Sroor *et al. Applied Biological Chemistry* (2022) 65:68
https://doi.org/10.1186/s13765-022-00737-3

KSABC
The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

Biochemical and histopathological studies of sulfonylurea derivative as a new chemotherapeutic agent against liver cancer in free- and nano-coated forms

Farid M. Sroor[1*], Wahid M. Basyouni[1], Hanan F. Aly[2], Eman A. Younis[2], Karima F. Mahrous[3] and Ahmed A. Haroun[4]

Abstract

The most frequent type of primary liver cancer is hepatocellular carcinoma (HCC), accounting for approximately 90% of primary liver cancers and a third leading cause of cancer deaths. In the current study, the synthesized compound **3** was re-formulated using tetraethyl orthosilicate (TEOS) with weight ratio (1:1) via sol-gel technique. The prepared material has been examined using Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray elemental analysis (EDX), and scanning and transmission electron microscopes (SEM and TEM). Herein, we investigate the mode of action of **3** as potent anti-liver cancer in vivo as normal and nano-forms. Rats were given a single dosage of 50 mg/kg b.wt. of HCC through an intraperitoneal injection (ip). A single dosage of CCl4 (2 ml/kg IP) was also given to rats 2 weeks later. Several liver, tumor and oxidative stress biomarkers were detected including liver enzymes; alanine and aspartate aminotransferases (ALT and AST), alkaline phosphatse (ALP), gamma glutamyl transferase (GGT), glutathione (GSH), lipid peroxide (MDA), catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), α-fetoprotein and α-L-Fucosidase. Hepatic pathological pictures were also performed for the documentation of the presence of HCC and supported the biochemical results. Moreover, the DNA damage in liver tissues of male rats using comet assay was studied. The results showed that the HePG2 (− ve) group of rats exhibited a significant reduction ($P < 0.05$) in DNA damage values (9.30 ± 0.89) relative to other treatment groups. Nevertheless, the DNA damage values in the HePG2 (+ ve) and 5-flurouracil groups were significantly higher ($P < 0.01$) compared to the HePG2 (− ve) group. Additionally, HePG2 (coated **3**) and HePG2 (**3**) groups exhibited significant decrease in the DNA damage compared to those in HePG2 (+ ve) group.

Keywords: Sulfonylurea derivative, Cytotoxicity, Anti-HePG2, Comet assay, Nano-size, Sol-gel technique, *in-vivo* study

Introduction

The liver cancer is adding and growing serious trouble to public health burden encyclopedically. Liver cancer is the world's fifth most frequent cancer and the alternate leading cause of cancer-related mortality [1, 2]. The most frequent type of primary hepatic cancer is hepatocellular carcinoma (HCC), accounting for 90% of all cancers and representing a major health problem globally. Patients with chronic liver disorders like hepatitis B or hepatitis C virus-induced hepatic cirrhosis are often tested for HCC [3, 4]. Sorafenib is the only small-molecule medication approved to treat advanced HCC and is therefore recommended as the first-line systemic treatment in such patients. However beside the presence of major side effects, it has only little survival advantage [5–8]. As

*Correspondence: faridsroor@gmx.de; fm.sroor@nrc.sci.eg

[1] Organometallic and Organometalloid Chemistry Department, National Research Centre, Cairo 12622, Egypt
Full list of author information is available at the end of the article

a result, the development of new small-molecule inhibitors of HCC is critically needed. Egypt occupied the third in Africa and 15th most populous country worldwide in ranking of the hepatocellular carcinoma (HCC). Owning to the increasing of HCC patients twofold over a decade, the health authorities in Egypt consider that HCC is the most challenging health problem in the country [9].

Urea and sulfonylurea derivatives have been reported as active compounds against solid tumors in vivo [8, 10, 11]. Although their mechanisms of action are unknown, it's worth mentioning that there is currently no clear data on the impacts of sulfonylureas on the growth of cancer. [12]. Several scientific articles developed from Sorafenib have lately been investigated into the design, synthetic technique, mechanistic features, structure–activity relationships (SAR), and interaction with biological systems of urea derivatives as possible antibacterial, antifungal, anticonvulsant, antidiabetic, anticancer, and antiviral-Because few publications on the same subject have been published, there is contradictory information regarding the true function of various urea and sulfonylurea derivatives on cancer cell development [13, 14].

The previous studies in our laboratory have investigated the effect of novel sulfonylurea derivatives against HePG2 cell line. The (4-methyl-*N*-((3-propionyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)benzenesulfonamide), **3** Fig. 1, showed potent anticancer activity versus HePG2 cell line with $IC_{50}=4.25$ µM compared to 5-fluorouracil with $IC_{50}=316.25$ µM as reference drug [13].

As a result, and as a follow-up to our past research into the preparation of biologically active organic compounds [13, 15–19] and encouraged by the potent bioactivity of 4-methyl-N-((3-propionyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)benzenesulfonamide **3** and in continuation to investigate the mode of action of compound **3**, the current study represents the treatment of liver cancer using compound **3** as normal- and nano-form after coating with silica as in vivo study.

Materials and methods

Chemistry

Synthesis of (3)

Compound **3** was prepared as we reported in our previous work [13]. To a solution of 1-(2-amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)propan-1-one **2** (1 mol) in acetonitrile, the 4-tolylisocyanate **1** (1.1 mol) was added while stirring at room temperature. The reaction mixture was stirred for two hours. The completion of reactions was monitored by TLC on silica gel coated aluminum sheets. The obtained precipitate was filtered off, washed with cold acetonitrile (two times) dried well, and recrystallized from ethyl acetate/acetonitrile (3:1) to give **3** [13].

Yield: (87%) as a white solid; m.p 130–132 °C. IR (KBr, cm^{-1}): 3331, 3275 (NH), 1684 (C=O), 1342, 1163 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.80 (1H, s, NH), 7.86 (2H, d, $J=8$ Hz, Ar–H), 7.46 (2H, d, $J=8$ Hz, Ar–H), 4.20 (2H, m, CH_2, Et), 3.86 (4H, m, CH_2, C_6H_8), 2.61 (4H, m, CH_2, C_6H_8), 2.40 (3H, s, CH_3), 1.26 (3H, t, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 165.3, 149.0, 147.0, 144.8, 137.1, 131.0, 130.2, 127.9, 126.3, 111.6, 60.7, 26.3, 24.1, 22.9, 22.7, 21.5, 14.6. Anal. For $C_{19}H_{22}N_2O_4S_2$:

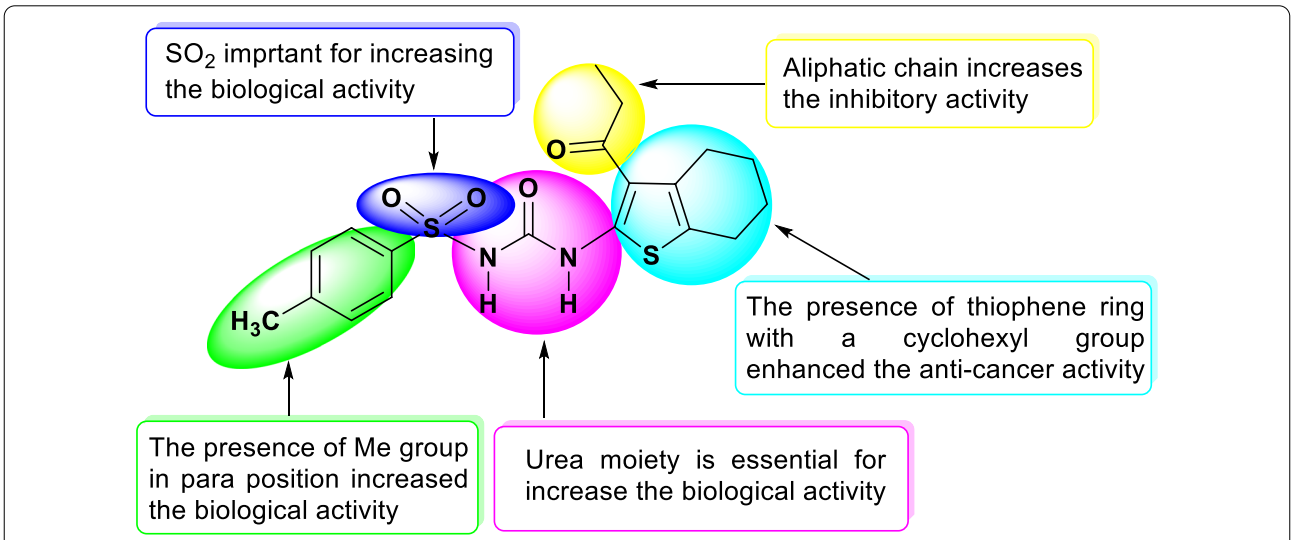


Fig. 1 Structure of 4-methyl-*N*-((3-propionyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)-benzenesulfonamide **3** (CAS registry number is 2770999–78-3**)**.[13]

Calcd. C, 56.14; H, 5.46; N, 6.89. Found: C, 55.70; H, 6.00; N, 6.78.

Re-formulation of the compound 3 using sol-gel technique

The compound **3** was formulated in nanoscale particles as described previously by our group [20]. The sol–gel method was used in the preparation of the nanomaterials through hydrolysis and poly-condensation of tetraethyl orthosilicate as source of SiO_2 containing HCL as catalyst [21] in the presence of the as-prepared sulfonylurea **3**. The silicate solution with the molar ratio TEOS:C_2H_5OH:H_2O:HCl 1:6:8:0.6 was stirred for 2 h. After refluxing, 100 mg of the compound **3** was added and further sonicated for 3 h at 50 °C. The sol was cast and gelled into plastic molds and kept at room temperature for 3 days. The prepared material was kept for further investigation.

In-vivo study

Ethical statement

All animal trial techniques were directed as per local and worldwide controls. The protocol of experiment was approved by Medical Research Ethics Committee, National Research Centre, Egypt (No. 19-193). Westar male albino rats weighing (150 ± 30 g), were supplied from animal house, National Research Centre (Dokki, Giza, Egypt), they were kept for 1 week to accommodate under constant environmental and nutritional conditions with free access to food and water.

Induction of hepatocellular carcinoma

Diethyl nitrosamine DEN was dissolved in corn oil and intraperitoneal injected with a single dose of 50 mg/kg body weight [22]. After two weeks, CCl_4 (2 ml/kg IP) were injected with a single dose for promotion of liver cancer [23].

Chemicals and drugs

5-Fluorouracil (5-FU) was purchased from S.X. Haipu Pharmaceutical Co., Ltd as ampoules (250 mg\10 ml) and rats were intraperitoneally injected by 75 mg/kg once per week for four successive weeks [24]. The kits used for the biochemical analysis were purchased from biodiagnostic Co. Cairo, Egypt. Reagents for ELIZA kit was obtained from Cloud–Clone Corp (USA). Diethyl nitrosamine (DEN) and CCl_4 for the induction of HCC was purchased from Sigma-Alderich Chemical Company (USA).

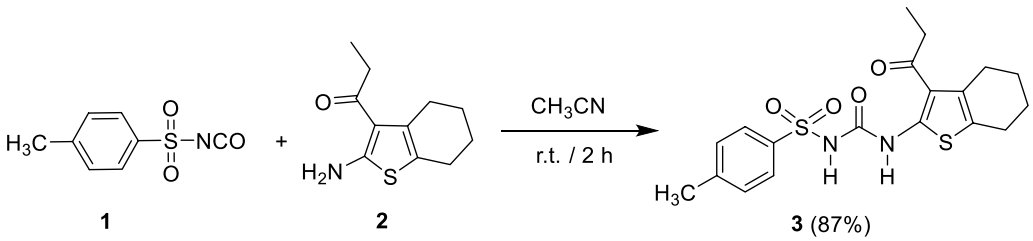
The levels of serum transferases (Reitman and Frankel) [25] and ALP (Belfiel dand Goldberg, 1971) [26] activities were determined as biochemical markers for the early hepatic damage using quantitative colorimetric commercial kits (Biodiagnostic, ARE), GSH (Beutler et al.) [27] were also detected using quantitative colorimetric kits (Biodiagnostic, ARE). Lipid peroxidation (MDA) was estimated according to Ohkawa et al. [28] γGGT was measured by the method of Szasz. [29] Catalase activity was measured according to the method of Aebi [1983], Serum alpha-fetoprotein (AFP) was determined by ELISA Biocheck kits (USA) (Abelev et al. [30]. Uotila et al. [31]. Chanand Miao et al. [32]). α-L-Fucosidase (AFU) was assayed using quantitative colorimetrically kit (Biodiagnostic, ARE) (Zielke et al.) [33]. Superoxide dismutase activity was measured by the method of Nishikimi et al. [34] and TAC (Ghiselli et al.) [35].

Experimental protocol

Fifty male Wistar rats were divided into 5 groups/10 rats each as follow: Group1; control group. Group2; was IP injected rats with a single dose of DEN; then, post two 2tweeks, carbon tetrachloride was IP injected with a single dose of and left for six months. The progress of HCC was assured Histopathologically. Groups 3 and 4; rats were treated orally with 50 mg/kgb.wt. of non-coated **3**

Table 1 Elemental analysis of the sulphonyl urea compound before and after coating with TEOS using sol–gel technique

Compound	Elemental analysis (atomic %)				
	C	N	O	S	Si
3	70.3	5.8	16.7	7.3	0
Coated **3**	47.2	8.0	28.7	1.6	5.2



1 2 **3** (87%)

Scheme 1 Synthesis of 4-methyl-N-((3-propionyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)-benzenesulfonamide **3**

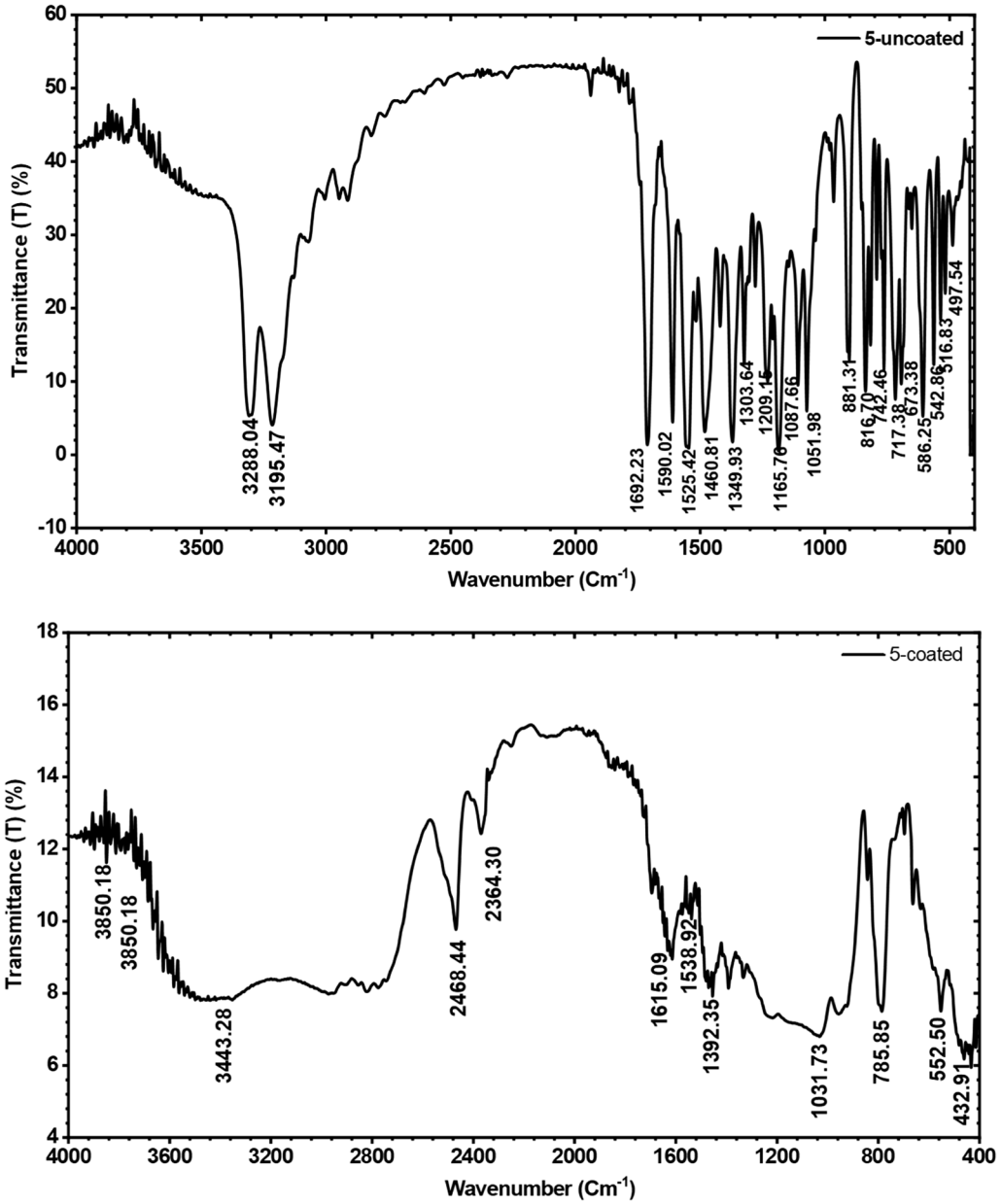


Fig. 2 FTIR spectra of the re-formulated compound **3** using sol–gel technique in comparison with the synthesized compound **3**

and coated **3** for four weeks. Group 5: rats were IP with 75 mg/kg 5-fluorouracil once a week for four weeks.

Animals were sacrificed by decapitation post six months CCl_4 injection; the blood was withdrawn by rupture of sublingual vein after light anesthesia by diethyl ether in clean and dry test tube, left 10 min to clot and centrifuged at 3000 rpm (4 °C) for serum separation. The separated sera were stored at − 20 °C for further assessment of liver enzymes, cholestatic, tumor markers, oxidative stress biomarkers. Hepatic tissue was homogenized in normal physiological saline solution (0.9% NaCl) (1:9 w/ v). The homogenate was centrifuged at 4 °C for 5 min at 3000 rpm. The supernatant was used for determination of antioxidant status. Sections of hepatic lobes were preserved in 10% formalin solution for pathological pictures of neoplastic nodules.

Statistical analysis

Statistical analysis was carried out using One Way Analysis of Variance (ANOVA, Version 8), computer program (mean ± SD, n = 10), combined with co-state

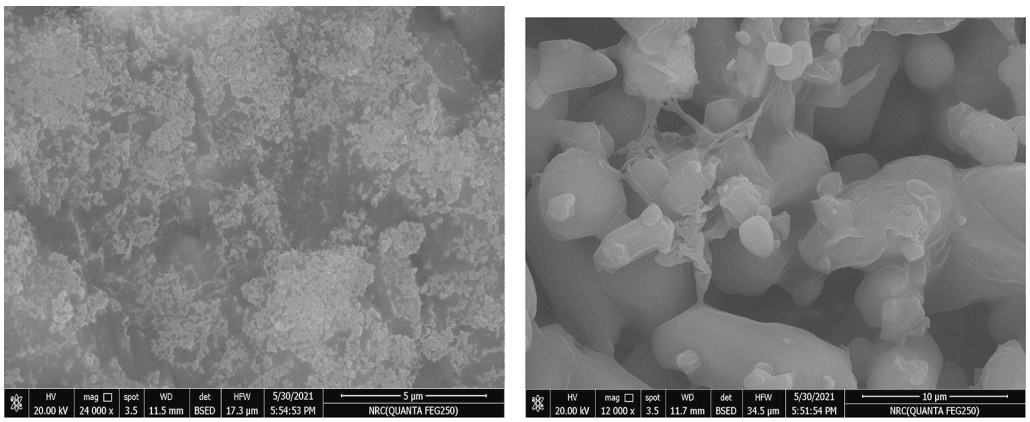


Fig. 3 SEM micrographs of the re-formulated compound **3** using sol–gel technique in comparison with the synthesized compound **3**

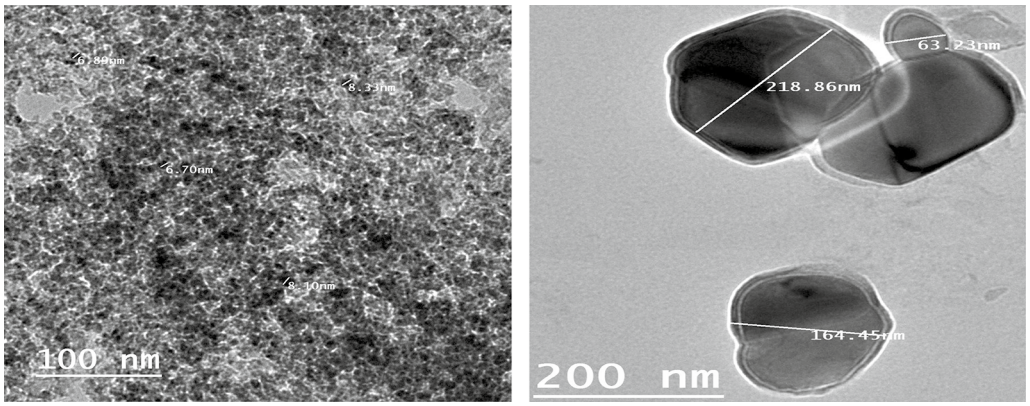


Fig. 4 TEM images of the re-formulated compound **3** using sol–gel technique in comparison with the

computer program, where different letters are significant at $P \leq 0.05$.

Acute toxicity

Thirty mice were used in this study, six mice in each concentration. Serial concentrations from 50, 100, 200, 400 and 800 mg/kg b.wt. were used.

Table 2 Acute study of different concentrations of compound **3**

50 mg/kg b.wt	100 mg/kg b.wt	200 mg/kg b.wt	400 mg/kg b.wt	800 mg/kg b.wt
No died	No died	50% died	All died	All died

Dose and route of administration

Same doses of non-coated **3** and coated **3** were used in the current study (50 mg/kg b.wt.; $1/4LD_{50}$) and supplemented orally for one month post CCl_4 and the documentation of the occurrence of HCC by biochemical and histopathological examination.

Comet assay

Comet assay was performed referring to the protocol developed by Blasiak et al. [36] with minor modifications. Liver tissues of each treatment were homogenized and mixed with low-melting-point agarose (ratio of 1:10 v/v), then pipetted to pre-coated slides with normal-melting-point agarose. The slides were kept flat at 4 °C for 30 min in dark environment. The third layer of low melting point

Table 3 Effect of the synthesized chemical compound **3** and its coated form on the levels of AST, ALT, ALP and GGT in DEN-induced HCC in rats and therapeutic groups

Parameters	Groups				
	Control	Induced-HCC	Treated-HCC (3)	Treated-HCC (coated 3)	Treated-HCC (5-flurouracil)
AST(U/l)	75.00 ± 3.90[a]	259.0 ± 6.88[b]	158.60 ± 4.20[c]	120.90 ± 3.00[d]	130.00 ± 5.10[d]
% of change	–	+ 245.33	+ 111.47	+ 101.20	+ 73.33
% of improvement	–	–	133.87	184.13	172.00
ALT (U/l)	49.02 ± 3.10[e]	129.00 ± 4.00[a]	79.00 ± 3.23[d]	65.40 ± 2.50[g]	63.00 ± 3. 80[g]
% of change	–	+ 163.160	+ 61.20	+ 33.42	+ 28.52
% of improvement	–	–	102.04	129.74	134.64
ALP(U/l)	42.19 ± 3.90[h]	257.67 ± 9.60[b]	95.00 ± 5.00[f]	67.00 ± 3.78[k]	80.40 ± 2.0[n]
% of change	–	+ 510.74	+ 125.18	+ 58.81	+ 90.57
% of improvement	–	–	385.57	451.93	420.17
GGT (U/L)	10.23 ± 0.23[a]	40.22 ± 3.90[b]	22.60 ± 2.11[c]	17.78 ± 1.20[d]	25.70 ± 3.90[e]
% of change	–	+ 293.16	+ 120.92	+ 73.80	+ 151.22
% of improvement	–	–	172.24	219.35	141.94

Data were expressed as means ± SD (n = 8).

Groups with similar letters are not significantly different; while, those with different Letters are significantly different at $P \leq 0.05$

Table 4 Effect of the synthesized chemical compound **3** and its coated form on the levels of antioxidant biomarkers in DEN-induced HCC in rats and therapeutic groups

Parameters	Groups				
	Control	Induced-HCC	Treated-HCC (3)	Treated-HCC (coated 3)	Treated-HCC (5-flurouracil)
GSH(µg/mgprotein)	3340.00 ± 67.00[a]	1340.00 ±	2200.00 ± 20.22[c]	2700.00 ± 50.00[d]	2650.00 ± 21.10[d]
% of change	–	− 23.90[b]	− 34.13	− 19.17	− 20.66
% of improvement	–	59.88	25.75	40.72	39.22
MDA(µg/mgprotei)	39.20	119.00 ± 4.00[f]	69.00 ± 4.88[g]	55.44 ± 3.22[g]	53.70 ± 2. 69[g]
% of change	± 2.90[e]	+ 99.95	+ 76.02	+ 41.43	+ 36.99
% of improvement	–	–	127.55	162.14	166.58
SOD(µmol/mgprotein)	1890.00 ± 50.07[h]	500.00 ± 20. 12[i]	1090.00 ± 45.00[j]	1345.90 ± 34.20[h]	1410.87 ± 44.00[h]
% of change	–	− 73.54	− 42.33	− 28.84	+ 25.40
% of improvement	–	–	31.22	44.70	48.15
CAT (U/g tissue)	276.00 ± 14.00[k]	52.20 ± 3.00 l	133.78 ± 10.00[m]	180.00 ± 9.00[n]	167.00 ± 7.00[n]
% of change	–	− 81.10	− 51.53	− 34.78	− 39.50
% of improvement	–	–	29.55	46.30	41.59
TAC (U/g tissue)	2.67	0.69 ± 0.010[p]	1.29 ± 0.02[o]	1.66 ± 0.03[o]	1.54 ± 0.06[o]
% of change	± 0.12[o]	− 100.75	− 51.69	− 37.83	− 42.32
% of improvement	–	–	22.47	36.33	31.84

GSH glutathione, *MDA* malondialdehyde, *SOD* superoxide dismutase, *CAT* catalase, *TAC* total antioxidant capacity

Data were expressed as means ± SD (n = 10).

Groups with similar letters are not significantly different; while, those with different Letters are significantly different at $P \leq 0.05$.

agarose was then pipetted on slides, left to solidify at for 30 min 4 °C. The slides were transferred to pre-chilled lysis solution, kept for 60 min at 4 °C. After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 min. Slides were subjected to an electrophoresis run at 0.8 V/cm, 300mAmps at 4 °C for 30 min. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air-dried. Ethidium bromide was used for slides stain then and visualized by using Zeiss epifluorescence microscope (510–560 nm, barrier filter 590 nm) with a magnification of ×400. 100 cells per animal were scored (Fairbairn et al.) [37]. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1 × and 2 × the nuclear diameter; and class 3 = tail longer than 2 × the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus (Collins et al.) [38].

Table 5 Effect of the synthesized chemical compound **3** and its coated form on the levels of α-Fetoprotein and α-L-Fucosidase in DEN-induced HCC in rat and therapeutic groups

Biomarkers/Groups	α-Fetoprotein ng/ml	α-L-Fucosidase (AFU) U/L
Control	0.60 ± 0.01^{a}	2.22 ± 0.03^{a}
Induced-HCC % change	2.80 ± 0.03^{b} +366.66	12.90 ± 1.20^{b} +481.10
Treated-HCC (**3**) % change % improvement	1.45 ± 0.05^{c} +141.67 225.00	8.80 ± 0.70^{c} +296.39 184.69
Treated -HCC (Coated **3**) % change % improvement	1.15 ± 0.03^{d} +91.67 275.00	7.00 ± 0.20^{d} +215.32 265.77
Treated-HCC (5-fluro-uracil) % change % improvement	1.30 ± 0.02^{e} +116.60 250.00	8.56 ± 1.00^{e} +285.59 195.50

Data were expressed as means ± SD (n = 10).

Groups with similar letters are not significantly different; while, those with different Letters are significantly different at $P \leq 0.05$

Statistical analysis

All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [39] followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean ± SEM. All statements of significance were based on probability of $P < 0.05$.

Results

Chemistry

The treatment of 4-tolylsulfonyl isocyanate **1** with 1-(2-amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)propan-1-one **2** in dry acetonitrile afforded the title compound 4-methyl-N-((3-propionyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)-benzenesulfonamide **3** as shown in Scheme 1 [13].

Characterization of the re-formulated compound 3

As shown in Table 1, the coated sulfonylurea derivative **3** has Si content about 5.2 (atomic %) compared to the uncoated **3** (0%) (in the TEOS). On the other hand, the elements percentage of C, N, O, and S in the coated **3** are 47.2, 8.0, 28.7 and 1.6% respectively, while, in the case of uncoated **3** they are 70.3, 5.8, 16.7 and 7.3%, respectively. As expected, both of Si and O were increased while the other ones were decreased after the treatment of the organic compound 3 with TEOS using sol–gel technique, these data are in agreement with our previous work [40]. From these data we can confirm the preparation of coated 4-tolylsufonyl urea 3 using sol–gel technique.

IR spectra of the coated **3** and uncoated **3** compounds showed that, the peaks of 1160, 808 and 563 cm^{-1} could be attributed to the Si–O–Si, Si–C ($Si–CH_2$) and C–O–C functional groups, respectively. In addition the band at 777 cm^{-1} associated to Si–OH is also appeared as expected from the hydrolysis of the TEOS as shown in Fig. 2. The results confirmed the formation of the salinized structure according to the hydrolysis and condensation of the TEOS during the sol–gel reaction.

As depicted in Fig. 3, it can be noticed that the obtained silica coated 4-tolylsulfonyl urea **3** agglomerated nanoparticles in different morphology rather than that in case of uncoated **3**. It can be concluded that sol–gel preparation of the 4-tolylsulfonyl urea **3** resulted in new formulation in nano-sized structure. Figure 4 shows the morphology and the particle size diameter of the re-formulated coated **3** relative to that in the case of uncoated **3**. It can be noticed that, when sol–gel process using TEOS was conducted in very diluted aqueous medium, the particle size was decreased after sol–gel preparation around 6–8 nm with spherical-like shape structure according to Greasley et al [41].

Biological evaluation

In vivo study

Acute toxicity study As depicted in Table 2, no mortality were appeared post 48 h. up to 100 mg. LD_{50} was ascertained using 200 mg/kg b.wt. The mice were remained for another two weeks and no mortality were recorded for 50 and 100 mg/ kg b.wt.

AST, ALT, and ALP study The activity of the liver function enzymes AST, ALT, and ALP increased significantly in HCC rats with percentages of 245.33, 163.16, and 510.74%, respectively (Table 3). Noticeable improvement was observed in liver enzymes level post treatment with compound **3** and re-formulated **3**. Higher percentages of improvement were noticed in AST, ALT and ALP (184.13, 129.74 and 451.93%, respectively) post treatment HCC rats than non-coated **3** and standard drug. Significant increase in the GGT in HCC induced rats (+293.16), while noticeable amelioration in its level post treated HCC with the chemical compound **3** with percentages of amelioration 172.24% respectively. Coated **3** showed the highest amelioration results (219.35%, respectively) compared to standard drug (Table 3).

GSH, SOD, CAT, TAC study The results in Table 4 declared significant decrement in the antioxidant of HCC-induced rats with percentages -23.90, -73.54, -81.10 and -100.75%, respectively for GSH, SOD, CAT,

TAC. While noticeable increment in MDA level was recorded (99.95%). Treatment of carcinogenic rats with the synthetic chemical compounds **3** and its coated form presented therapeutic amelioration in all biomarkers examined with the highest percentages for coated one relatively to standard drug 40.27, 162.14, 44.70, 46.30 and 36.33% respectively for GSH, MDA, SOD, CAT and TAC (Table 4).

α-Fetoprotein and α-L-Fucosidase study According to Table 5, a significant elevation in the α-Fetoprotein and α-L-Fucosidase in hepatocarcinogenic rats (+366.66 and +481.10%, respectively). While noticeable improvements in their levels post treatment of HCC rats with the compound **3** (225 and 184.69% respectively). The coated **3** recorded the highest percentages of amelioration (275 and 265.77%, respectively), comparing with reference drug (195.50%) (Table 5).

Discussions

The current study clearly indicated no mortality was appeared post 48 h.up to 100 mg. LD_{50} was ascertained using 200 mg/kg b.wt. The mice were remained for another 2 two weeks and no mortality were recorded for 50 and 100 mg/ kg b.wt. as illustrated in Table 2. In the animal model, diethyl nitrosamine is a well-known hepatocarcinogenic agent [42]. Although DEN/CCl4 therapy was efficient in causing HCC in rats, compound 3 and coated 3 (administered post HCC induction) were beneficial in ameliorating the hepatocarcinogenic impact of DEN/CCl4. In comparison to the control group, the efficiency of DEN/CCl4 in producing hepatic dysfunction is assessed by increased AST and ALT. This increase may be related to enzymes leaking from necrotic and/or destroyed cells.This is supported by a number of previous studies and could be utilized as an indicator of HCC progress in DEN-intoxicated rats [43]. On the other hand, the ALP elevation in intoxicated rats (G3) may be illustrated on the basis of the mechanical blockage of bile ducts, which prevents the enzyme from being excreted, hence increasing the concentration in the blood [44]. Lowering in the ALP level post compounds and drug treatment (Table 3) might be due to relief in the mechanical obstruction of bile duct.

In terms of gamma-glutamyl transferase (GGT), this is a glycoprotein-based enzyme, which is more commonly found on the cell membrane of hepatocytes, and is used as a biomarker routinely for the diagnosis of liver injury [45]. GGT is utilized as indicator of the extracellular catabolism of glutathione which cause ROS production [45]. Glutathione plays an important role in cells protection against the free radicals resulted from metabolism. Transferation of glutamyl residue to an acceptor was catalyzed by GGT, helping in preserving adequate levels of glutathione GGT accumulation in the hepatic tissue and excess GGT secretion into the blood circulation was reported by the blockage of bile ducts or liver damage. As a consequence, the high level of GGT in serum can be used as a useful diagnostic tool hepatic or biliary destruction [45]. Our findings demonstrated a significant rise in GGT levels in DEN-intoxicated rats as compared to the control group (Table 3). This could be due to the rapid turnover of cancer cells, which leads to enzymes being released into the circulation of blood. In line with

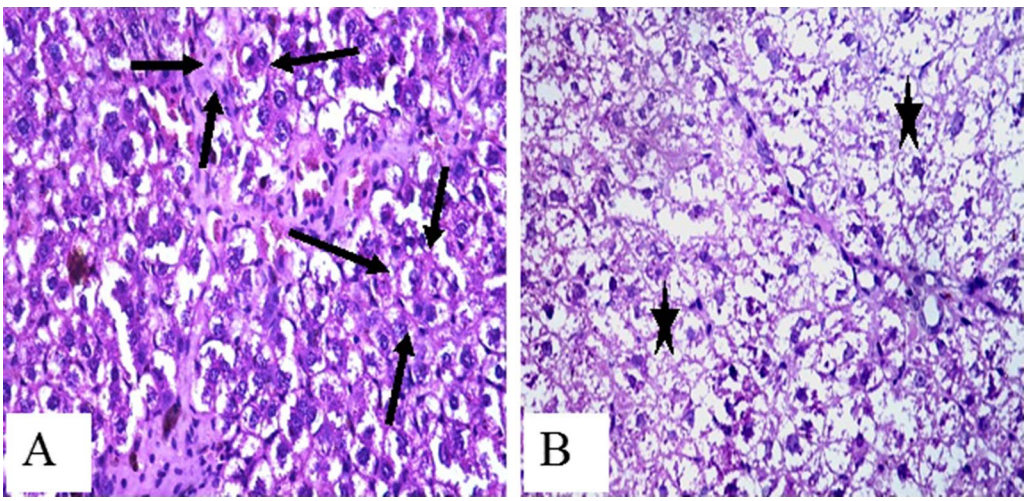


Fig. 5 Liver from control positive group showing; **A** HCC (Hepato Cellular Carcinoma); note the trabecular growth pattern of pleomorphic hepatocytes (arrows in borders) with absence of central veins and portal areas, (H&E X400) (Score Lesion:++++). **B** Massive necrosis of hepatocytes; note the massive disintegration and lysis of hepatocytes (*), (H&E X 400) (Score Lesion:++++)

our findings, Salau et al. [46] discovered that DEN injections caused a significant decrease in GGT activity in liver tissue while increasing enzyme activity in serum, resulting in plasma membrane damage. The results are similar to those of prior research, which found elevated levels of GGT serum activity, showing oxidative and cellular stress caused by glutathione depletion within the cells [44]. Further, Latief *etal*. [47], indicated that an increment in serum GGT activity in cancer-bearing rats is closely linked to the fast turnover of cancer cells, which elaborates the GGT enzyme into the blood. Moreover, Ahmed et al. and Shahat et al. [48, 49], found similar outcomes. This increase in GGT level suggested the progression of carcinogenesis, as the activity of the GGT enzyme is correlated to the level of tumor growth [45]. The same authors also linked the high GGT levels to gene expression upregulation in DEN-intoxicated rats. The improvement in GGT level post treatment of HCC rats with compounds may be due to the normalization in glutathione level and antioxidant enzymes besides decrease in MDA level (as shown in Table 4).

One of the most remarkable indicators of oxidative stress is the measurement of lipid peroxides as MDA, which is used to assess the oxidative stress in liver

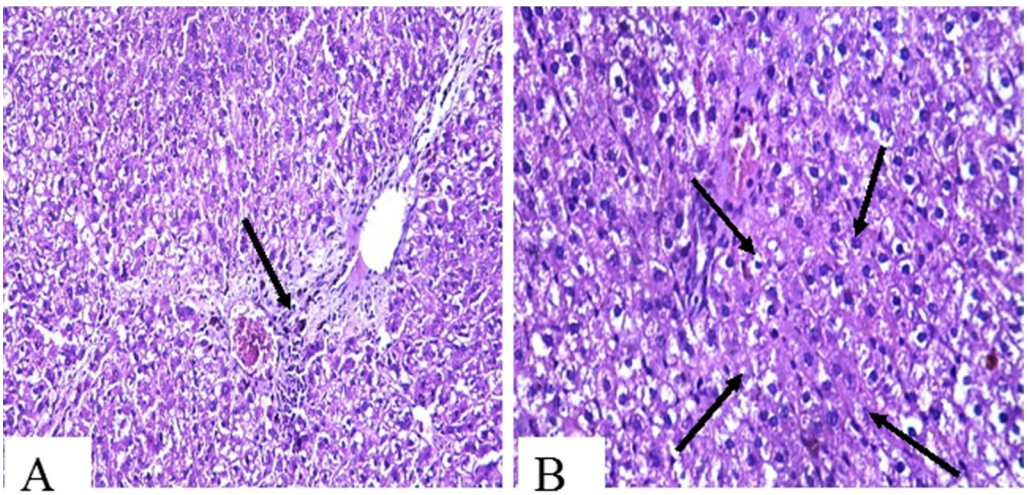


Fig. 6 Liver from compound (**3**) treated -cancer group showing; **A** Mono-Focal regenerated hepatic lobule with normal portal area (arrow), (H&E X200). (Score Lesion: + + +). **B** Higher magnification of the regenerated hepatic lobule; note the normal hepatocytes (arrows in borders), (H&E X400) (Score Lesion: + + +)

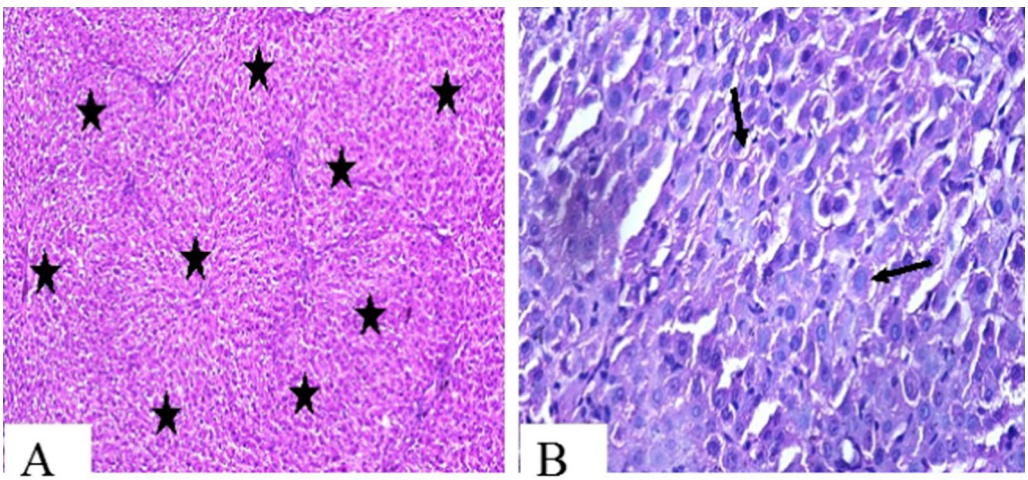


Fig. 7 Liver from coated compound (**3**) treated-cancer group showing; **A** Massive regenerated hepatic lobules (*) with normal portal areas, (H&E X200) (Score Lesion: +). **B** Higher magnification of the regenerated hepatic lobule; note the normal hepatocytes with normal cytoplasm and nucleus (arrows), (H&E X400) (Score Lesion: +)

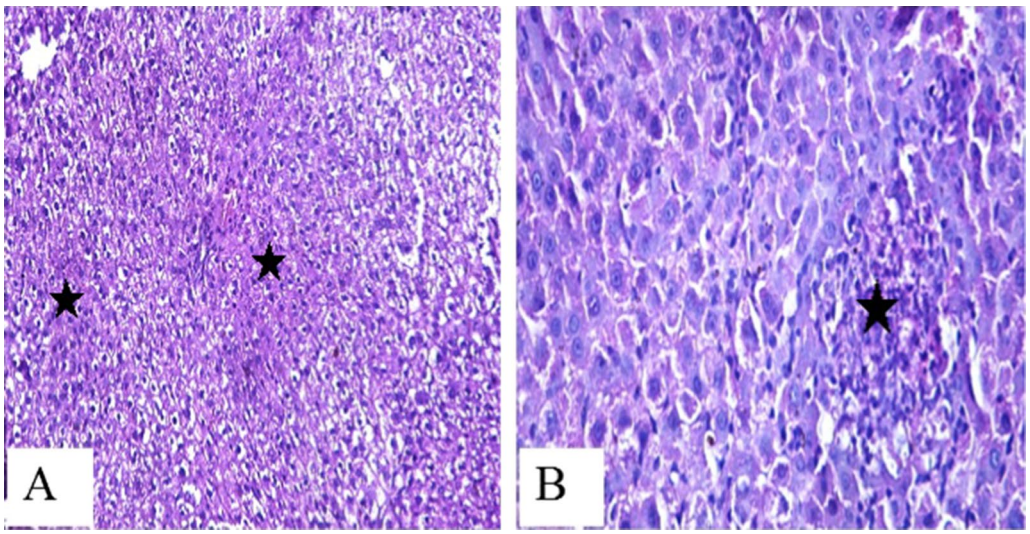


Fig. 8 Liver from Standard Drug treated- cancer group showing; **A** Multi-Focal regenerated hepatic lobule (*) with normal portal areas, (H&E X200) (Score Lesion: ++) **B** Higher magnification of the regenerated hepatic lobule together with focal area of necrosed hepatocytes (*), (H&E X400) (Score Lesion: ++)

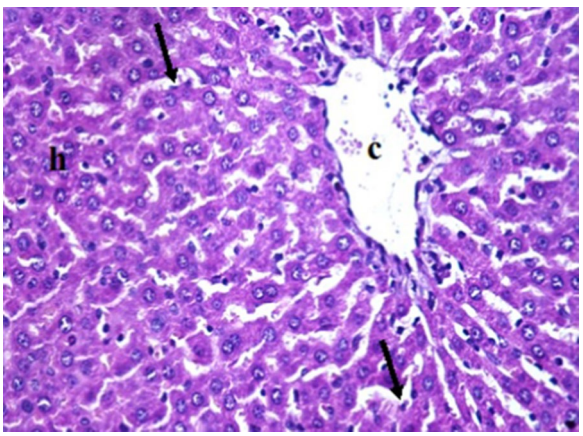


Fig. 9 Liver from control negative group showing normal hepatic parenchyma; note the polyhedral hepatocytes in organized hepatic cords (h) with normal central vein (c) and blood sinusoids (arrows), (H&E X400) (Score Lesion: 0)

damage [45]. The oxidative toxic impact of DEN/CCl4 may be seen in Table 4 as a significant rise in MDA with a significant drop in GSH as a non-enzymatic antioxidant, and CAT, GPx, SOD, and GST as the main antioxidant enzymes critical for MDA scavenging as an indicator of oxidative stress,. This finding was supported in the study of Zhang et al. [50]. which found that DEN can promote HCC through interactions with macromolecules like antioxidative enzymes, lipids, DNA, and DNA repair system enzymes. Furthermore, it is widely accepted that cytochrome P-450 biotransformation of CCl4 normally converts to trichloromethyl free radical (CCl3*) and trichloromethyl proxy free radical (CCl3OO*) as two metabolites responsible for ROS, LP, and inhibition of CAT, GST, SOD, and GPx enzymatic activities [45]. Also, the observed findings are in agreement with the findings of Hussein & Khalifa and Kadasa et al. [51, 52] who found significant inhibition of antioxidant enzyme activities and gene expression in DEN-induced rats compared to controls.

One of the fatal glycoproteins linked with tumors is alpha-fetoprotein (AFP). The AFP gene is reactivated in the liver of hepatocytes; during the early stage of hepatocarcinogensis, cytoplasmic AFP stimulates the proliferation of hepatic cancer cells. Extracellular AFP was also found to accelerate the proliferation of malignant hepatocytes that is mediated via the AFP receptor [45]. During cellular differentiation of hepatocytes, liver progenitor cells (LPC) also develop AFP [45]. An important role of the LPCs was showed in hepatic homeostasis and regeneration [45]. Elevation of serum AFP, as seen in the current study (Table 5), indicates LPC growth as a consequence of chronic liver damage or HCC development [45]. In comparison to control rats (Table 5), our research found a significant increase in AFP levels following injection with DEN/CCl4. This noticeable increase in AFP is symptomatic of hepatic destruction as well as HCC development. This finding is consistent with the findings of Kadasa et al [52] and Zhang et al. [50] who found AFP elevation in DEN-intoxicated rats. The carcinogenic effect of DEN and HCC induction was suggested by the elevated levels. AFP is utilized to distinguish between

HCC and chronic liver illnesses. In this context, Hussain et al. [51] discovered that throughout the metabolic biotransformation of DEN, pro-mutagenic products are released that are accountable for DEN's carcinogenicity impact.

One of the important useful HCC biomarker is alpha-l-fucosidase (AFU), due to its considered elevated levels in HCC patients with liver diseases, reported by several ones. [45] DEN is transformed to ethyl radical metabolites, which combine with DNA to cause mutations, leading to carcinogenesis [45]. When DEN/CCl4 intoxicated animals were compared to control rats, the present study found a significant increase in AFU level (Table 5). Abdallah and Khattab [53] detected an increase in the level of AFU in both the cytosol and the serum of DEN-treated animals, which was similar to our findings. In a parallel result, El-Attwa et al. [54] discovered a significant increase in the level of AFU that is strongly linked with tumor size. Moriwaki et al. [55] also found that fucosylation of sugar proteins increases throughout the development of hepatocarcinogensis, resulting in an elevation in enzyme level. According to Gan et al. [56], the level of the AFU enzyme is correlated to the growth of tumors.

Histopathological examination results

At sacrifice, number and size of liver tumors as well as tumor burden were recorded, and all liver tumors were processed for histological and immunohistological analyses. Rats-induced HCC showed trabecular growth pattern of pleomorphic hepatocytes with disappearance of central veins and portal areas with score lesion of grade (++++) Hepatocyte proliferation is accompanied by cytoplasmic edema, visible cellular injury, and mortality. Moreover, hepatocytes lose their regular form and arrangement, as well as vacuoles of various shapes and sizes, necrotic regions with mild cytoplasm, and nuclei that lose their vesicular look and become hyperchromatic (Fig. 5A). Also, massive necrosis of hepatocytes and lysis

Table 6 Rate of DNA damage in liver tissues of male rats exposed to different treatment using comet assay

Treatment	No. of cells		Class[¥] of comet				DNA damaged cells (mean ± SEM)
	Analyzed*	Total comets	0	1	2	3	
HePG2 (-ve)	300	28	273	19	7	2	9.30 ± 0.89^{c}
HePG2 (+ve)	300	75	226	34	23	18	24.93 ± 1.15^{a}
5-Fluorouracil	300	68	233	31	17	20	22.59 ± 1.20^{ab}
HePG2 (coated **3**)	300	51	250	21	19	11	16.94 ± 0.58^{b}
HePG2 (non-cated **3**)	300	59	242	19	26	14	19.61 ± 0.67^{b}

(¥): Class 0 = no tail; 1 = tail length < diameter of nucleus; 2 = tail length between 1 and 2X the diameter of nucleus; and 3 = tail length > 2X the diameter of nucleus

(*): No of cells analyzed were 100 per an animal

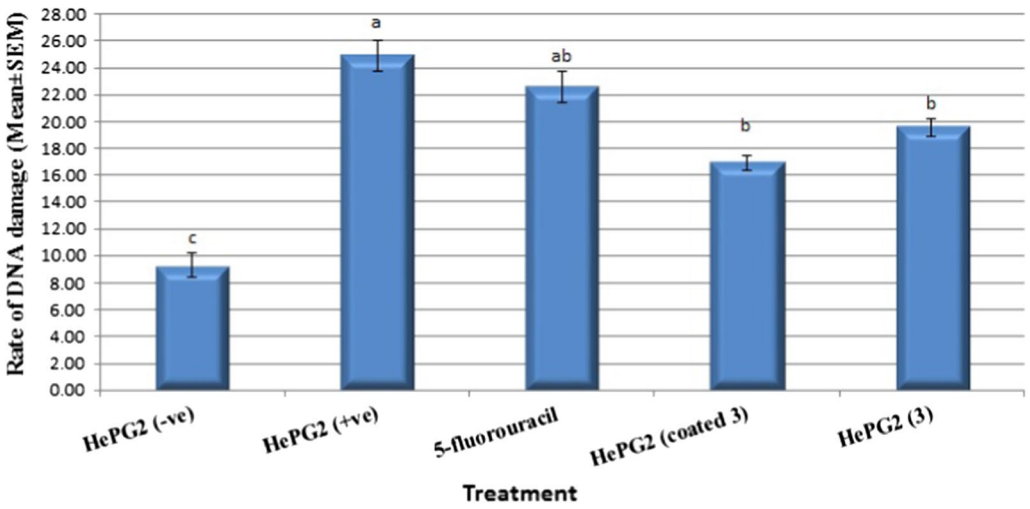


Fig. 10 The incidence of DNA damage in liver tissues of male rats exposed to different treatment using comet assay. Data are presented as mean ± SEM. [a,b,c,d,e] followed by different superscripts are significantly different ($P \leq 0.05$)

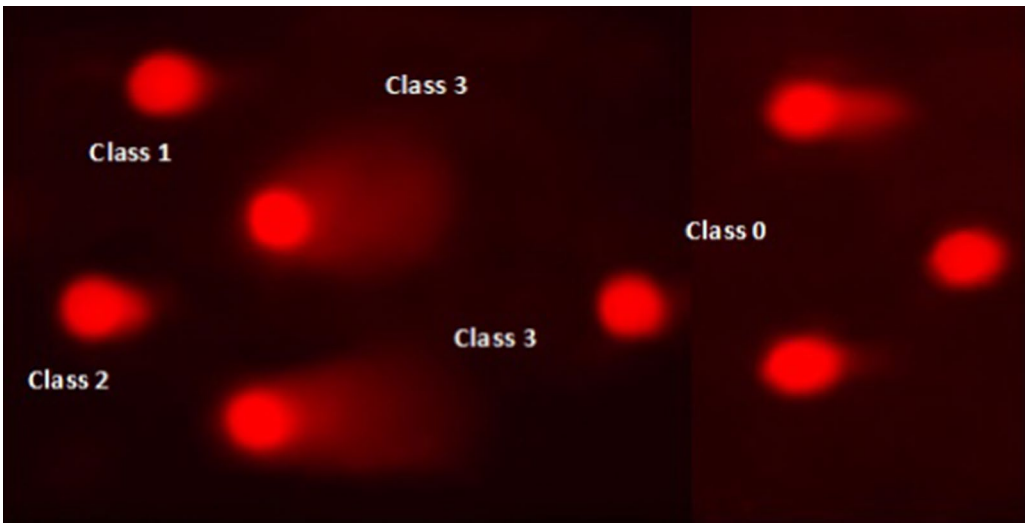


Fig. 11 Visual score of DNA damage (classes 0, 1, 2 and 3) using comet assay in liver tissues

of hepatocytes with high lesion score grade (++++) (Fig. 5B).

While, carcinogenic liver treated with compound (**3**) (treated-cancer group) declared mono-focal regenerated hepatic lobule with normal portal area with score lesion+++(Fig. 6A). Higher magnification of the regenerated hepatic lobule recorded normal hepatocytes with score lesion:+++(Fig. 6B). Additionally, HCC rats treated with the coated form of compound (**3**) (treated -cancer group) showed massive regenerated hepatic lobules with normal portal areas, with lesion score:+(Fig. 7A). Higher magnification of the regenerated hepatic lobule showed normal hepatocytes with normal cytoplasm and nucleous (Score Lesion:+) (Fig. 7A).

HCC rats treated with standard drug multi-focal regenerated hepatic lobule with normal portal areas, (Score Lesion:++) (Fig. 8A). However, higher magnification showed regenerated hepatic lobule together with focal area of necrosis hepatocytes (Score Lesion:++) (Fig. 8B) compared to normal control rats (Fig. 9).

The histopathological examination of livers in experimental rats supported our study. The histopathological examination of liver sections from DEN/CCl4-intoxicated animals revealed pleomorphic hepatocytes with the disappearance of central veins and portal areas, confirming the documentation of all biochemical alterations following DEN/CCl4 intoxication. Also, massive necrosis of hepatocytes and lysis of hepatocytes, hepatocytes were proliferated with cytoplasmic edema, apparent cellular damage and death. In addition, the normal shape and arrangement of hepatocytes were lost. Furthermore, a great variation in the sizes and shapes of vacuoles resulted, necrotic regions with little cytoplasm, whereas nuclei lost their vesicular look and were hyperchromatic (Fig. 5A & B). Excessive free radicals were thought to be the reason for this deterioration induced by DEN metabolism, which resulted in HCC. In line with the findings of the current study, Hussain et al. [51], Zhao et al. [57], Kadasa et al. [52]. and Vedarethinam et al. [58] investigated unformatted architecture showed by DEN-treated rats. Inflammatory cells were present along the central vein, as well as enlarged nuclear size in hepatic cells. The ameliorating effect of synthetic compounds, either **3** or coated, showed a better effect than standard drugs, which could be the result of the compounds' capacity to repair liver damage induced by DEN and regenerating liver cells. Thus the plasma membrane retains its strength, preventing enzymes leakage and tumor markers. Moreover, the biochemical results are confirmed by marked amelioration in hepatic tissue post treatment the HCC-induced rats with compound **3** and coated one with higher noticeable amelioration of formulated coated compound **3** than either non coated or standard drug (Figs. 6, 7, and 8) compared to control (Fig. 9).

The ameliorating effect of synthetic compounds either **3** or coated one showed better effect than standard drug which may be resulted from the ability of compounds to repair the hepatic damage caused by DEN and regenerating liver cells. Thus the plasma membrane retains its strength, preventing enzymes leakage and tumor markers. Moreover, the biochemical results are confirmed by marked amelioration in hepatic tissue post treatment the HCC-induced rats with compound **3** and coated one with higher noticeable amelioration of formulated coated compound **3** than either non coated or standard drug (Figs. 6, 7, and 8) compared to control (Fig. 9).

Comet assay

As shown in Table 6 and Figs. 10 and 11, DNA damage in male rats' liver tissues subjected to different treatments was assessed using a comet assay. The results showed that the HePG2 (-ve) group of rats exhibited a significant reduction ($P < 0.05$) in DNA damage values (9.30 ± 0.89) relative to other treatment groups. Nevertheless, the DNA damage values in HePG2 (+ve) were significantly higher ($P < 0.01$) and the 5-flurouracil groups compared to the HePG2 (-ve) group. Additionally, HePG2 (coated **3**) and HePG2 (non-coated **3**) groups exhibited significant decrease in the DNA damage compared to those in HePG2 (+ve) group.

Acknowledgements
Farid Sroor greatly appreciate the financial support of this work by the National Research Centre (NRC) under project number 12010102.

Author contributions
Concept and design: FS. Search and collection of the data: all authors. Analysis of data and interpretation: all authors. Statistical analysis: HA. First draft of the manuscript: FS. All authors contributed to the content of the report and reviewed further drafts. The authors take full responsibility for the scope, direction, and content of the report. All authors read and approved the final manuscript.

Funding
Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). This work was supported by the National Research Centre (NRC) under project number 12010102.

Availability of data and materials
All data generated or analyzed during this study are included in this published article. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. All data and the materials related to this work and used in this study are available to any qualified researcher upon reasonable request addressed to Farid Sroor.

Declarations

Ethics approval and consent to participate
This article has animals study and carried out with ethical permission no. 19-193 in the National Research Centre, Cairo (NRC). Moreover, the authors confirm that all methods were performed in accordance with the guidelines and regulations of the Medical Research Ethics Committee in the NRC. The authors are confirming that + the current study is reported in accordance with ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

Author details
[1]Organometallic and Organometalloid Chemistry Department, National Research Centre, Cairo 12622, Egypt. [2]Department of Therapeutic Chemistry, National Research Centre, Cairo 12622, Egypt. [3]Cell Biology Department, National Research Centre, Dokki 12622, Egypt. [4]Chemical Industries Research Division, National Research Centre, Cairo, Egypt.

Received: 30 April 2022 Accepted: 16 October 2022
Published online: 03 November 2022

References

1. Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul J-L, Schirmacher P, Vilgrain V (2018) EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 69:182–236
2. Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, Al-Raddadi R, Alvis-Guzman N, Amoako Y, Artaman A, Ayele TA, Barac A, Bensenor I, Berhane A, Bhutta Z, Castillo-Rivas J, Chitheer A, Choi J-Y, Cowie B, Dandona L, Dandona R, Dey S, Dicker D, Phuc H, Ekwueme DU, Zaki MES, Fischer F, Fürst T, Hancock J, Hay SI, Hotez P, Jee SH, Kasaeian A, Khader Y, Khang Y-H, Kumar GA, Kutz M, Larson H, Lopez A, Lunevicius R, Malekzadeh R, McAlinden C, Meier T, Mendoza W, Mokdad A, Moradi-Lakeh M, Nagel G, Nguyen Q, Nguyen G, Ogbo F, Patton G, Pereira DM, Pourmalek F, Qorbani M, Radfar A, Roshandel G, Salomon JA, Sanabria J, Sartorius B, Satpathy M, Sawhney M, Sepanlou S, Shackelford K, Shore H, Sun J, Mengistu DT, Topór-Madry R, Tran B, Ukwaja KN, Vlassov V, Vollset SE, Vos T, Wakayo T, Weiderpass E, Werdecker A, Yonemoto N, Younis M, Yu C, Zaidi Z, Zhu L, Murray CJL, Naghavi M, Fitzmaurice C (2017) The burden of primary liver cancer and underlying etiologies From 1990 to 2015 at the global, regional, and national level. JAMA Oncol 3:1683
3. Chen K-F, Chen H-L, Tai W-T, Feng W-C, Hsu C-H, Chen P-J, Cheng A-L (2011) Activation of phosphatidylinositol 3-Kinase/Akt signaling pathway mediates acquired resistance to Sorafenib in hepatocellular carcinoma cells. J Pharmacol Exp Ther 337:155–161
4. Zhang B, Wang N, Zhang C, Gao C, Zhang W, Chen K, Wu W, Chen Y, Tan C, Liu F, Jiang Y (2017) Novel multi-substituted benzyl acridone derivatives as survivin inhibitors for hepatocellular carcinoma treatment. Eur J Med Chem 129:337–348
5. Kudo M, Finn RS, Qin S, Han K-H, Ikeda K, Piscaglia F, Baron A, Park J-W, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng A-L (2018) Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. The Lancet 391:1163–1173
6. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J-F, de Oliveira AC, Santoro A, Raoul J-L, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz J-F, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359:378–390
7. Lee Y-S, Jung YK, Kim JH, Cho SB, Kim DY, Kim MY, Kim HJ, Seo YS, Yoon KT, Hong YM, Lee J-H, Lee HW, Yim HJ, Jang BK, Jang ES, Jang JY, Hwang SY (2020) Effect of urea cream on sorafenib-associated hand–foot skin reaction in patients with hepatocellular carcinoma: a multicenter, randomised, double-blind controlled study. Eur J Cancer 140:19–27
8. Chen F, Fang Y, Zhao R, Le J, Zhang B, Huang R, Chen Z, Shao J (2019) Evolution in medicinal chemistry of sorafenib derivatives for hepatocellular carcinoma. Eur J Med Chem 179:916–935
9. Rashed WM, Kandeil MAM, Mahmoud MO, Ezzat S (2020) Hepatocellular Carcinoma (HCC) in Egypt: a comprehensive overview. J Egypt Natl Cancer Inst. 32:5
10. Catalano A, Iacopetta D, Sinicropi MS, Franchini C (2021) Diarylureas as antitumor agents. Appl Sci 11:374
11. Bai C, Wang H, Dong D, Li T, Yu Z, Guo J, Zhou W, Li D, Yan R, Wang L, Wang Z, Li Y, Ren L (2021) Urea as a by-product of ammonia metabolism can be a potential serum biomarker of hepatocellular carcinoma. Front Cell Dev Bio. https://doi.org/10.3389/fcell.2021.650748
12. Pasello G, Urso L, Conte P, Favaretto A (2013) Effects of sulfonylureas on tumor growth: a review of the literature. Oncologist 18:1118–1125
13. Sroor FM, Othman AM, Tantawy MA, Mahrous KF, El-Naggar ME (2021) Synthesis, antimicrobial, anti-cancer and in silico studies of new urea derivatives. Bioorg Chem 112:104953
14. Sroor FM, Abdelmoniem AM, Abdelhamid IA (2019) Facile synthesis, structural activity relationship, molecular modeling and in vitro biological evaluation of new urea derivatives with incorporated isoxazole and thiazole moieties as anticancer agents. ChemistrySelect 4:10113–10121
15. Fathi EM, Sroor FM, Mahrous KF, Mohamed MF, Mahmoud K, Emara M, Elwahy AHM, Abdelhamid IA (2021) Design, synthesis, in silico and in vitro anticancer activity of novel bis-furanyl-chalcone derivatives linked through alkyl spacers. ChemistrySelect 6:6202–6211

16. Sroor FM, Aboelenin MM, Mahrous KF, Mahmoud K, Elwahy AHM, Abdelhamid IA (2020) Novel 2-cyanoacrylamido-4,5,6,7-tetrahydrobenzo[b]thiophene derivatives as potent anticancer agents. Arch Pharm 353:2000069
17. Sroor FM, Basyouni WM, Tohamy WM, Abdelhafez TH, El-awady MK (2019) Novel pyrrolo[2,3-d]pyrimidine derivatives: design, synthesis, structure elucidation and in vitro anti-BVDV activity. Tetrahedron 75:130749
18. Sroor FM, Khatab TK, Basyouni WM, El-Bayouki KAM (2019) Synthesis and molecular docking studies of some new thiosemicarbazone derivatives as HCV polymeraseinhibitors. Synth Commun 49:1444–1456
19. Khatab TK, El-Bayouki KAM, Basyouni WM, Sroor FMA (2013) An Efficient Synthesis of Biopertinent Dihydropyrimidine (thi) one derivatives via three-component one-pot synthesis catalyzed by tetrachlorosilane. Egypt J Chem 56:291–305
20. Sroor FM, Abbas SY, Basyouni WM, El-Bayouki KAM, El-Mansy MF, Aly HF, Ali SA, Arafa AF, Haroun AA (2019) Synthesis, structural characterization and in vivo anti-diabetic evaluation of some new sulfonylurea derivatives in normal and silicate coated nanoparticle forms as anti-hyperglycemic agents. Bioorg Chem 92:103290
21. Sakka S (1994) The current state of sol-gel technology. J Sol-Gel Sci Technol 3:69–81
22. Cayama E, Tsuda H, Sarma DSR, Farber E (1978) Initiation of chemical carcinogenesis requires cell proliferation. Nat 275:60–62
23. Al-Rejaie SS, Aleisa AM, Al-Yahya AA, Bakheet SA, Alsheikh A, Fatani AG, Al-Shabanah OA, Sayed-Ahmed MM (2009) Progression of diethylnitrosamine-induced hepatic carcinogenesis in carnitine-depleted rats. World J Gastroenterol 15:1373
24. Abdel-Hamid NM, Morsy MA (2010) Novel biochemical pathways for 5-fluorouracil in managing experimental hepatocellular carcinoma in rats. J Membr Bio 234:29–34
25. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63
26. Belfield A, Goldberg DM (1971) Revised assay for serum phenyl phosphatase activity using 4- amino - antipyrine. Enzyme 12:561–573
27. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888
28. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
29. Szasz G (1974) New substrates for measuring gamma-glutamyl transpeptidase activity. Z Klin Chem Klin Biochem 12:228
30. Abelev GI (1974) alpha-Fetoprotein as a marker of embryo-specific differentiations in normal and tumor tissues. Immunol Rev 20:3–37
31. Uotila M, Ruoslahti E, Engvall E (1981) Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. J Immunol Methods 42:11–15
32. Miao YC, Chan DW (1986) Affinity chromatographic separation of alpha-fetoprotein variants: development of a mini-column procedure, and application to cancer patients. Clin Chem 32:2143–2146
33. Zielke K, Okada S, O'Brien JS (1972) Fucosidosis: diagnosis by serum assay of alpha-L-fucosidase. J Lab Clin Med 79:164–169
34. Nishikimi M, Appaji Rao N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46:849–854
35. Ghiselli A, Serafini M, Natella F, Scaccini C (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radical Biol Med 29:1106–1114
36. Blasiak J, Arabski M, Krupa R, Wozniak K, Zadrozny M, Kasznicki J, Zurawska M, Drzewoski J (2004) DNA damage and repair in type 2 diabetes mellitus. Mutat Res Fundam Mol Mech Mutagen 554:297–304
37. Fairbairn DW, Olive PL, O'Neill KL (1995) The comet assay: a comprehensive review. Mutat Res Rev Genet Toxicol 339:37–59
38. Collins A, Dusinska M, Franklin M, Somorovska M, Petrovska H, Duthie S, Fillion L, Panayiotidis M, Raslova K, Vaughan N (1997) Comet assay in human biomonitoring studies: reliability, validation, and applications. Environ Mol Mutagen 30:139–146
39. Institute SAS (1982) SAS user's guide: statistics. SAS Institute, Cary, N.C.
40. Haroun A, Gospodinova Z, Krasteva N (2021) Amino acid functionalization of multi-walled carbon nanotubes for enhanced apatite formation and biocompatibility. Nano Biomed En 13:380
41. Greasley SL, Page SJ, Sirovica S, Chen S, Martin RA, Riveiro A, Hanna JV, Porter AE, Jones JR (2016) Controlling particle size in the Stöber process and incorporation of calcium. J Colloid Interface Sci 469:213
42. Chang W, He W, Li P-P, Song S-S, Yuan P-F, Lu J-T, Wei W (2016) Protective effects of Celastrol on diethylnitrosamine-induced hepatocellular carcinoma in rats and its mechanisms. Eur J Pharma 784:173–180
43. Kim N-H, Heo J-D, Kim TB, Rho J-R, Yang MH, Jeong EJ (2016) Protective Effects of Ethyl Acetate Soluble Fraction of Limonium tetragonum on Diethylnitrosamine-Induced Liver Fibrosis in Rats. Biol Pharm Bull 39:1022–1028
44. Wiwanitkit V (2001) High serum alkaline phosphatase levels, a study in 181 Thai adult hospitalized patients. BMC Family Practice. https://doi.org/10.1186/1471-2296-2-2
45. Mohamed NZ, Aly HF, El-Mezayen HA, El-Salamony HE (2019) Effect of co-administration of Bee honey and some chemotherapeutic drugs on dissemination of hepatocellular carcinoma in rats. Toxi Rep 6:875–888
46. Salau A, Yakubu M, Oladiji A (2015) Hepatoprotective effects of aqueous root bark extracts of Anogeissus leiocarpus, Terminalia avicennioides, and their mixture in diethylnitrosamine-treated rats. Drug Dev Ther 6:93
47. Latief U, Husain H, Mukherjee D, Ahmad R (2016) Hepatoprotective efficacy of gallic acid during Nitrosodiethylamine-induced liver inflammation in Wistar rats. J Basic Appl Zool 76:31–41
48. Shahat AA, Alsaid MS, Kotob SE, Husseiny HA, Al-Ghamdi AAM, Ahmed HH (2016) Biochemical and histological evidences for the antitumor potential of Teucrium Oliverianum and Rhazya stricta in chemically-induced hepatocellular carcinoma. Afr J Tradit, Complementary Altern Med 13:62
49. Ahmed HH, Shousha WG, Shalby AB, El-Mezayen HA, Ismaiel NN, Mahmoud NS (2014) Curcumin: a unique antioxidant offers a multimechanistic approach for management of hepatocellular carcinoma in rat model. Tumor Biol 36:1667–1678
50. Zhang Q, Yang J, Wang J (2016) Modulatory effect of luteolin on redox homeostasis and inflammatory cytokines in a mouse model of liver cancer. Onco Lett 12:4767–4772
51. Hussain T, Siddiqui HH, Fareed S, Vijayakumar M, Rao CV (2012) Evaluation of chemopreventive effect of Fumaria indica against N-nitrosodiethylamine and CCl4-induced hepatocellular carcinoma in Wistar rats. Asian Pac J Trop Med 5:623–629
52. Kadasa NM, Abdallah H, Afifi M, Gowayed S (2015) Hepatoprotective effects of curcumin against diethyl nitrosamine induced hepatotoxicity in albino rats. Asian Pac J Cancer Prev 16:103–108
53. Abdallah IZA, Khattab HAH (2004) Protective role of lycopene against diethylnitrosamine induced experimental hepatocarcinogenesis. Egypt J Hosp Med 16:1–13
54. Attwa MH (2015) Guide for diagnosis and treatment of hepatocellular carcinoma. World J Hepatol 7:1632
55. Moriwaki K, Noda K, Nakagawa T, Asahi M, Yoshihara H, Taniguchi N, Hayashi N, Miyoshi E (2007) A High Expression of GDP-Fucose Transporter in hepatocellular carcinoma is a key factor for increases in fucosylation. Glycobiology 17:1311–1320
56. Gan Y, Liang Q, Song X (2014) Diagnostic value of alpha-l-fucosidase for hepatocellular carcinoma: a meta-analysis. Tumor Biol 35:3953–3960
57. Zhao J-A, Peng L, Geng C-Z, Liu Y-P, Wang X, Yang H-C, Wang S-J (2014) Preventive effect of hydrazinocurcumin on carcinogenesis of Diethylnitrosamine-induced hepatocarcinoma in male SD Rats. Asian Pac J Cancer Prev 15:2115–2121
58. Vedarethinam V, Dhanaraj K, Ilavenil S, Arasu M, Choi K, Al-Dhabi N, Srisesharam S, Lee K, Kim D, Dhanapal T, Sivanesan R, Choi H, Kim Y (2016) Antitumor Effect of the Mannich Base(1,3-bis-((3-Hydroxynaphthalen-2-yl) phenylmethyl)urea) on Hepatocellular Carcinoma. Molecules 21:632

Publisher's Note