoreaction in any hepatocytes, Bar =6.25 µm, CD34 immnostain.



Figs.(9&10): Sections of the cirrhotic liver of mice administered with GT extract at a dose 600mg/kg/bw/d for one month expressing normal strong CD34 immunostain in endothelial cells of blood sinusoids (thick arrow) approximately similar to control and appearance a lot of intense brown oval cells (thin arrows). CD34 immnostain, Bar =25& 6.25 µm, respectively.



Figs.(11&12): Sections of the cirrhotic mice administered with MO extract at a dose 400mg/kg/bw/d for one month expressing the improvement of CD34 immunostain in endothelial cells of blood sinusoids (thick arrows) and appearance of moderate brown oval cells (thin arrows). Bar = 25&6.25 µm.

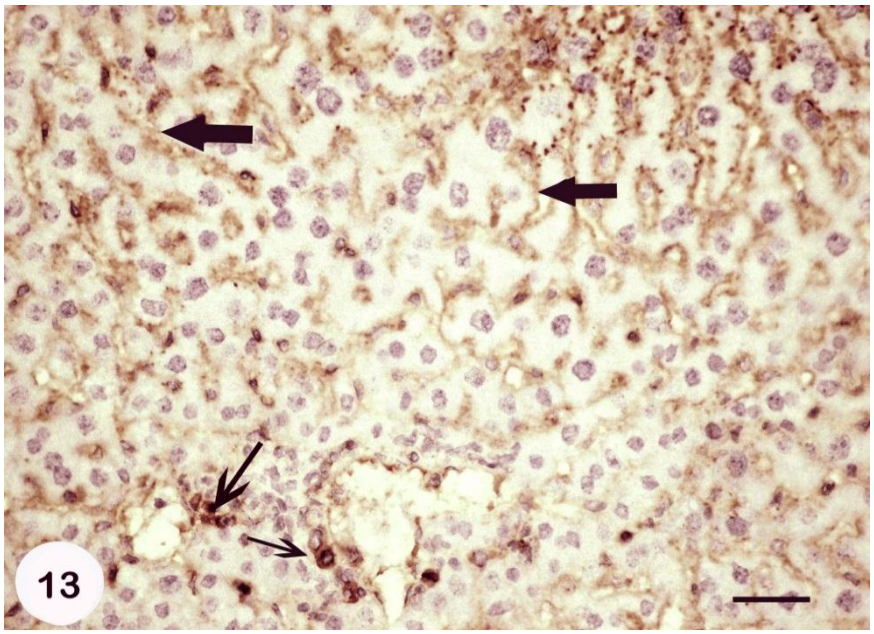


Fig.(13): Section of the cirrhotic liver of a mouse administrated with a mixture of GT and MO extracts demonstrating low expression of CD34 in the endothelia (thick arrows) and appearance of few brown oval cells (thin arrows). CD34 immnostain, Bar = 6.25 µm.

Discussion

The present results expressed intense AFP immunoreactivity in the hepatocytes of cirrhotic mice group that induced by CCl4 in comparable to control mice group which expressed weak AFP immunostain in the hepatic tissues. Cirrhotic mice administrated with GT extract were more effective than MO extract or a mixture of both to improve and decrease AFP immunostain expression in hepatic cells in comparable to cirrhotic mice.

CCl4 is one of the most commonly used to induce liver injury as fibrosis and cirrhosis in the experimental studies that associated with oxidative stress and free radicals due to the formation of trichloromethyl free radical (·CCl3) which was formed by the action of the mixed function of the cytochrome P450 oxygenase system. This free radical reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl3OO·). Both radicals are capable of binding to proteins or lipids, thus initiating tissue lipids peroxidation, inflammation and hepato-toxification **(19& 24).**

Arrieta *et al.* (29) illustrated the progressive elevation of AFP in patients with liver cirrhosis is useful for the diagnosis of hepatocellular carcinoma (HCC). By IHC, AFP immunoreaction was expressed surrounding cirrhotic tissues and increased in HCC tissues than cirrhotic tissues **(30). Kuwuahara *et al.* (31)** demonstrated that a fast and sudden elevation of AFP in patients with liver lesions detected more HCC when compared to other elevation patterns in patients with cirrhosis and hepatic lesions. **Sahin (32)** recorded the increment level of AFP in the carcinogenic animal confirmed the presence of HCC.

Moreover, the hepatotoxicity and degeneration in liver tissues of rats indicated by the elevation of AFP levels in serum **(33).** The serum AFP measurement may be useful as a sensitive marker for early detection of hepatocellular carcinoma **(34).** Furthermore, **Zhao *et al.* (35)** and **Tawfek *et al.* (36)** elucidated that anti-AFP confirmed that the liver tumors induced by CCl4 definitely originated from the hepatocytes. CCl4 stimulated the increase of serum AFP levels that has been reported in several diseases including HCC **(21).** AFP has a function to activate phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signal pathway to stimulate expression of

metastasis-related factor. A highly levels of AFP is associated with intrahepatic metastasis **(37).** AFP is the major serum protein in the developing of mammalian foetus produced at high levels by foetal liver and visceral endoderm of the yolk sac and at low levels by foetal gut and kidney **(38).**

The present result demonstrated the cirrhotic mice administrated with GT or MO extracts or a mixture of GT and MO extracts expressed improvements and decrement of AFP immunoreaction in the hepatic cells in comparable to cirrhotic mice. However, GT extract was more effective than MO extract or a mixture of both to improve AFP in cirrhotic mice.

The reduction of AFP serum level in liver cancer pateints treated with GT was recorded by many authors **(39& 40)**. AFP serum levels decreased after treatment with GT to hepatorenal toxicity in rat **(41). Sadek *et al*. (42)** illustrated the decrement of AFP level after administration of MO to HCC in rats because of preventing of tumor generation. AFP was decreased in response to treatment due to the low specificity of AFP for HCC **(43).**

GT contains polyphenols and catechins that have antioxidative and hepatoprotective effects. It is found that (45-90%) of GT are polyphenols **(8&44).** MO has (65.1-66.8%) phenolic compounds that act as antioxidant **(45).** Antioxidants prevent oxygen free radicals of CCl4 induced hepatocyte damage and prevent lipopolysaccharide-induced liver injury **(24)**, and prevent oxidative damage of a tissue indirectly by enhancing natural defenses of cell and/or directly by scavenging the free radical species **(46).** GT is more effective than MO may probably due to its components. However, the administration of high level of antioxidants than usual has a negative impact on health **(47).** Thus the administration of a mixture of GT+MO extracts to mice with liver cirrhosis inducer less curation may due to more antioxidant..

The present results demonstrated the normal strong positive expression of CD34 immunostain in endothelial cells of the blood sinusoids and many hepatocytes as well as that observed in the control groups that received GT or MO extracts separately or co-administrated together. Cirrhotic mice group showed no CD34 immunostain expression in great lobules of hepatic tissues, and few hepatic lobules expressed weak CD34 immunostain in endothelial cells and hepatocytes. While, the administration of cirrhotic mice with GT or MO extracts or a mixture of both recovery the expression of strong CD34 immunostain in endothelia and appearance of intense brown oval cells, but GT extract was more effective than MO alone or GT+MO extracts to improve CD34 protein of the cirrhotic liver of mice.

Similarly, **Tátrai *et al*. (48)** and **Ding *et al*. (49)** demonstrated a weakly staining of CD34 antibody in sinusoidal epithelium in HCC. Most HCC cells showed diffuse CD34 staining pattern in contrast to the negative expression pattern in surrounding non-tumor liver cells (hepatitis cells, cirrhosis cells, or normal liver cells) **(50&51).** The immobilized-stressed rats for 5 and 30 days expressed an obvious decrement of CD34 expression in the liver sections **(52).** CD34 immunostaining was aided in the correlation of the sinusoidal capillarization or neo-vascularization to dedifferentiation of the liver tissue during cirrhosis **(53).**

The herbal compound "*Diwu Yanggan*" increase the percentage of CD34/CD45 double positive cells in 2-acetyl amino fluorene/partial hepatectomy mice **(54).** CD34-positive stem cells have effective method to induce therapeutic angiogenesis in animal models of myocardial, peripheral, and cerebral ischemia, and in the treatment of heart and vascular disease in human being **(55).**

CD34 is a highly glycosylated sialomucin expressed on a variety of cells, ranging vascular endothelial cells to haematopoietic stem cells, deponding on its glycosylation state. CD34 has been shown to promote proliferation of the haematopoietic progenitor cells and lymphocyte adhesion to vascular endothelium via binding to L-

selectin. Also, CD34 is required for mucosal inflammatory disease development **(56&57).**

In conclusion, administration of either GT or MO extracts or co- mixture of them to mice with liver cirrhosis illustrated the recovery and improvements of hepatic AFP and CD34 but GT extract alone was more effective than MO extract alone or GT+MO to improve liver cirrhosis.

References

(1) **Bruha, R; Dvorak, K. and Petrtyl, J. (2012):** Alcoholic liver disease. W. J. Hepatol., 4(3): 81-90.

(2) **Gao, B. and Bataller, R. (2011):** Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology, 141(5): 1572-1585-1572.

(3) **Rowe, I. A. (2017):** Lessons from epidemiology: the burned of the liver disease. Dig. Dis., 35: 304-309.

(4) **Elgharably, A.; Gomaa, A. I.; Crossey, M. M. E.; Norsworthy, P. J.; Waked, I. and Taylor-Robinson, S. D. (2017):** Hepatitis C in Egypt – past, present, and future. Int. J. Gen. Med., 10: 1–6.

(5) **Franciscus, A. and Chief, E. I. (2015):** What is cirrhosis? HCSP, 6: 1-3.

(6) **Jayakeerthana, S. (2016):** Benefits of green tea: A review. J. Pharm. Sci. & Res., 8(10): 1184-1187.

(7) **Graham, H. N. (1992):** Green tea composition, consumption, and polyphenol chemistry. Prev. Med., 21: 334–350.

(8) **Naumovski, N.; Blades, B. L. and Roach, P. D. (2015):** Food inhibits the oral bioavail ability of the major green tea antioxidant epigallocatechin gallate in humans. Antioxidant, 4: 373-393.

(9) **Anwar, F. and Bhanger M. I. (2003):** Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. J. Agric. Food Chem., 51: 6558-6563.

(10) **Prabhu, K.; Murugan, K.; Nareshkumar, A.; Ramasubramanian, N. and Bragadeeswaran, S. (2011):** Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). Asian Pacif. J. Trop. Boimed., 1(2): 124-129.

(11) **Anwar, F.; Ashraf, M. and Bhanger, M. I. (2005):** Interprovenance variation in the composition of *Moringa oleifera* oil seeds from Pakistan. J. Am.Oil Chem. Soc., 82: 45-51.

(12) **Aslam, M.; Anwar, F.;Nadeem, R.; Rashid, U.; Kazi, T. G. and Nadeem, M. (2005):** Mineral composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan. Asian J. Plant Sci., 4: 417-421.

(13) **Anjorin, T. B.; Ikokoh, P. and Okolo, S. (2010):** Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria Int. J. Agric. Biol., 12: 431-434.

(14) **Gopalakrishnanb, L.; Doriyaa, K. and Kumara, S. D. (2016):** *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Sci. Human Wellness, 5(2): 49–56.

(15) **Mahajan, S. G. and Mehta, A. A. (2008):** Effect of *Moringa oleifera Lam*. Seed extract on ovalbumin-induced airway inflammation in guinea pigs. Inter. J. Toxicol., 20: 897-909.

(16) **Leone, A.; Spada, A.; Battezzati, A.; Schiraldi, A.; Aristil, J. and Bertoli, S. (2016):** *Moringa oleifera* seeds and oil: characteristics and uses for human health. Int. J. Mol. Sci., 17(12): 2141.

(17) **Saini, R. K.; Sivanesan, I. and Keum, Y. (2016):** Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. Biotechenology, 6(2): 203.

(18) **Arabshahi, D. S.; Devi, V. and Urooj, A. (2007):** Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. Food Chem., 100: 1100-1105.

(19) **El-bakry, K.; Toson, E.; Serag, M. and Aboser, M. (2016):**

Hepatoprotective effect of *Moringa oleifera* leaves extract against carbon tetrachloride induced liver damage in rats. W. J. Pharm. Pharmaceutical. Sci., 5(5): 76-89.

(20) Wong, G. L. H.; Chan, H. L.Y.; Tse, y.; Chan, H.; Tse, C.; Lo, A.O. S. and Wong, V. W.S. (2014): On-treatment alpha-fetoprotein is a specific tumor marker for hepatocellular carcinoma in patients with chronic hepatitis B receiving entecavir. Hepatology, 59(3): 986–995.

(21) Mizejewski, G. J. (2015): Alpha-fetoprotein (AFP) and inflammation: is AFP an acute and/or chronic phase reactant? Hematol. Thrombo. Dis., 3(1): 1-9.

(22) Nielsen, J. S. and Mcnagny, K. M. (2008): "Novel functions of the CD34 family". J. Cell Sci., 121 (22): 3683–3692.

(23) Sidney, L. E.; Branch, M. J.; Dunphy, S. E.; Dua, H. S. and Hopkinson, A. (2014): "Concise review: evidence for CD34 as a common marker for diverse progenitors". Stem Cells, 32 (6): 1380–1389.

(24) Sakaida, I.; Terai, S.; Yamamoto, N.; Aoyama, K.; Ishikawa, T.; Nishina, H. and Okita, K. (2004): Transplantation of bone marrow cells reduces CCl4 induced liver fibrosis in mice. Hepatology, 40: 131-1304.

(25) Thomas, J. and Thomas, G. (2013): Effect of catechin rich green tea (*Camellia sinensis*) extracts on obesity triggered hepatic steatosis in rats fed with HFCS. Int. J. Pharm. Bio. Sci., (4): 525 – 532.

(26) Sharifudin, S. A.; Hidayate, M. T.; Haidayate, M.T.; Hairuszah, I.; Moklas, M. A. and Arulselvan, P. (2013): Therapeutic potential of *Mornga oleifera* extracts against acetaminophen-induced hepatotoxicity n rats. Pharm. Biol., 51(3): 279-288.

(27) Hus, M.; Raine, L. and Fanger, H. (1981): Use of avidin- biotin peroxidase complex (ABC) in immunoperoxidase techniques. A comparision between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem., 29: 557-580.

(28) Arrieta, O.; Cacho, B.; Morales-Espinosa, D.; Ruelas-Villavicencio, A.; Flores-Estrada, D. and Hernández-Pedro, N. (2007): The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. BMC Cancer, 7(28): 1471-2407.

(29) Caruso, R. A. (1991): Hepatoid gastric adenocarcinoma. A histological and immunohistochemical study of a case. Eur. J. Basic Appl. Histochem., 35(2):203-209.

(30) Chen, X.; Yamamoto, M.; Fujii, K.; Nagahama, Y.; Ooshio, T.; Xin, B.; Yoko Okada, Y.; Furukawa, H. and Nishikawa, Y. (2015): Differential reactivation of fetal/neonatal genes in mouse liver tumors induced in cirrhotic and non-cirrhotic conditions. Cancer Sci., 106 (8): 972–981.

(31) Kuwuahara, T.; Sakai.; Majima, Y.; Hirai, K. and Tanikawa, K. (1993): Serial changes in serum alpha-fetoprotein prior to detection of hepatocellular carcinoma in liver cirrhosis. Hepatogastroenterology, 40: 347–351.

(32) Sahin, P. (2005): The effect of diethylnitrosamine on the levels of sialic acid, lipid-bound sialic acid and enzyme activities of transferase in rat serum. Turk. J. Vet. Anim. Sci., 29: 607-612.

(33) Sarhan, N. A. Z.; El-Denshary, E. S.; Hassan, N. S.; Abu-Salem, F. M. and Abdel-Wahhab, M. A. (2012): Isoflavones-enriched soy protein prevents ccl4-induced hepatotoxicity in rats. ISRN. Pharmacol., 10: 5402

(34) Malati, T. (2007): Tumor markers: an overview. Indian J. Clinic. Biochem., 22 (2): 17-31.

(35) Zhao, X.; Fu, J.; Xu, A.; Yu, L.; Zhu, J.; Dai, R.; Su, B.; Luo, T.; Li, N.;

Qin, W.; B Wang, B.; Jiang, J.; Li, S.; Chen, Y. and Wang, H. (2015): Gankyrin drives malignant transformation of chronic liver damage-mediated fibrosis via the Rac1/JNK pathway. Cell Death and Disease, 6: 1-10.

(36) Tawfek, N. S.; Al Azhary, D. B.; Abuel Hussien, B. K. and Abd El-geleel, D. M. (2015): Effects of *Cassia fistula* and *Ficus carica* leaf extracts on hepatocarcinogenesis in rats. Middle East J. Appl. Sci., 5(2): 462-479.

(37) Tolleshaug, H.; Kolset, S. O. and Berg, T. (1985): The influence of cellular ATP levels on receptor-mediated endocytosis and degradation of asialoglycoproteins in suspended hepatocytes. Biochem. Pharmacol., 34:1639-1645.

(38) Jain, S.; Gautam, V. and Naseem, S. (2011): Acute-phase proteins: As diagnostic tool. J. Pharm. Bioallied. Sci., 3(1): 118–127.

(39) Baba, y.; Sonoda, J.; Hayashi, S.; Tosuji, N.; Sonoda, S.; Makisumi, K. and Nakajo, K. (2012): Reduction of oxidative stress in liver cancer patients by oral green tea polyphenol tablets during hepatic arterial infusion chemotherapy. Exp. Ther. Med., 4(3): 452–458.

(40) Essam, R.; EL Sharkawi, F.; Shabon, M. and Khaled, H. (2016): The effect of high dose of green tea on therapeutic action of gamma radiation in liver carcinoma. Int. J. Adv. Res., 4(12), 254-262.

(41) Abozalam, S. B.; Salama, A. A.; Arbid, M. S.; Shoka, A. A. and Abd El-Latif, H. A. (2016): Effect of barley, green tea and doxorubicin against n-dimethylnitrosamine induced hepatorenal toxicity in rats. Curr. Sci. Int., 5(4): 386-399.

(42) Sadek, K. M.; Abouzed, T. K.; Abouelkhair, R. and Nasr, S. (2017): The chemo-prophylactic efficacy of an ethanol *Moringa oleifera* leaf extract against hepatocellular carcinoma in rats. J. Pharmaceutical Biol., 55(1): 1458-1466.

(43) Walhof, C. M.; Van Sonderen, L.; Voûte, P. A. and Delemarre, J. F. (1988): Half -life of alpha-fetoprotein in patients with a teratoma, endodermal sinus tumor or hepatoblastoma. Pediatr. Hematol. Onco., l 5: 217-227.

(44) Chacko, S. M.; Thambi, P. T.; Kuttan, R. and Nishigaki, I. (2010): Beneficial effects of green tea: A literature review. Chin. Med., 5: 13-18.

(45) Siddhuraju, P. and Becker, K. (2003): Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera Lam*) leaves. J. Agric. Food Chem., 51: 2144-2155.

(46) Verma, A. R.; Vijayakumar, M.; Mathela, C. S. and Rao, C. V. (2009): *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food Chem. Toxicol., 47: 2196–2201.

(47) Clee, B. (2013): Why those antioxidants could be causing you more harm than good. Health, 22:14.

(48) Tátrai, P.; Somoracz, A. and Batmunkh, E. (2009): Agrin and CD34 immunohistochemistry for the discrimination of benign versus malignant hepatocellular lesions. Am. J. Surg. Pathol., 33:874–885.

(49) Ding, T.; Xu, J. and Zhang, Y. (2011): Endothelium-coated tumor clusters are associated with poor prognosis and micrometastasis of hepatocellular carcinoma after resection. Cancer, 117: 4878–4889.

(50) Yao, S.; Zhang, J.; Chen, H.; Sheng, Y.; Zhang, X.; Liu, Z. and Zhang, C. (2013): Diagnostic value of immunohistochemical staining of GP73, GPC3, DCP, CD34, CD31, and reticulin staining in hepatocellular carcinoma. J. Histochem. Cytochem., 61(9): 639–648.

(51) Wang, X.; Ren, H.; Zhao, T.; Chen, J.; Sun, W.; Sun, Y.; Ma, W.; Wang, J.; Gao, C.; Gao, S.; Lang, M.; Jia, L.

and Hao, J. (2014): Stem cell factor is a novel independent prognostic biomarker for hepatocellular carcinoma after curative resection. Carcinogenesis, 35(10): 2283–2290.

(52) El–Desouki, N. I.; Gabry, M. S.; Nagi, H. M.; and Mohamed, I. H. (2017): Role of diazepam on stress- related changes in rat liver cytoskeletal Intermediate filaments and CD34 expression. RJPBCS, 8(1): 1614-1625.

(53) Gligorijević, J.; Djordjević, B.; Petrović, A.; Radirević A. and Stojanović, S. (2010):** Expression of CD34 in cirrhotic liver-reliance to dedifferentiation. Vojnosanit. Pregl., 67(6):459-462.

(54) Zhao, B.; Li, H.; Gao, X.; Ye, Z. and Cheng, S. (2015): The herbal compound "*Diwu Yanggan*" modulates liver regeneration by affecting the hepatic stem cell microenvironment in 2-acetylaminofluorene/partial hepatectomy rats. Evidence-Based Complementary Alter. Med.; 2015: 1-7 .

(55) Mackie, A. R. and Losordo, D. W. (2011): CD34-positive stem cells in the treatment of heart and vascular disease in human beings. Tex. Heart Inst. J., 38(5): 474-485.

(56) Gold, M.; Blanchet, M. R.; Samayawardhena, L. A.; Bennett, J.; Maltby, S.; Pallen, C. J. and McNagny, K. (2010): CD34 function in intracellular signaling and mucosal inflammatory disease development. Allergy Asthma Clin. Immunol., 6(3): 15.

(57) Scherberich, A.; Di Maggio, N. and McNagny, K. M. (2013): A familiar stranger: CD34 expressiosn and putative functions in SVF cells of adipose tissue. W. J. Stem Cells, 5(1): 1-8.