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Chemopreventive Effect of *Momordica charantia* Extract Against Chemically-Induced Hepatocellular Carcinoma in Experimental Animals.

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ABSTRACT

Hepatocellular carcinoma (HCC) is still a complicated health problem worldwide. It represents the third reason for tumor associated deaths and the fifth most frequent cancer with an increasing of mortality rate. Therefore, searching for a chemopreventive agent is an important approach for HCC management. This study was designed to inspect the chemopreventive potential of *Momordica charantia* (MC) extract(s) on diethylnitrosamine (DENA)-induced hepatocarcinogenicity in albino western rats. This target was undertaken through preparing different extracts (aqueous, methanolic, ethanolic and chloroformic) of MC and screening their effect on hepatocarcinoma HepG2 cell line then the most active extract was used for combating liver cancer in animal model. At the beginning, the different extracts were prepared and their effect against HepG2 cells was examined and from this preliminary experiment the methanolic extract was found to be the superior extract against HepG2 cells and throughout the study. The methanolic extract of *Momordica charantia* (MEMC) was used in the animals after determination its safe dose which represents 1/10 of lethal dose. Then the rats were divided into six groups (n=10): Control group; DENA group; MEMC group; three groups received MEMC extract after, simultaneous- and before-treated with DENA. At the end of the experiment, the body weight, liver weight and relative liver weight (RLW) were estimated. Biochemical parameters as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and alpha fetoprotein (AFP) were evaluated in serum along with histopathological investigations of liver tissues. The result revealed that in DENA-treated group, there was a decrease in the body weight and an increase in the liver weight and the RLW. Also, liver enzymes (AST, ALT, and ALP) activities and AFP level were significantly increased as compared to control group. However, treatment with MEMC ameliorated this decrease in body weight as well as alleviated the increase in liver weight, RLW, liver enzymes activities and AFP level in after-, simultaneous- and before-treated groups when compared with DENA-treated group. This improvement in biochemical results were also supported when morphological injury as nodule incidence and multiplicity, as well as histopathological impairment were minimalized in MEMC treated groups. The improvement in before-treated group was more pronounced than after- and simultaneous-treated groups which indicated that MEMC had a chemoprotective effect on rats bearing HCC. In conclusion it was demonstrated from the results that MEMC could be developed as a promising chemopreventive agent for liver cancer through repressing the tumor load, normalizing the biochemical parameters and improving the morphological and histological investigations, but the exact mechanism need to be interpreted in another promising elucidation.

Keywords: Hepatocellular carcinoma; Diethylnitrosamine; Chemoprevention; *Momordica Charantia*; Histological investigations.

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INTRODUCTION

Hepatocellular carcinoma represents the fifth frequent tumor and the third reason for cancer-related deaths worldwide [1]. The incidence rate of HCC is increased especially in developing countries, where East, South East, Asia and sub-Saharan Africa represent the highest HCC rates [2,3]. In Egypt, a doubling in the HCC incidence rate has been demonstrated in the past 10 years. This has been attributed to two kinds of risk factors that contribute in the etiology or progression of that disease. Firstly, environmental risk factors which include biological factors, e.g., hepatitis B and C virus infections, dietary pollution, e.g., aflatoxin B1 [4], drugs, medications [5], smoking [6], exposure to chemicals such as pesticides [7] and schistosomiasis [8].

In addition, nitrosamines are among these environmental risk factors which result from using pharmaceutical products, agricultural chemicals, tobacco products and cosmetics [9]. They are formed endogenously by ingestion of nitrate and nitrite compounds which are used for preservation as flavors and color fixers and by presence of nitrosatable precursors as primary amines in the stomach acidic condition [10]. About 1 mg/day of nitrosamines is intaken from food in humans [11]. As a genotoxic carcinogen, DENA is well desired inducer for liver cancer through oxidative stress which composes major pro-mutagenic alkyl DNA adducts that can impede base-pairing, stimulate chromosomal aberrations, generate micronuclei and sister chromatid exchanges and obstruct tumor suppressor gene p53, leading to hepatocarcinogenesis development [12]. Secondly, host-related risk factors involve obesity [13], diabetes mellitus [14], hemochromatosis [15], severe alpha-1 antitrypsin deficiency [16], nonalcoholic fatty liver disease [17], autoimmune hepatitis [18], and hereditary tyrosinemia [19].

Chemoprevention, by definition it is a strategy of cancer management in which the ability to prevent, retard or reverse its incidence strongly by administration of one or more non-toxic naturally occurring agent which is safer, multi-targeting, less expensive and immediately available and/or synthetic agent. Chemoprevention becomes an attractive and alternative approach for controlling malignancy [20]. Nature represents a rich source of medicinal plants that having valuable benefits in treatment of different diseases [21]. *Momordica Charantia* (MC) (bitter melon, bitter gourd, balsam pear or Karela) is one of these natural traditional medicinal plants with different biological activities. It is a member of the family *Cucurbitaceae* and widely cultivated in many tropical and subtropical areas such as Asia, Africa and South America. Fruits and seeds of MC contain charantin (a steroid glycoside) [22,23], which used as antimalarial, immunomodulatory, antipsoriatic, antiviral, anthelmintic and antimicrobic remedy [24]. The fruits of this plant is also contain mormordin, vitamin C, carotenoids, flavanoids, essential amino acids, vitamin A, folic acid and polyphenols [25,26]. The seeds of this plant contain two proteins, α and β momorcharin, where these proteins act as immunosuppressive agent without having any cytotoxic effect, and they also modulate the activity of both α and β lymphocytes [27].

Because there were no previous studies dealt with the using of MC extract as a chemoprevention for HCC, the above ideas had prompted this investigation to search for a potent and cost-effective treatment of liver cancer. Therefore, this study was aimed to execute an inquiry about the protective and therapeutic effects of MC extract(s) on diethylnitrosamine (DENA)-induced hepatocarcinogenicity in experimental animals, via preparing different extracts (aqueous, methanolic, ethanolic and chloroformic extracts) of MC as a natural substance and screening their effect on HepG2 cell line and on liver cancer model.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used in this study were of the highest purity and analytical grade, and purchased from Sigma-Aldrich chemical Co. (Deisenhofer, Germany).

Plant materials and extracts preparation

Fruits of MC were purchased from the local market in the Kingdom of Saudi Arabia. Green, unriped fresh whole fruits of MC were dried, powdered and the powder was used for the extraction into different solvents such as water, methanol, ethanol and chloroform in a ratio of 1:10. The mixture was carried out at 50°C in the dark for 1 h with stirring at regular intervals. The extract was then filtered and evaporated to

dryness under reduced pressure using a rotary evaporator to produce the yield, which was 90, 74, 10, 56 mg of powder per 1g of dried whole fruit for water, methanol, ethanol, and chloroform respectively. The extract was then kept at -80°C until use [28].

***In vitro* cytotoxicity**

The effect of aqueous, methanolic, ethanolic and chloroformic extracts from fruits of MC against liver cancer HepG2 cell line was evaluated by using SRB assay according to Skehan et al. [29]. Briefly, HepG2 cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h. Tested extracts and doxorubicin (as standard reference drug) were dissolved in DMSO at 1 mg/ml and diluted to the appropriate volumes then added to the cells (6 wells were prepared for each individual dose). Cells were incubated for 48 h. at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by washing with 1% acetic acid. Color intensity was measured in an ELISA reader at 564 nm. The concentration required for 50% inhibition of cells viability (IC_{50}) was calculated.

Determination of median lethal dose LD_{50} of the methanolic extract

The methanolic extract of *Momordica charantia*, MEMC (the most active extract against HepG2 cells) was tested on control rats to determine the LD_{50} using the method described by Wilbrandt [30]. Briefly, 60 fasted adult male albino western rats were randomly divided into six groups of 10 per group. Each group was separately administrated once daily for a period of 4 weeks with doses ranging from 0-1000 mg/kg b.w. by oral gavaging in a value of 1 ml/kg body weight. The fasted animals were then provided with food and water after the administration. The mortality of the animals was observed up to one month post-treatment. The LD_{50} of the MEMC was calculated and 1/10 of the LD_{50} will be used throughout the study as a safe dose.

Animals

The present study was conducted on 60 adult male albino western rats (average body weight of 130 ± 20 g) were obtained and bred in the Animal House Colony of National Research Centre, Dokki, Giza, Egypt. Animals were allowed 7 days for acclimatization at 24ºC with 12 h light – dark cycle. They were provided with commercial standard diets and tap water *ad libitum*. All experiments inclusive of animal handling and sacrificing were conducted strictly in conformation with standard guidelines of institutional ethics committee for animal's care at the National Research Centre, Egypt.

Experimental design

After acclimatization for 1 week on based diet, the experimental animals were weighed and randomly divided into 6 groups, with 10 animals in each group for a study period of 16 weeks. Control group in which rats were given the vehicle only. DENA-treated group in which HCC was induced in rats by intraperitoneal (i.p.) injection of DENA in saline at a dose of 200 mg/kg body weight once, 2 weeks later rats were received CCl_4 (as a promoter for carcinogenesis) subcutaneously (3 ml/kg/week) continued for 10 weeks and then the animals were given the vehicle till the end of the experiment [31]. The methanolic extract of *Momordica charantia* (MEMC)-treated group in which rats were treated daily by oral gavage with 40 mg/kg body weight (1/10 of the LD_{50}) for 30 days, then the animals were given the vehicle till the end of the experiment. After-treated group in which rats were treated with the MEMC after DENA injection. Simultaneous-treated group in which rats were treated with the extract starting with the first injection of DENA and then the animals were given the vehicle till the end of the experiment. Before-treated group in which rats were pretreated with the extract before the administration of DENA and then the animals were given the vehicle till the end of the experiment.

At the end of the experiment (16 weeks) the animals were weighed, anesthetized, blood was collected from the retro-orbital plexus of all studied groups, allowed for clotting at room temperature, then sera were separated by centrifugation at 3000 rpm for 15 minutes and stored in aliquots at -20ºC to be used for biochemical analysis. Animals were dissected then the liver lobes were excised, photographed, their weights were recorded, relative liver weight (RLW) was calculated and other sections of them were used for the histopathological examination.

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Biochemical analysis

The liver enzymes (ALT, AST and ALP) activities were determined spectro- photometrically according to the manufacturer's instructions, using reagent kits obtained from Biomerieux (France). In addition, AFP concentration was determined as a tumor marker using ELISA kit obtained from Immunospec Corporation, USA.

Histopathological investigations

All biopsies were fixed in formalin, embedded in paraffin, and sectioned by microtome with a thickness of 5µm. Routine specimen processing involved staining slides with hematoxylin and eosin (H&E) or Masson trichrome in some liver sections.

RESULTS

The cytotoxicity effect of the MC extracts against HepG2 cells revealed that the IC_{50} value of the four extracts (aqueous, methanolic, ethanolic and chloroformic) were 24.00, 4.10, 29.25 and 21.50 µg/ml respectively as shown in figure 1. Moreover, the data revealed that the methanolic extract was the most potent anticancer agent with IC_{50} (4.10 µg/ml) near to the reference drug, doxorubicin (IC_{50}: 3.73 µg/ml).

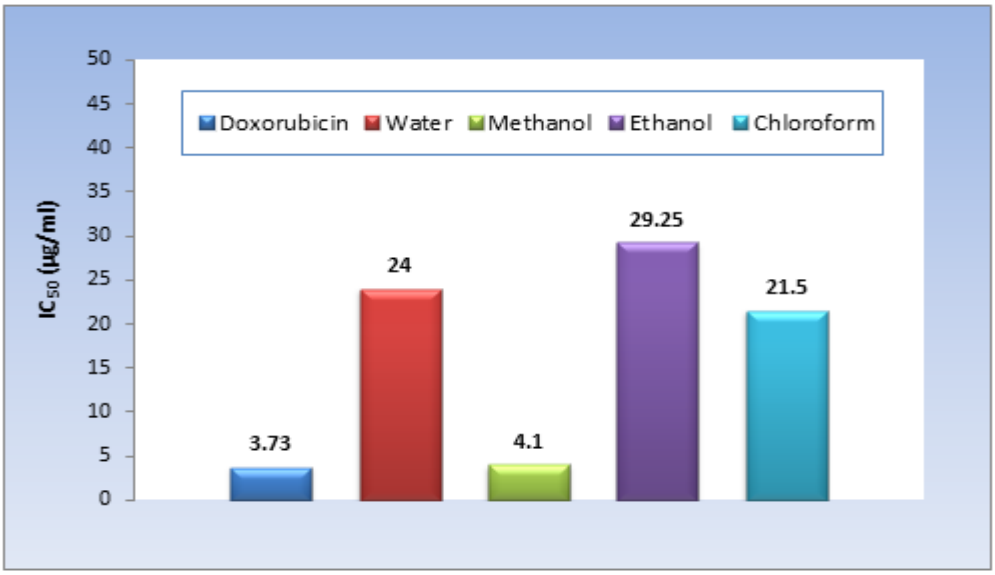

Figure 1: The cytotoxic effect of doxorubicin and different extracts (water, methanol, ethanol and chloroform) on HepG2 cells

The initial and final body weights, as well as liver weight and RLW of rats were illustrated in figures 2 - 4. The results showed an obvious decrease between final and initial body weight by 13% in DENA-treated group. Treatment with MEMC ameliorated body weight where it increased in after-, simultaneous- and before-treated groups by 9%, 7% and 11% respectively. The amelioration in before-treated group is more noticed than after and simultaneous-treated group (Figure 2). While the liver weight significantly ($P<0.0001$) increased in DENA-treated group as compared to control group. Treatment with MEMC showed a significant decrease of liver weight by 26%, 16% and 28% respectively in after-, simultaneous- and before-treated groups as compared to DENA-treated group (Figure 3). In addition the RLW showed a significant ($P<0.0001$) increase in DENA-treated group as compared to control group. Treatment with MEMC showed a significant ($P<0.0001$) decrease of RLW by 31%, 22% and 35% respectively in after-, simultaneous- and before-treated groups as compared to DENA-treated group (Figure 4).

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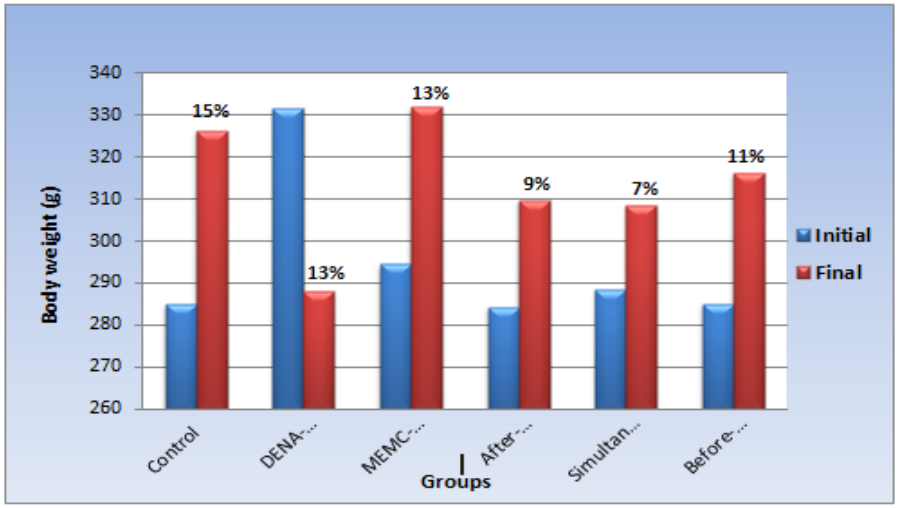

Figure 2: The percent of change in body weight (g) between initial and final body weights in different study groups

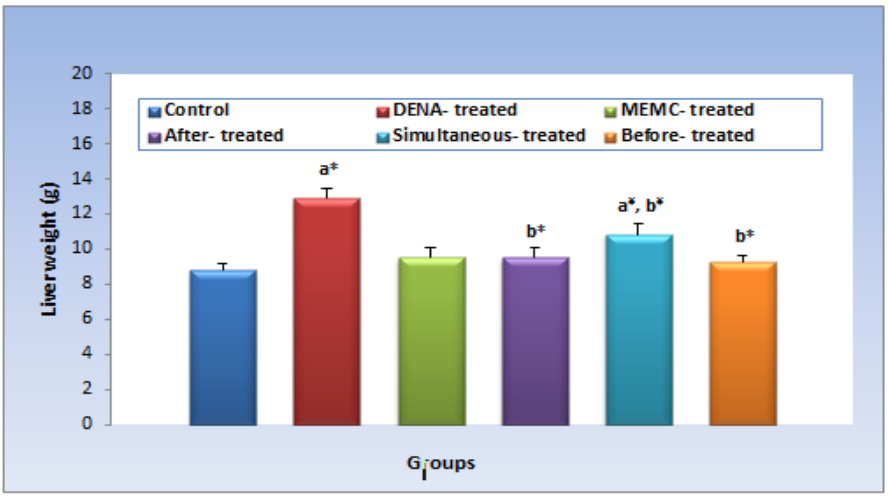

Figure 3: The effect of MEMC on liver weight (g). Data are expressed as mean values ± SE. a and b are significant differences from control and DENA-treated groups respectively at * p<0.0001, ¥ p<0.05

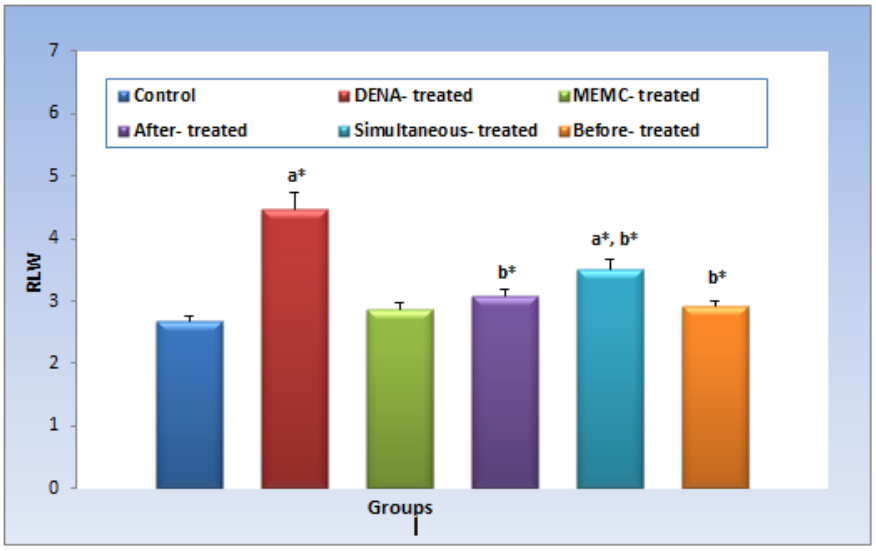

Figure 4: The effect of MEMC on relative liver weight (RLW). Data are expressed as mean values ± SE. a and b are significant differences from control and DENA-treated groups respectively at * p < 0.0001

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The effect of MEMC on the activities of liver enzymes were illustrated in table 1. The current study showed a highly significant ($p<0.0001$) elevation in the activities of serum AST, ALT and ALP of DENA-treated group as compared to the control group. Otherwise, there was a significant ($p<0.0001$) decrease in the activities of these enzymes in after-, simultaneous-, and before-treated groups as compared to DENA-treated group. Administration of MEMC alone has no marked impact on the previous enzymes when compared to the control group.

Table 1: The serum activities of liver enzymes in different studied groups

Groups	AST (U/ml)	ALT (U/ml)	ALP (U/L)
Control	58.17 ± 3.49	19.43 ± 1.11	136.09 ± 9.46
DENA-treated	106.33 ± 4.90^{a*}	114.67 ± 11.68^{a*}	361.46 ± 15.70^{a*}
MEMC-treated	60.20 ± 2.58	21.00 ± 1.14	145.39 ± 14.48
After-treated	69.86 ± 5.07$^{a¥,b*}$	27.86 ± 2.30$^{a¥,b*}$	210.26 ± 11.87$^{a¥,b*}$
Simultaneous-treated	77.33 ± 4.03$^{a¥,b*}$	30.43 ± 2.38$^{a¥,b*}$	243.16 ± 16.23^{a*,b*}
Before-treated	60.29 ± 2.29^{b*}	21.88 ± 1.95^{b*}	150.53 ± 11.70^{b*}

Data are expressed as mean values ± SE. a and b are significant differences from control and DENA-treated groups respectively at $^{*}p < 0.0001$, $^{¥}p < 0.01$ and $^{¥}p < 0.05$.

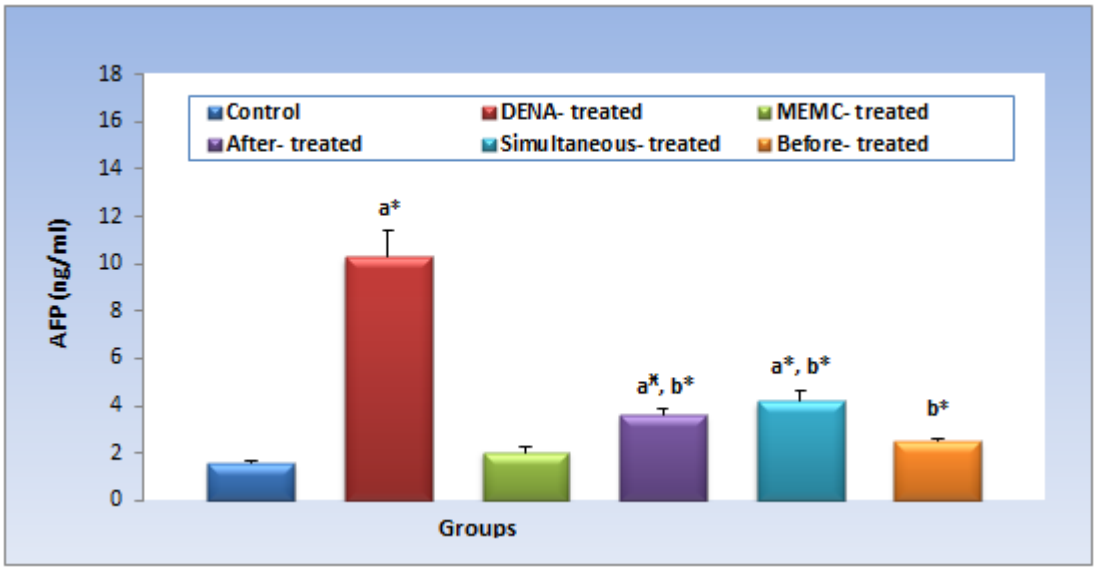

Figure 5: The effect of MEMC on serum AFP concentration (ng/ml) in different groups. Data are expressed as mean values ± SE. a and b are significant differences from control and DENA-treated groups respectively at $^{*}p < 0.001$, $^{¥}p < 0.01$

As shown in figure 5**,** while AFP concentration was significantly ($P<0.0001$) higher in the DENA-treated group (7-fold) as compared to control group, the AFP levels were significantly ($P<0.0001$) reduced in after-, simultaneous-, and before-treated groups with approximately 65%, 59% and 76% reduction respectively as compared with DENA-treated group. It is noteworthy that rats treated with MEMC alone showed non-significant changes in serum AFP level as compared to control group.

The morphological examination of rat liver tissues **that were taken** from different studied experimental groups was illustrated in figure 6. The figure showed that there were no morphological changes appeared in both normal control group (Figure 6A) and MEMC-treated group (Figure 6B) with the absence of any nodules. While the liver of DENA-treated group (Figure 6C) was showed enlargement in size with large number of hepatic nodules which could be seen by naked eyes, where, they were scattered on the peripheral surface of the liver. The administration of MEMC improved the morphology of liver with a reduction in the number of nodules in before-, after- and simultaneous-treated groups as compared to DENA-treated group as shown in Figures 6D, 6E and 6F, respectively. The most improvement was noticed in before-treated group (Figure 6D), where most nodules disappeared and nearly the liver had a normal morphology, while after-treated group had rare tiny nodules lower than those in the liver of simultaneous-treated group.

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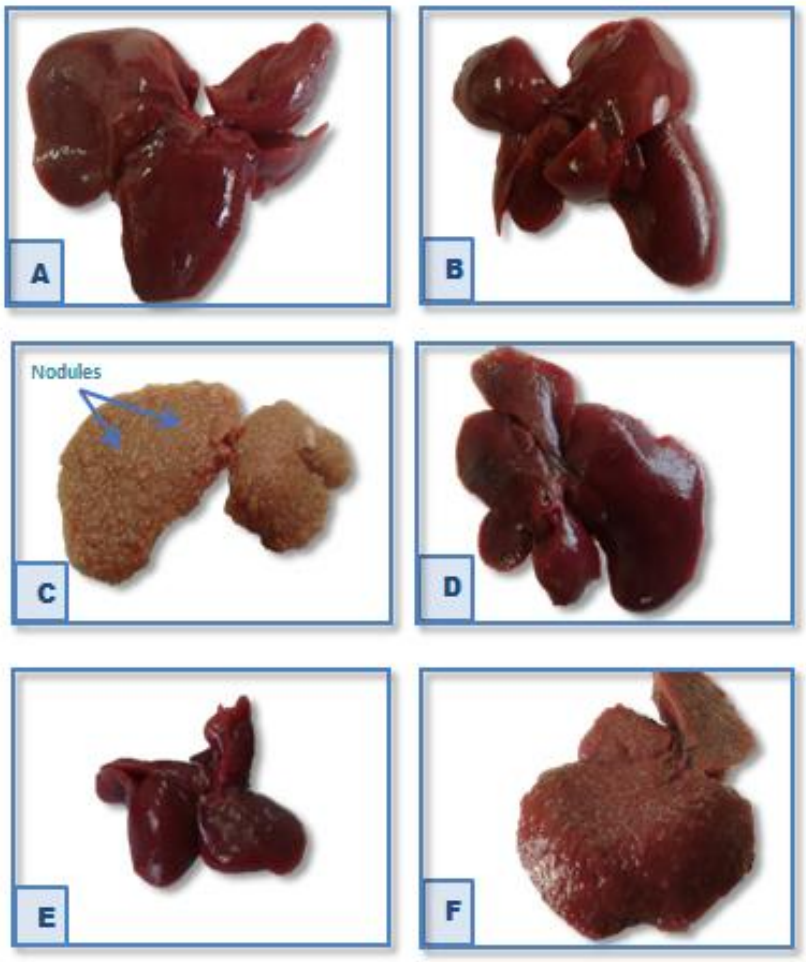

Figure 6: Macroscopic appearance of rat liver tissues from different studied groups, where: (A) Control group, (B) MEMC-treated group, (C) DENA-treated group, (D) Before-treated group, (E) After-treated group, (F) Simultaneous-treated group

Histopathological investigations of liver tissues in different studied groups were illustrated in figure 7. It was found that liver sections of control group showed normal cellular architecture with small uniform nuclei and granulated cytoplasm (Figure 7A). MEMC-treated group showed normal liver architecture where hepatic cells appeared as those of control group (Figure 7B). Some liver sections from DENA-treated group showed well differentiated HCC grade I, where different changes were appeared as loss of hepatic lobular architecture, high nuclear/cytoplasmic (N/C) ratio, nuclear hyperchromasia, multinucleated hepatocytes, increased thickness of liver cell plate and occasional formation of glandular structures liver (Figure 7C). Other sections from DENA-treated group showed a cirrhotic nodule with partially lost hepatic lobular architecture where the hepatocytes are surrounded by dense fibrous septa with many hepatocytes showed ballooning degeneration (Figure 7D). Treatment with MEMC extract showed improvement in the hepatic pattern. Liver sections from rats treated with MEMC before DENA showed mild parenchymal inflammation, mild vascular congestion, microvesicular and macrovesicular steatosis, as well as restoring of hepatic architecture (Figure 7E and 7F). Liver section from After-treated group showing vascular congestion and drop out cells (Figure 7G). While, simultaneous-treated group showing partially lost hepatic lobular architecture with bridging fibrosis, but no dysplasia or malignancy was detected (Figure 7H).

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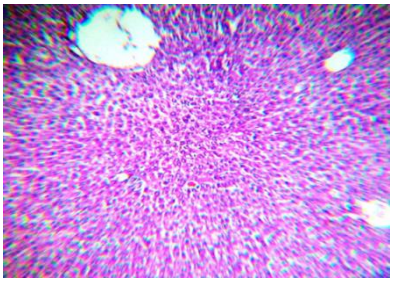
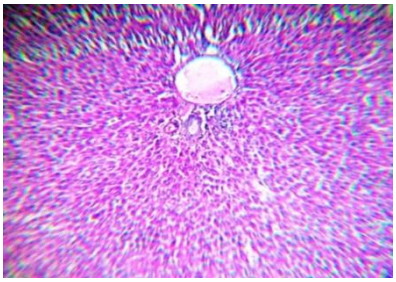
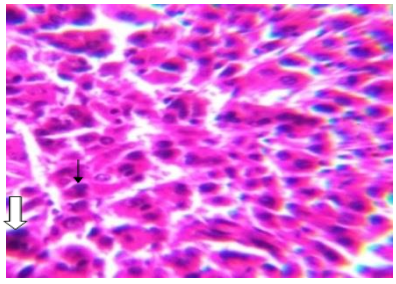


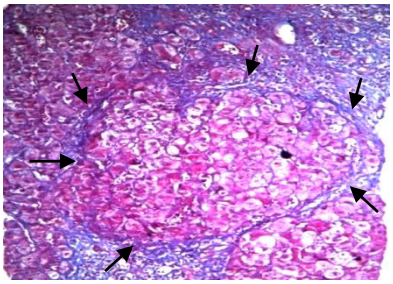
(**A**) Liver section from control group (H&E, X100).



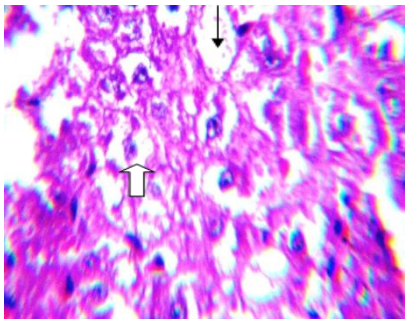
(B) Liver section from MEMC-treated group (H&E, X400).



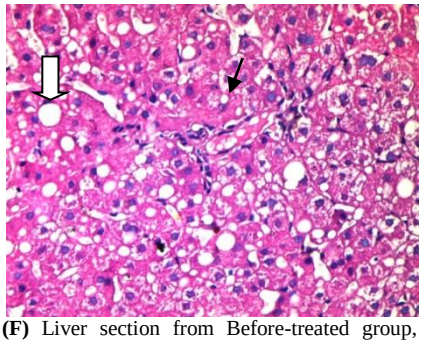
(C) Liver section from DENA-treated group, thin arrow refers to hyperchromasia and thick arrow refers to multi-nucleated hepatocytes (H&E, X100).



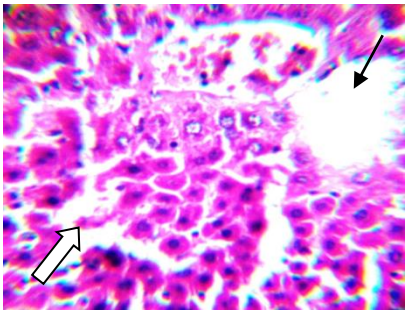
(D) Liver section from DENA-treated group**,** arrows refer to dense fibrous septa (Masson trichrome, X100).



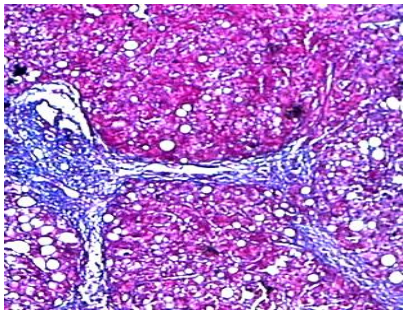
(E) Liver section from Before-treated group, thin arrow refers to mild vascular congestion and thick arrow refers to ballooning degeneration of the hepatocytes (H&E, X400).



(F) Liver section from Before-treated group, thin arrow refers to microvesicular steatosis and thick arrow refers to macrovesicular steatosis (H&E, X100).



(**G**) Liver section from After-treated group, thin arrow refers to vascular congestion and thick arrow refers to drop out cells (H&E, X200).



(H) Liver section from Simultaneous-treated group (Masson trichrome, X100).

Figure 7: Histopathological investigation of rat liver tissues from different studied groups

DISCUSSION

HCC is one of the most common malignancies worldwide with an increasing in incidence rate around the world [32]. Although there are different therapeutic opportunities for HCC in its early stages, which including liver resection, transplantation, chemotherapy and radiotherapy, there are a special need to

innovate or develop new strategies for cancer treatment [33,34]. *Momordica charantia* fruits contain many beneficial substances that have different biological activities. It has anti-inflammatory, anti-diabetic, antimicrobial and anti-tumor activities [35].

The principal aim in this work is to prepare different extracts (aqueous, methanolic, ethanolic and chloroformic) of MC as a natural substance and screening their effect *in vitro* on hepatocarcinoma cell line and to combat tumorigenesis *in vivo* liver cancer model. The potential cytotoxicity of aqueous, methanolic, ethanolic and chloroformic extracts from fruits of MC against liver cancer HepG2 cell line was evaluated. From the results it was clear that the methanolic extract of MC fruits had the highest cytotoxic activity (IC_{50}: 4.10 µg/ml) than aqueous, ethanolic and chloroformic extracts, which was near to the cytotoxic activity of the standard drug doxorubicin (IC_{50}: 3.73 µg/ml). In the *in vivo* study, liver cancer was induced in rats by treatment with DENA (as genotoxic carcinogen) and CCl_4 (as a promoter for carcinogenesis). The MEMC extract was tested against liver cancer when treated after-, simultaneous- and before-DENA.

The results of the study seem to provide support for the chemopreventive potential of MEMC against DENA- induced hepatocarcinogenesis in rats. In DENA-treated group there was a reduction in the body weight which is an indicator for low appetite and minimal food intake that could be related indirectly to the declining of the hepatic function. In addition, the increase in the liver weight could be occurred due to the nodules and tumors formation in the liver following carcinogen exposure [36]. MEMC administration showed its anticancer effectiveness by increasing appetite and reducing tumor incidence, this was obviously appeared in MEMC-treated groups which showed an increase in the body weight and a decrease in the liver weight when compared to DENA-treated group. Relative liver weight (RLW) which is the outcome of liver weight over final body weight is a principal parameter in appreciating the pathological condition of the liver [37]. Therefore, in our results RLW was significantly increased in DENA-treated group when compared to control group referred the liver tumorigenesis induced by that carcinogen, besides the significant reducing of the rats RLW by MEMC administration which was a marker for the pathological amelioration of the liver.

Hepatic damage caused by DENA- induced hepatocarcinogenesis reflects instability of liver cells metabolism which leads to distinctive changes in the hepatic enzymes activities. A significant elevation ($p< 0.0001$) in AST, ALT and ALP enzymes activities in DENA-treated group as compared to control group indicating that DENA could induce a damaged effect on liver tissues. The elevation in enzymes activities is due to the rupture in the architecture of cell membrane and the leakage and liberation of enzymes into the serum as a result of carcinogenesis, necrosis and toxicity [38,39]. The overproduction of these enzymes in tumor cells may cause increased permeability of the cell membrane which leads to such drastic rise of enzymes in serum [40]. DENA induced elevation in AST and ALT activities in serum [41]. ALP elevation in serum is correlated with any pathological interference with the flow of bile and discharge of total bilirubin (TBL), indicating non-specific change in the plasma membrane permeability or integrity [42,43]. On the other hand, treatment of rats with MEMC resulted in a significant decrease ($p<0.0001$) in the activities of liver enzymes as compared to DENA-treated group, where the most improvement effect was shown in before-treated group. These results suggested that MEMC may have potential protective and therapeutic effects against DENA-induced liver damage beside the stabilization of both plasma membrane and hepatobiliary dysfunction of rat liver. There was no effect on liver enzymes activities between normal and MEMC-treated groups, indicating the nontoxic effect of MEMC administration.

It was reported that hepatocarcinogens as DENA caused an elevation in AFP level (which is widely used as tumor marker for diagnosis of HCC) associated with the increment in tumor growth and progression [44]. The immature liver cells in fetus normally made this unique immunomodulatory glycoprotein (65 kDa) [45], but the reinitiating of AFP synthesis by neoplastic hepatocytes due to the enhancement of transcription or posttranslational modification of AFP gene indicates for HCC diagnosis [46]. In consist with this finding the result of the present study revealed that, AFP level was significantly increased ($P<0.0001$) in the serum of DENA-treated group (7- folds) as compared to control group. Otherwise, the level of AFP was significantly decreased ($P<0.0001$) in after-, simultaneous- and before-treated group as compared to DENA-treated group, indicating the antitumor effect of MEMC. The highest amelioration effect was occurred in before-treated group than both after- and simultaneous-treated groups. The obvious decrease in the AFP level can be explained by the fact that MC containing many phenolic antioxidants [47] which can inhibit NF-κB activity that is known to modulate the expression of COX-2 gene [48,49], that is thought to downregulate the AFP synthesis

[50]. It is obviously that no remarked changes on AFP level between normal and MEMC-treated groups indicating the safe use of MEMC.

In addition, in the present study the biochemical results were confirmed by the morphological and histopathological investigations as morphological examination of different experimental studied group demonstrated that both control and MEMC-treated groups showed normal liver morphology, while DENA-treated group showed increased size of liver with large number of nodules on the outer surface of liver. Treatment with MEMC improved the morphological changes where the number of nodules on the liver surface was reduced as compared to DENA-treated group, which indicated the antitumor effect of MEMC on rats. The most improvement was noticed in before-treated group, where it had a normal morphology compared to after- and simultaneous-treated groups.

These morphological findings were in agreement with the histopathological examination where, liver sections of control group showed normal cellular architecture with small uniform nuclei and granulated cytoplasm. MEMC-treated group showed normal liver architecture where hepatic cells appeared as control group, indicating the non-toxic nature of MEMC. Sections of DENA-treated group showed loss of hepatic lobular architecture, enlarged nuclei with high N/C ratio where neoplastic cells were smaller than normal cells with granular cytoplasm, nuclear hyperchromasia, multi-nucleated hepatocytes, increased thickness of liver cell plate and occasional formation of glandular structures. In addition, some liver sections from DENA-treated group showed a cirrhotic nodule with partially lost hepatic lobular architecture where the hepatocytes are surrounded by dense fibrous septa with many hepatocytes showed ballooning degeneration. It was mentioned that DENA and CCl_4 administration can induce different proliferative and neoplastic lesions in rat liver [51].

On other hand, treatment with MEMC improved the carcinogenic changes in after-, simultaneous- and before-treated groups. The improvement in before-treated group was more effective than that in after- and simultaneous-treated groups, where, before-treated group showed mild parenchymal inflammation with mild vascular congestion, microvesicular and macrovesicular steatosis, as well as restoring of normal architecture. The after-treated group showed minor changes in the form of vascular congestion and drop out cells. While, simultaneous-treated group showed partially lost hepatic lobular architecture with bridging fibrosis, but no dysplasia or malignancy was detected.

CONCLUSION

From the present study, it is concluded that the methanolic extract of *Momordica charantia* (MEMC) could be considered as promising chemopreventive agent endowed with cytotoxic action against liver cancer and with no toxic effect on normal cells. This cytotoxic action appeared via repressing the tumorigenesis incidence, restoring the biochemical parameters and improving the morphological through preventing the multiplicity of neoplastic nodules, as well as histological investigations. In addition, it had a protective effect rather than therapeutic effect against DENA-induced hepatocarcinogenicity in rats. Future elucidation is required to interpret the detailed mechanism of action which may result in identification of potent molecules from MEMC.

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