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Therapeutic activity of sour orange albedo extract and abundant flavanones loaded silica nanoparticles against acrylamide-induced hepatotoxicity

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ABSTRACT

The current research aims to demonstrate the therapeutic effect of sour orange albedo extract (SOAE) and two flavanones loaded-tetraethylorthosilicate (TEOS) using sol-gel technique, in adose100 mg/kg body weight taken orally or45 days against acrylamide (ACR)toxicity in rats. This was achieved through measuring the activities of specific biochemical parameters related to liver functions in tissue of ACR intoxicated rats as compared to normal one. Liver functions included alanine and aspartate aminotransferases, antioxidants and oxidative stress biomarkers; superoxide dismutase, catalase, glutathione and lipid peroxide (malondialdehyde, MDA). Moreover, histological examination of liver was performed to confirm the biochemical findings. The present results clearly indicated disturbances in all biochemical parameters, such as increase in the liver function enzyme activities and MDA level. Results of ATPase enzyme activities revealed significant decrease in ACR intoxicated rats and liver biomarker enzymes declared significant decrease. On the other hand, treatment of intoxicated rats with the previous different nano-particles natural product demonstrated improvement in all biochemical parameters under investigation.

1. Introduction

Functionalized nanoparticles have received considerable attention in recent years as they display unique properties and are recently considered as a promising materials in a variety of medical applications [1].Carbon nanotubes (CNTs) - based materials are used for molecular delivery systems due to be highly biocompatible. Several studies have shown that extract could be capsulated and grafted with functionalized CNTs to form nanocomposites [2]. The connection between extract and functionalized CNTs leads to increase the activity compared with plant extract alone. It was found that, metformin hydrochloride (MET)-loaded poly-lactic-co-glycolic acid (PLGA) nanoparticles (NPs) at the dose of 2 mM markedly decrease cancer volume which considered a promising approach for glioblastoma multiforme treatment [3]. Further, Kuskov et al. [4], detected a new nanoparticles of amphiphilic poly-*N*-vinylpyrrolidone which can be used as good carriers for novel, highly efficacy, systems of hydrophobic drug delivery such as indomethacin. Silymarin loaded Poly (3-HydroxyButyrate-co-3-HydroxyValerate) (PHBHV) nanocarriers markedly reduced viability of HT-29 cells post 6 and 24 h of medications. Also, *in vivo* study demonstrated that the Silymarin loaded PHBHV nanocarriers are significantly capable of to penetrate 3D micro tumors and decrease their size [5].Polymer nanocarriers based on amphiphilic thiooctadecyl-terminated poly-*N*-vinyl-2-pyrrolidone were produced and loaded with a model hydrophobic drug, curcumin which reveal two various distributions of size with characteristic various biochemical effect. This result enables the use of intranuclear delivery by reducing the size of polymeric carriers, which consider a novel process for therapies of cancer [6].

The pharmacological results of natural products from plants showed their content of valuable sources of antifibrotic and hepatoprotective agents against liver injury and liver fibrosis [7,8].The processing of citrus by products is considered a rich source of natural flavonoids, owing to the large amount of citrus peel produced, a high amount of phenolic compounds [7]. Phenolic compounds as flavonoids including flavones, flavanones and isoflavones, possess various pharmacological

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actions and therapeutic applications [9]. The flavanones naringenin and hesperetin, that sour orange (*Citrus aurantium* L.) contain high amounts of them, exhibit wide range of physiological activities such as inflammatory, estrogenic, anticarcinogenic, hepatoprotective, neuroprotective, scavenger of peroxynitrite, and antioxidative properties [10,11].Since the finding that, acrylamide (ACR)is naturally formed during home cooking and industrial processing of many foods, much effort has been (andstillisbeing)put in to understand mechanism of its formation and on evaluation of its toxicity and potential human health consequences [12]. Administration of ACR is markedly elevated the levels of malondialdhyde(MDA) and total protein, while decreased glutathione(GSH),superoxide dismutase(SOD), catalase, succinate dehydrogenase(SDH),lactate dehydrogenase(LDH) and ATPase levels in liver. ACR also increase aspartate and alanine aminotrasferases (AST and ALT) level in serum [13].Oxidative damage and dysfunctions of mitochondria have been determined to be principle mechanisms in various chemical-induced cell damage and diseases of degeneration [10]. It refers to enhanced generation of reactive oxygen species/reactive nitrogen species and/or depletion of antioxidant defense system, causing an imbalance between pro-oxidants and antioxidants [14]. Hence, the present study is designed to examine the two abundant flavanones isolated from sour orange to ameliorate hepatotoxicity induced by acrylamide in Wistar rats in comparison with standard drug silymarin.

2. Materials and methods

2.1. Fruit materials

The fruits of *Citrus aurantium L.* (Rutaceae) were collected from a private farm Banha, Qalyubia governorate, Egypt. All the fruits were of eating quality and without blemishes or damage. The identity was confirmed by Treas Labib, Herbarium Section, El-Orman Botanical Garden, Giza, Egypt. The pericarp region (peel)is separated from the edible part. The white, spongy in inner part of the peel, called the mesocarp, or albedo was separated, dried at room temperature and grinded.

Thin-layer chromatography (TLC) was performed on precoated plates of GF254 silica gel (Fluka). The chromatograms were visualized under UV light at 254 and 366 nm before and after ammonia vapour exposure, as well as AlCl3 (R1) or aldehyde/sulfuric acid (R2) spraying. The systems of solvent (v/v)were as follows:S1,acetic acid/water(3:7);S2,*n*-butanol/aceticacid/water(4:1:5,toplayer).All chemicals used in the current work were of high analytical grade, Sigma products (USA).UV spectra were measured in methanol solutions and with different diagnostic UV shift reagentsonaShimadzuUV240spectrophotometer. The NMR spectra were record dat300 (1 H) and75 (13C) MHz,on a Varian Mercury 300 NMR spectrometer and δ-values are reported as ppm relative to tetramethylsilane (TMS) as internal standard. TMS in the convenient solvent. Preparation of the nano-materials based on tetraethoxyortho silicate (TEOS).

2.2. Isolation of abundant flavanones

Part(300 g)of the dried alcoholic extract was then partitioned between 500 mLof9:1 ethanol-water and 500 of mL *n*- hexane, in aliquots of approximately 50 g at a time. The ethanol/water layer was evaporated to a thick brown residue and partitioned with methylene chloride (3 × 500 mL).The methylene chloride-soluble fraction (185 g)was chromate graphed on SiCC using n-hexane gradient elution with ethyl acetate. The fraction eluted with20–50%ethyl acetate in n-hexane was selected for further study. This sub fraction was further done separated by repeatedchromatographed on Si CC and more purified by flash chromatographs. ASub-fraction afforded precipitate and syrup- like mother liquor. Naringenin(42.5 mg) and hesperetin(35.9 mg)were isolated from this precipitate using different columns viz., SiCC using methylene chloride/methanol and Sephadex LH-20 using *n*-butanol/*iso*-propanol/water (40:11:29v/v/v).The purity of the compounds is being checked by TLC using solvent systems S1(Rf;0.86,0.79),S2 (Rf;0.18,0.23),respectively and spray reagents(R1 andR2).The chromatographic properties of compounds(color under UV light, change with ammonia vapour and responses towards spray reagents can suggest their flavonoid aglycone characters. Their identification were done on the basis of chromatographic properties,1H and 13CNMR spectroscopic data with literature values [15,16].

2.3. Preparation of loaded silica nanoparticles

The sol gel method was used in the preparation of the nanomaterial's through hydrolysis and poly condensation of TEOS as sources of SiO2containing HCl as catalyst, in the presence of citrus extracts, naringenin and hesperetin. The silica solutions with the molar ratio; TEOS:C2H2OH:H2O:HCl1:6:8:0.6, were stirred for1hr.After reflux for 2 h,2 mg citrus extract were added and sonicated followed by addition of 2.0 mmol benzoin methyl ether. First, the plant extract moieties were cross linked by means of the sol-gel process, followed by UV photo initiated radical reaction of the carboxylic groups. The sol was cast and gelled into plastic molds and kept at room temperaturefor3days.The ageing and drying of the prepared gels were carried out at 800 °C for 48h [17–19].

2.4. Characterization of the prepared nanomaterial

Characterization of the prepared nanomaterial was carried out by Perkin-Elmer Fourier Transform Infrared

Spectroscopy (PEFTIRS) with resolution 4 cm-1, scale range 4000-400, data interval:0.964cm-1and scanning speed 2mm/sec. Also, High Resolution Transmission Electron Microscope (TEM) JEOL-2100 was used. Particle size and Zeta potential was measured by Malvern zeta sizer Nano system.

2.5. In vitro radical scavenging assay

The antiradical activity of the extract was estimated according to the procedure described by Ilhami et al. [20] using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution.

2.6. Animals

Ninety male Wistar rats, aged8–12weeks and weighing 100–120 g, provided by National Research Centre Animal House, Cairo, Egypt. Rats were housed in a temperature 26–29 °C, with affixed cycle of light/dark to acclimatize for two weeks. They were allowed free access to food and water. This study had been approved (Approval no: 13115) by National Research Centre Ethical Committee. The rats were classified into nine groups of ten rats each.

2.7. Ethics

Guidelines of Ethics of Medical Ethical Committee of the National Research Centre, Egypt are used in handling with animals and demonstrated for being sure that the animals not suffer at any stage of the experiment (Approvalno:13115).

2.8. Dose regimens and route of administration

Intraperitoneal (i.p) injection of ACR suspended in H2O in a dose of 50 mg/kg b.wt. five times weekly for ten consecutive days [21]. SOAE, hesperetin and nanoparticles were administered orally five times weekly for45days post-ACR induction in adose100 mg/kg BW [22,23]. The standard herbal reference drug, silymarin, was given orally five times weekly for45 days post-ACR treatment in a dose 50 mg/kg BW

[23].

2.9. Experimental design

The normal control group has received normal diet (Group1) and groups 2–4 will be normal healthy rats orally administrated with loaded silica nanoparticles: citrus, naringenin and hesperetin for 6weeks, respectively. Group5will be injected with toxic dose of ACR for 10 days and served as intoxicated group. Group's 6-8willbe orally administrated with loaded silica nanoparticles of citrus, naringenin and hesperetin post-ACR administration respectively for six weeks. Group 9 will be orally administrated with silymarin post-ACR administration for six consecutive weeks.

2.10. Liver marker enzymes

Liver function tests of alanine and aspartate aminotransferases (ALT and AST) and were conducted [24] using colorimetric diagnostic kits.

2.11. Total protein

The total serum content of protein was evaluated according to Bradford [25].

2.12. Determination of oxidative stress biomarkers

2.12.1. Catalase

Catalase enzyme was determined by colorimetric method in tissue homogenate as previously illustrated by Aebi [26].The following equation showed the reaction of catalase with water which was stopped with inhibitor of catalase after one min.

In the presence of peroxide (HRP), rem

$$2H_2O_2 \xrightarrow[2H_2O_2+O_2]{} \text{Catalase}$$

In the presence of peroxide (HRP), remaining H2O2reacts with3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) and4-aminophenazone(APP)to form a chromophore.

$$2H_2O_2 + \text{DHBS} + \text{AAPHRP} \rightarrow \text{Quinoneimine Dye} + 4H_2O$$

2.12.2. Glutathione

In the liver homogenate, glutathione (GSH) was assayed [27].

The development of a relatively yellow color when the sulfhydryl compounds were mixed with 5,5′-dithiobis-2-nitrobenzoic acid and this was the base of this method.

2.12.3. Malondialdehyde

Buege and Aust reported the method of malondialdehyde (MDA) estimation in liver tissue [28]. A complex series of reactive carbonyl compounds were resulted from the decomposition of malondialdehyde (unstable compound). Malondialdehyde has been used as an indicator of lipid peroxidation process.

2.13. Estimation of lactate and succinate dehydrogenases

Liver biomarker enzymes lactate and succincate dehydrogenises (LDH and SDH) activities were also estimated in hepatic tissue [29,30].

2.14. Estimation of superoxide dismutase and ATPase

Nishikimi et al. [31] recorded the method of assay of superoxide dismutase. Superoxide determination is based on the oxidation of NADH mediated by radical of super oxide through many reactions involving thiol oxidation and univalent O2reduction. The method of Matteucci et al. [32] illustrated the assay of ATPase.

2.15. Histopathological analysis

Formaldehyde (10%) was used to fix liver slices which were embedded in paraffin. Thickness of sections (5 mm) was stained with hematoxylin and eosin. The pathological changes were recorded using a light microscope [33].

2.16. Statistical analysis

SPSS computer program was used for statistical analysis. The program was coupled with Co-state computer program. The unshared letters were significant at $P \leq 0.05$.

3. Results

3.1. Characterization of the prepared nanomaterial's

3.1.1. FTIR

All spectra showed nearly the same characterization peaks. It was observed that characteristic bands at 34202915,

2622,1730,1644,1392,1218,1100,966and796thatcorrespondtoO-H,CH3,C–O,C═C,C−H(band),C−O(str), = C−H(band),O−H(band)respectively. The characteristic peaks of silicate (Si−O−Si,Si−O−C,Si−C) were observed in range 796–1100 cm-1. This confirmed the reaction of sol-gel (Fig. 1)

3.1.2. TEM, particle size and zeta potential

It was observed that silicate particles were agglomerated and filled with the natural materials. Moreover, they had particles size around 327, 835 and 370 nm with different molecular weight(302 g/molfor *hesperetin* and 272 g/mol for naringenin), respectively (Figs. 2 and 3). In addition *zeta* potential values are around 3,6 and 6.4mv, respectively. This may be due to the absence of the anionic groups outside the particles. In other words, if the particles in suspension have alarge positive zeta potential they will tend to repel each other and there is no tendency to flocculate. Consequently, they are not stable in the medium.

3.1.3. In vitro DPPH scavenging activity of the different selected nanomaterials

It was observed that nanoparticles of naringenin had the highest antioxidant activity at the concentration0.1 μg/mL, followed by hesperetin and finally SOAE at the concentration 0.1μg/mL (Fig. 4).

3.2. Effect of samples loaded with silicate nanoparticles on liver enzyme activities

Table1clearly indicated insignificant difference in ALT and AST enzyme activities in normal rats treated with SOAE, hesperetin and naringenin loaded silica nanoparticles comparing with normal untreated one. However, ACR-intoxicated rats showed significant ($P \leq 0.05$), increase in liver enzyme activities; ALT (295.10%) and AST (65.71%),as compared to normal control rats. On the other hand,

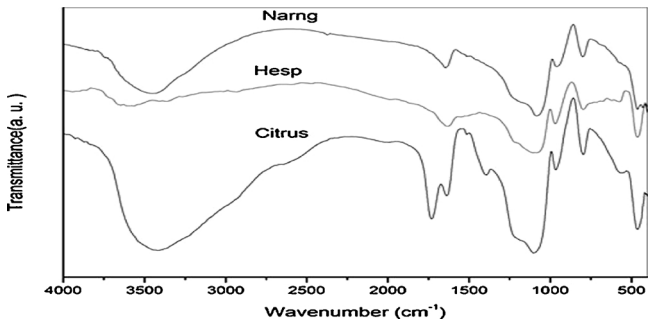

Fig. 1. Characteristic peaks of prepared nanomaterials.

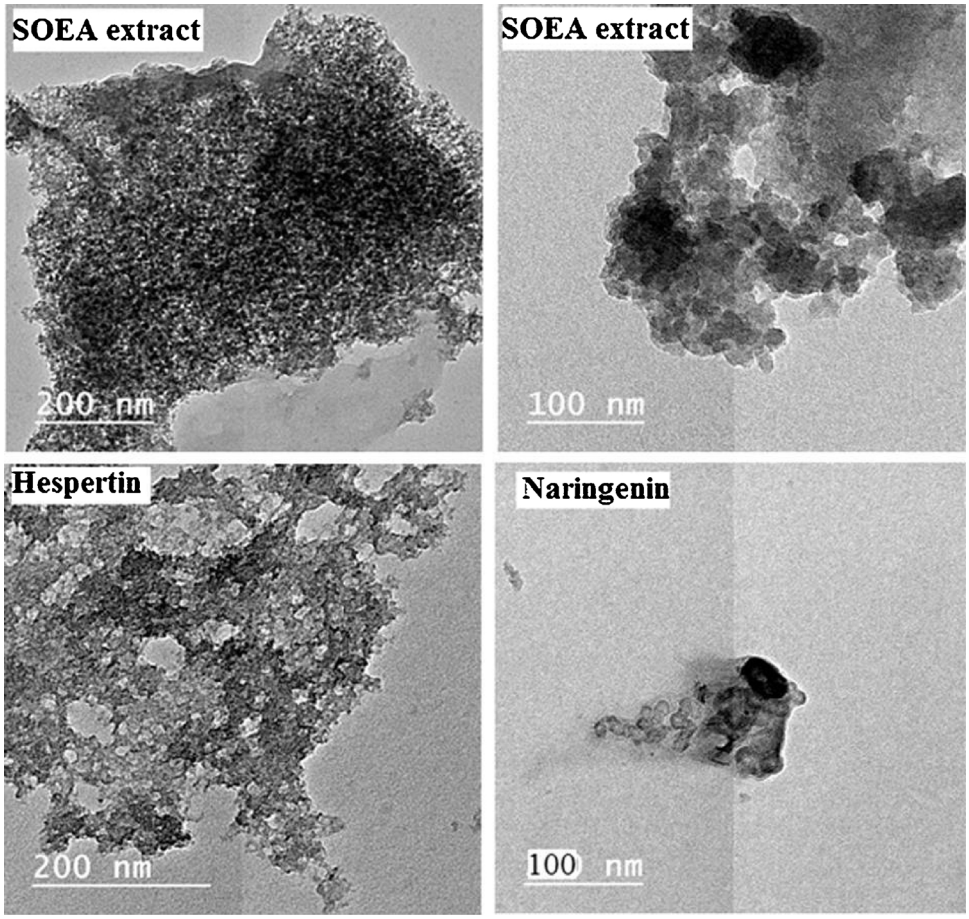

Fig. 2. TEM, particle size and Zeta potential.

intoxicated rats treated with SOAE, hespertin nanoparticles as well as silymarin as reference drug declared an insignificant change in ALT enzyme activity while, naringenin nanoparticle showed significant ($P \leq 0.05$)increase in ALT activity (45.11%).In addition, treated rats with citrus, naringenin nanoparticles and silymarin post-ACR treatment showed insignificant($P \leq 0.05$)difference in AST and ALT activities comparing with normal healthy rats(Table1).With respect to the percentages of improvement, ALT enzyme activity recorded improvement percentage932.71%,in hesperetin nanoparticle administered post-ACR, followed by SOAE (303.11%),then silymarin(283.00%).While, naringenin nanoparticle recorded the lowest amelioration percentage (250.00%).Moreover, AST enzyme activity showed improvement percentage78.70%, in SOAE nanoparticle administered to ACR-intoxicated rats, followed by naringenin nanoparticle(74.81%), then silymarin (69.70%).While,hesperetin nanoparticle recorded the lowest improvement percentage (66.11%)(Table1).

3.3. Effect of samples loaded silica nanoparticles on hepatic ATPase activity and total protein content

It's noticeable that ATPase activity and total protein content, exhibited insignificant change upon treated normal control rats with SOAE and the two flavanones nanoparticles comparing to normal control rats. However, ACR-intoxicated rats declared obvious reduction in hepatic ATPase activity and total protein content by 40.64 and70.80%, respectively comparing with normal healthy rats (Table2). Treatment of challenged rats with SOAE, naringenin nanoparticles and silymarin indicated significant decrease in hepatic ATPase activity by 16.00,11.21and24.60%, respectively for while hesperetin nanoparticle showed insignificant change. Further, ACR-treated rats with nanoparticles of SOAE, hesperetin, naringenin and silymarin demonstrated significant decrease in total protein content by 36.30,51.40,41.50 and 44.70%,respectively (Table2).Hence, hesperetin nanoparticle recorded improvement by 37.11%, followed by naringenin nanoparticle (29.42%), then SOAE (24.61%).While silymarin declared the lowest improvement percentage(16.00%). Regarding to SOAE nanoparticle administered post-ACR, it demonstrates amelioration percentage 34.51%, in total protein content followed by naringenin nanoparticle (29.31%), then silymarin (26.22%). While, hesperetin nanoparticle showed the lowest Improvement percentage (19.42%) (Table2).

3.4. Effect on the antioxidants and oxidative stress biomarkers

3.4.1. Antioxidants effect of samples loaded silica nanoparticles on SOD and catalase activities in ACR intoxicated rats

It's obvious from Table 3 that, an insignificant difference in SOD activity in normal control rats treated with the selected nanomaterial, while significant increase in catalase enzyme activity was detected upon treatment of normal control rats with the nanoparticles of all investigated samples as compared to normal untreated one. Remarkable reduction in SOD and catalase activities was noticed in ACR- intoxicated rats with percentages71.29 and 65.70% respectively. Treatment of intoxicated rats with sour orange albedo extract, hesperetin nanoparticles as well as silymarin exhibited significant decrease in liver SOD activity by 38.81,18.83and35.50%respectivelywhile,naringenin nanoparticle showed insignificant decrease in liver SOD activity. Also, catalase activity recorded significant increase reached to 53.11,102.81, 63.91 and 67.00%, respectively post-ACR treatment comparing with normal control rats. Regarding to the recorded changes in liver SOD

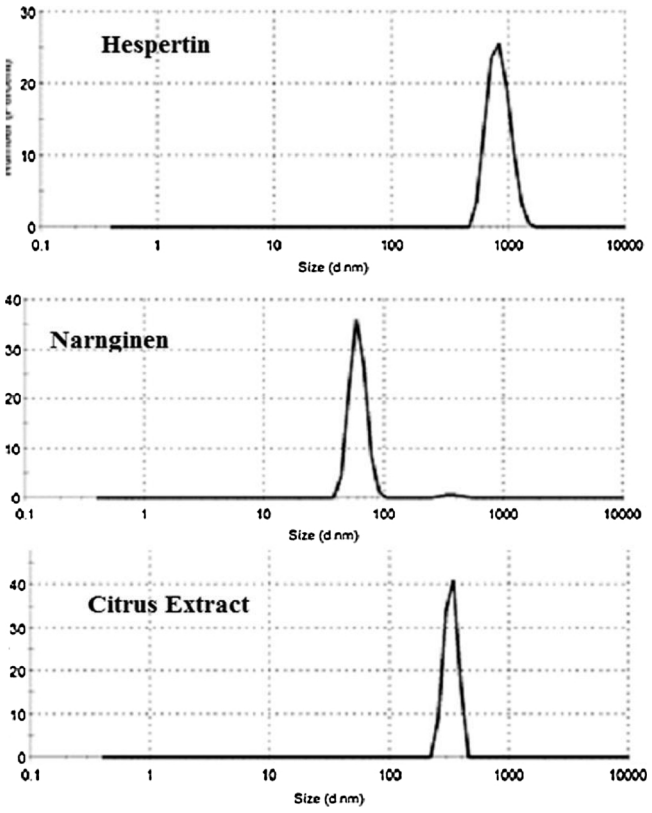

Fig. 3. Particle size and Zeta potential of SOEA extract, Hespertin and Naringenin.

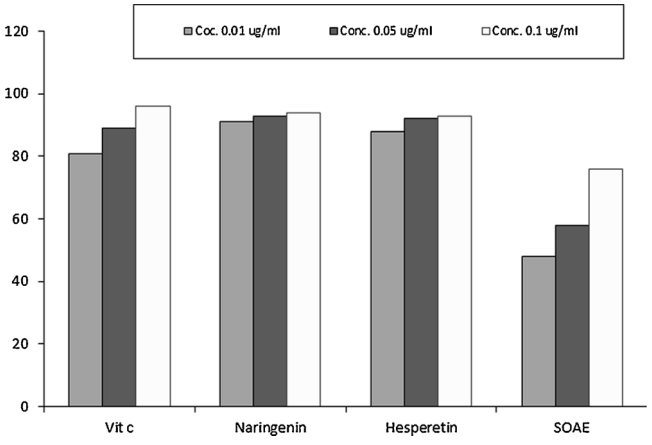

Fig. 4. DPPH scavenging activity of the different selected nanomaterials.

activity, SOAE nanoparticle treated intoxicated rats demonstrated improvement percentage 60.60%,followed by naringenin(55.70%),and hesperetin nanoparticles(52.52%).While silymarin recorded the lowest percentage of amelioration35.81%.For catalase enzyme activity, hesperetin nanoparticle recorded improvement percentage168.42%,followed by silymarin(132.60%),and naringenin nanoparticle (129.60%).While, SOAE nanoparticle recorded the lowest improvement percentage in catalase enzyme activity(118.80%)(Table 3).

3.4.2. Antioxidants effect of different samples loaded silica nanoparticles on GSH and MDA levels in ACR intoxicated rats

Table 4 revealed significant increase in GSH level in normal rats treated with SOAE(40.31%)and hesperetin nanoparticles (62.90%),while, an insignificant change in GSH level upon treated normal control rats with naringenin nanoparticle comparing to normal

Table 1
Effect of SOAE and flavanones loaded silica nanoparticles on liver enzyme activities in the different experimental groups.

Groups	Control	Controls-Treated			Acrylamide	Acrylamide-Treated			
Parameters		SOAE	Hes	Nar		SOAE	Hes	Nar	Sily
ALT (U\L)	28.75 ± 4.55cd	26.25 ± 3.60d	25.50 ± 3.60d	26.30 ± 2.50ded	113.80 ± 5.00a	27.80 ± 2.00cd	26.50 ± 1.70d	41.80 ± 2.70b	32.30 ± 2.70c
Mean ± SD		8.69	11.30	8.52	295.10	3.52	8.00	45.11	12.20
%a						75.57	76.71	63.27	71.62
%b						303.11	932.71	250.00	283.00
%c									
AST (U\L)	143.50 ± 12.20b	136.50 ± 1.30b	135.50 ± 2.40b	136.30 ± 3.00b	237.80 ± 2.20a	124.80 ± 3.00	143.00 ± 2.60b	130.50 ± 2.40b	137.80 ± 3.60b
Mean ± SD		4.87	5.57	5.01	65.71	13.01	0.35	9.11	4.00
%a						47.52	39.87	45.12	42.05
%b						78.70	66.11	74.81	69.70
%c									

%a:% change as compared to norml control rats,%b:%change as compared to acrylamide intoxicated rats,%c:%of improvement. Hes: Hesperetin, Nar:Naringenin, Sily: Silymarin.
Data are means ± SD of ten rats in each group. Data are expressed as Unit/mL. SPSS (version 8) and Co-Stat computer programs are used in statistical analysis where un-matched letters between groups are significant at $P \leq 0.05$.

Table 2
Effect of SOAE and flavanones loaded silica nanoparticles on hepatic ATPase activity and total protein content in the different experimental groups.

Groups Parameters	Control	Controls-Treated			Acrylamid e	Acrylamide-Treated			
		SOAE	Hes	Nar		SOAE	Hes	Nar	Sily
ATPase	12.50 ± 0.50ab	11.20 ± 0.90ab	10.97 ± 0.75ab	11.67 ± 1.02ab	7.42 ± 0.35e	10.50 ± 0.39ab	12.05 ± 1.27ab	11.10 ± 0.60ab	9.42 ± 0.22d
Mean ± SD		10.41	12.20	6.60	40.64	16.00	3.60	11.21	24.60
%a						41.51	62.40	49.60	26.96
%b						24.61	37.11	29.42	16.00
%c									
Total protein	27.32 ± 3.51f	25.67 ± 1.21f	23.92 ± 1.13f	24.40 ± 1.54f	7.97 ± 1.17g	17.40 ± 1.32cd	13.27 ± 1.61e	15.97 ± 1.63cd	15.12 ± 1.23cd
Mean ± SD		6.04	12.45	10.71	70.80	36.30	51.40	41.50	44.70
%a						118.32	66.50	100.38	89.71
%b						34.51	19.42	29.31	26.22
%c									

%a:%change as compared to normal control rats,%b:%change as compared to acrylamide intoxicated rats,%c:% of improvement. Hes: Hesperetin, Nar:Naringenin, Sily:Silymarin.
Data are represented by Means ± SD of ten rats in each group. Data are expressed as μmol/g wet weight for ATPase. While mg / g liver tissue was used for total protein content. SPSS (version 8) and CO-state computer programs were used in statistical analysis where un-matched letters between groups are significant at $P \leq 0.05$.

one. Moreover, ACR intoxicated rats demonstrated significant reduction in liver GSH while, significant increase in MDA levels by 59.40 and 383.40%, respectively. Treatment of intoxicated rats with nanoparticles of SOAE, hesperetin and silymarin markedly increase GSH level by 46.50, 74.31 and 14.20% respectively, while naringenin nanoparticle showed insignificant change. In addition, treatment of intoxicated rat with SOAE, naringenin nanoparticles as well as silymarin demonstrated insignificant increase in liver MDA level. Hesperetin nanoparticle showed significant increase in MDA level (49.32%), as compared to normal control rats (Table 4). Liver GSH level, recoded improvement percentage133.70%, for hesperetin nanoparticle administered post-ACR followed by SOAE nanoparticle (105.91%), and silymarin (73.61%). While, naringenin nanoparticle treatment recorded the lowest improvement percentage (62.00%)(Table 4). For MDA level, silymarin administered post-ACR showed improvement percentage 377.41%,followed by SOAE(372.61%),and naringenin nanoparticle (360.70%).While hesperetin nanoparticle recorded the lowest improvement percentage (334.21%).

3.5. Effect of different samples loaded silica nanoparticles on LDH and SDH enzyme activities in the different experimental groups

Table 5 demonstrated significant increases in LDH and SDH activities in normal rats treated with SOAE and hesperetin nanoparticles, while an insignificant change was detected with naringenin nanoparticle. Intoxicated rats with ACR recorded significant decrease in LDH and SDH activities by77.61 and58.30%, respectively comparing to normal rats. Challenged rats medicated with nanoparticles of SOAE, naringenin and silymarin showed insignificant change in liver LDH activity. However, hesperetin declared significant increase in LDH activity (58.00%).Rats treated with nanoparticles of albedo extract, hesperetin, naringenin and silymarin administrated post-ACR recorded significant increase in SDH enzyme activity by 69.22, 123.61, 53.11 and115.91%, respectively as compared to normal control rats (Table5).The recorded changes in liver LDH enzyme activity, hesperetin nanoparticle administered post-ACR showed improvement percentage by 135.60,followed by silymarin(91.90%), then naringenin nanoparticle(80.21%), while, for SOAE nanoparticle, it showed the lowest improvement percentage(72.30%).In addition, SDH enzyme activity, hesperetin nanoparticle recorded improvement percentage 181.90%,followed by silymarin(174.20%),then SOAE nanoparticle (127.61%),while, naringenin nanoparticle recoded the lowest improvement percentage (111.40%).

3.6. Histopathological analysis

Liver section of control rats showed liver with preserved (intact) lobular hepatic architecture and insignificant pathology changes (Photo 1).

While, liver section of ACR intoxicated rats showed lobular hepatic architecture with mild inflammation, mild hydropic degeneration, thin fibrous tissue bands and congested blood vessels(Photo 2).

However, liver section of control rats treated with nanoparticles of SOAE, naringenin and hesperetin showed liver with preserved (intact) lobular hepatic architecture and insignificant pathology changes (Photos 3–5Photo 3).

On the other hand, rats treated liver with nanoparticles of SOAE post-ACR administration showed preserved (intact) lobular hepatic architecture with mild interlobular inflammation and blood congestion (Photo 6).

Also, liver section of rats treated with nanoparticles of naringenin post-ACR administration showed liver with preserved (intact) lobular hepatic architecture and mild micro-and macrovesicular steatotic changes mild interlobular inflammation (Photo 7).

While, intoxicated rats treated with hesperetin nanoparticles post-ACR showed liver with preserved (intact) lobular hepatic architecture, mild interlobular inflammation and sinusoidal congestion (Photo 8).

4. Discussion

The present results clearly demonstrated significant elevation in liver enzyme activities, while significant reduction in total protein content in ACR intoxicated rats. AST and ALT are used in the specific liver diseases diagnosis. These enzymes of aminotransferases are implicated in the transamination reaction. In addition, such enzymes also considered as remarkable biomarkers of liver function, particularly ALT [34].Detection of ALT and AST in plasma has been reported to be promising indicator of liver injury [35].The obtained results are in agreement with those reported by Gowri et al. [36]who attributed the elevation of AST and ALT activities to liver parenchymal cells damage [37].However, Bilgin et al. [38] related the increment in activities to the liver cell membrane damage lead to leakage of enzymes into the circulation; cytolysis process. Moreover, Hassan and Youssef [39], found that, rats received ACR showed significant increase in serum AST, ALT activities and this increment could be attributed to the damage in liver tissue as well as destruction in red blood corpuscles [40].With respect to total protein content, the current results demonstrated significant decrease in total protein content in the ACR induced rats. This observation is in accordance to the results of several authors [41,42],

Table 3
Antioxidants effect of SOAE and flavanones loaded silica nanoparticles on SOD and catalase enzyme activities in acrylamide-intoxicated rats.

Groups Parameters	Control	Controls-Treated			Acrylamide	Acrylamide-Treated			
		SOAE	Hes	Nar		SOAE	Hes	Nar	Sily
SOD	376.27 ± 78.55a	335.85 ± 28.01a	375.47 ± 14.08a	275.22 ± 17.83c	108.00 ± 9.09e	230.22 ± 39.76c	305.40 ± 58.16a	317.50 ± 45.45a	242.77 ± 21.22c
Mean +SD		10.72	0.28	26.90	71.29	38.81	18.83	15.61	35.50
%a						113.17	182.78	194.03	124.79
%b						32.48	52.52	55.70	35.81
%c									
Catalase	13.70 ± 2.52d	21.53 ± 1.19c	24.75 ±	22.70 ±	4.70 ±	20.97 ±	27.77 ± 3.13c	22.45 ±	22.87 ±
Mean +SD		57.21	0.20C	2.60c	1.51e	2.15c	102.81	1.32c	1.37c
%a			80.72	65.71	65.70	53.11	490.85	63.91	67.00
%b						34.62	168.42	377.66	386.60
%c						118.80		129.60	132.60

%a:%change as compared to normal control rats,%b:%change as compared to acrylamide intoxicated rats,%c:%of improvement. Hes: Hesperetin, Nar:Naringenin, Sily: Silymarin.
Data are represented by Means ± SD of ten rats in each group. Data are expressed as μmol/mg protein for SOD and unit/g tissue of brain for catalase enzyme. SPSS (version,8), and Co-Stat computer programs are used in statistical analysis where unmatched letters between groups are significant at $P \leq 0.05$.

Table 4
Antioxidants effect of SOAE and flavanones loaded silica nanoparticles on GSH and MDA levels in acrylamide intoxicated rats.

Groups Parameters	Control	Controls-Treated			Acrylamide	Acrylamide-Treated			
		SOAE	Hes	Nar		SOAE	Hes	Nar	Sily
GSH	757.5 ± 9.09e	1062.5 ± 35.94c	1233.75 ± 21.36b	727.50 ± 123.38e	307.50 ± 35.00f	1109 ± 24.51c	1320 ± 53.54a	777.50 ± 17.07e	865.00 ± 26.45d
Mean +SD		40.31	62.90	4.00	59.40	46.50	74.31	2.60	14.20
%a						260.81	329.27	152.85	181.30
%b						105.91	133.70	62.00	73.61
%c									
MDA	64.20 ± 2.52c	63.75 ± 4.11c	61.00 ± 1.82c	62.65 ± 1.92c	310.40 ± 33.54a	71.15 ± 1.84c	95.82 ± 4.85b	78.8 ± 10.99bc	68.05 ± 10.37c
Mean +SD		0.70	4.98	2.41	383.40	10.80	49.32	22.81	6.00
%a						77.08	69.13	74.61	78.08
%b						372.61	334.21	360.70	377.41
%c									

%a:% change comparing to normal rats,%b:%change comparing to acrylamide intoxicated rats,%c:%of improvement. Hes: Hesperetin, Nar:Naringenin,Sily:Silymarin.
Data are represented by Means ± SD of ten rats in each group. Data are expressed as μg/mg protein for glutathione and malondialdehyde. SPSS and Co-Stat computer programs are used in statistical analysis (version,8),where unmatched letters between groups are significant at $P \leq 0.05$.

Table 5
Effect of SOAE and flavanones loaded silica nanoparticles on LDH and SDH enzyme activities in the different experimental groups.

Groups / Parameters	Control	Controls-Treated			Acrylamide	Acrylamide-Treated			
		SOAE	Hes	Nar		SOAE	Hes	Nar	Sily
LDH	497.77 ± 132.38ab	480.02 ± 114.62ab	443.50 ± 82.08a	413.62 ± 20.16a	111.60 ± 12.09e	471.65 ± 62.44ab	536.55 ± 101.09ab	510.77 ± 63.01ab	569.30 ± 65.87ab
Mean +SD		3.57	10.9	17.00	77.61	5.21	7.79	2.63	14.42
%a						322.63	380.78	357.68	410.13
%b						72.30	85.37	80.21	91.90
%c									
SDH	326.20 ± 47.05b	333.20 ± 50.04b	340.00 ± 42.75b	336.10 ± 20.11b	135.91 ± 42.00e	551.90 ± 42.00c	429.31 ± 45.68a	499.30 ± 42.07c	404.21 ± 26.89a
Mean +SD		2.13	4.23	3.11	58.30	69.22	31.60	53.11	23.98
%a						306.11	215.88	267.40	197.41
%b						127.61	89.95	111.40	82.25
%c									

%a:% change comparing to normal contro lrats,%b:%change comparing to acrylamide intoxicated rats,%c:%of improvement. Hes: Hesperetin, Nar: Naringenin, Sily: Silymarin.
Data are represented by means ± SD of ten rats in each group. Data is expressed asμmol/mg protein/min for LDH and SDH enzyme activities. SPSS (version8) and Co-Stat computer programs, are used in statistical analysis, where unmatched letters between groups are significant at $P \leq 0.05$.

which found that, liver cirrhosis is accompanied by a fall in whole-body protein turnover which may be due to elevation in deamination of amino acids and impairment in cellular proteins building.

Sherlock and Dooley [43] as curtained perturbation in protein synthetic machinery in the liver. Furthermore, the lower protein level that is observed in ACR induced rats might be also related to formation of the toxic *N*-nitroso compounds lead to suppressing of oxidative phosphorylation as mentioned by Anthony et al. [44]. Also, thenitroso-compounds interact with cellular DNA, RNA and protein resulted in biochemical and physical alterations of these macromolecules [45]. The generation of free radicals as indicated by increased LPO, and decreased antioxidants are considered to play an important role in the toxic effects of ACR compound [46]. Treatment of ACR intoxicated rats with naringenin nanoparticle exhibited improvement percentages by74.81, 250.00 and 29.31%respectively for AST, ALT and total protein content. However, ACR intoxicated rats treated with hesperetin nanoparticle demonstrated enhancement percentage reached to 66.11, 932.71and19.42%, respectively. In addition, ACR intoxicated rats treated with SOAE nanoparticle showed improvement percentages78.70,303.11 and 34.51%, respectively. These ameliorations of the three nanoparticles may be attributed to several beneficial effects, including improvement of vascular function [47], cardioprotective effects [48] neuroprotective effects [49] stimulation of uptake of glucose in adipocytes and hepatocytes [50], protection against oxidative damage of tissue, and efficacy as an antioxidant agents, all of which provide a promising strategy for the treatment of hepatic disorders [51]. Thus, the healing role of naringenin, hesperetin and SOAE nanoparticles may be related to their antioxidant effect that preserved liver enzymes and architectures [52]. In the present study, phytochemical screening of the SOAE shows the presence of various phytochemicals. The extract was observed to contain steroids, flavonoids, saponins, tannins, carbohydrates, and terpenoids. Alkaloids and cardiac glycosides are reported as minor constituents. Generally, phytochemicals are known to confer certain health benefits such as anti-inflammatory, cardioprotective and neuroprotective effects [53,54]. Citrus fruits are an abundant source of various flavonoids, which have been possessed different pharmacological effects and therapeutic usefulness [55]. Some of these, have antioxidant effect and inhibit free radical-mediated mechanisms due to their phenolic structures. Among these citrus flavonoids are naringenin and hesperetin. Naringenin and hesperetin had recently received considerable attention as a dietary supplement. Naringenin has a critical effect as an antioxidant by controlling SOD, catalase, and GSH gene expression [56]. Besides, naringenin has been found to have several biological effects as antimicrobial, antimutagenic, anticancer and anti- inflammatory [57]. Hesperetin is known to act as an antioxidant, anti-inflammatory, and neuroprotective agent. Therefore, the present study has been constructed to illustrate the possible mechanisms of them in protecting physiological changes induced by toxicity of ACR in rats. The present study declared, significant decrease in GSH level, catalase and SOD antioxidant enzyme activities comparing with control rats. Significant increase in catalase, SOD activities and GSH level were detected in intoxicated rats treated with the three nanoparticles. In addition, the present data declared that, reduction in enzymatic/non- enzymatic antioxidants was associated with significant increase in MDA in ACR intoxicated rats comparing with normal control rats. The reduction in hepatic GSH level was noticed to be occurred after induction with ACR, GSH has a role in the mitochondrial function regulation and the mitochondria GSH low contents have been connected with a decreased tolerance of these organelles to destruction insults. Hence, elevating GSH pool may ameliorate function of mitochondria and regeneration of liver. In a disagreement with the present study, Halliwell [58] and Maiese et al. [14] found that, in the ACR intoxicated rats, marked decrease of oxidative destruction to macromolecules such as lipids and proteins combined with high content of GSH was detected. These alterations are indicative of low oxidative stress in ACR intoxicated rats.

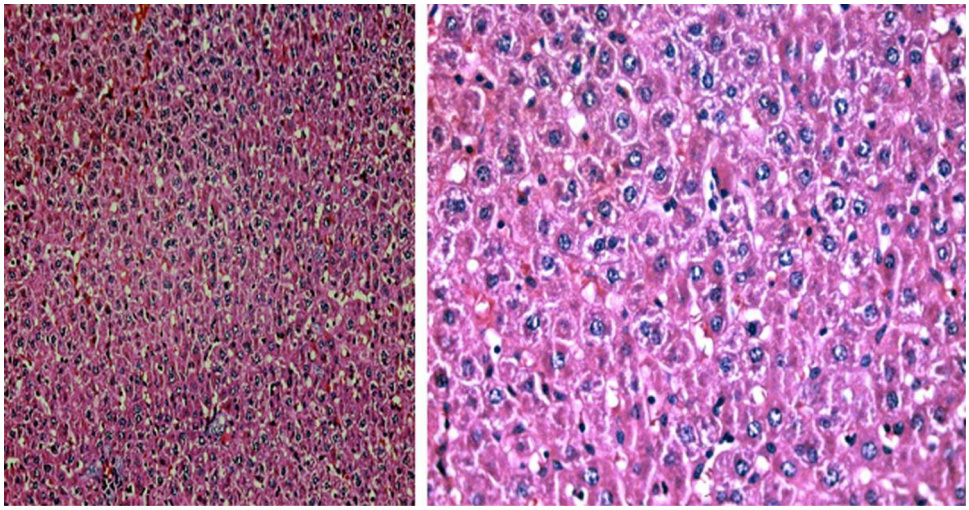

Photo 1. Hematoxylin and eosin stained section of liver of normal control rats showed preserved (intact)lobular hepatic architecture and insignificant pathology changes(H&E, x200, x 400).

Also, in contradictory with the present findings, significant decrease of lipid peroxidation after intoxication with ACR. The elevated lipid peroxide in the current research may be related to liver damage after ACR intoxication [59,60]. It has been suggested that during oxidative stress, the body has mechanism of defense to protect against damage elaborated by the free radical. This is attenuated by endogenous system of enzymatic and non-enzymatic antioxidants, superoxide dismutase, catalase as well as GSH.

An inverse association between the efficiency of the antioxidant system and lipid peroxidative event has been established. In the present study, it was probable that pre-treatment with different natural antioxidants as flavanones boosted the antioxidant system and showed antitoxic hepatoprotective action [61].

In the current study, silymarin, naringenin, hesperetin and SOAE supplementations markedly improved SOD, catalase activities and level of GSH by ROS scavenging activity as well as by inhibiting lipid peroxidation, speculating the properties of antioxidant of both flavanones. In addition, the present study shows that, significant decrease in liver marker enzymes LDH and SDH in ACR intoxicated rats liver while, these enzymes exhibited significant increase in intoxicated rats treated with the three selected nanoparticles. In concomitant with the current result, Ansari et al. [62] declared that one of the most characteristics sign of hepatic dysfunction is the marked reduction in the activity of LDH enzyme due to the enzyme leakage to the blood stream. The authors added that, the determination of LDH enzyme is considered as an important quantitative biomarker for the extent and type of hepatic damage. Singh and Reen [63] demonstrated that, toxicity of ACR elicit damage of membrane which leads to leakage of enzyme to blood stream and. Furthermore, toxicity resulted from ACR induced -LDH time dependent leakage in the medium, so that ACR- treated cells for 24 h showed an elevated leakage of LDH about 30%in the medium which increased to 43%. Further, ACR initiates mitochondrial impairment function as documented by the decreased SDH activity. This action either may be related to either inhibitory effect of ACR on this enzyme or decrease in the number of viable mitochondria per cell. These findings are also consistent with previous evidence of ACR induced mitochondrial degeneration in rodent models [64]. It is well accepted that depletion of energy and dysfunctions of mitochondria apart from oxidative stress are vital factors connected with most of the mechanisms of neuro-degeneration [65]. Considering liver mitochondria ATPase, the present study shows, significant decrease in this enzyme activity in ACR intoxicated rat. ATPase microsomes are the major cellular biosynthetic sites [66]. The inhibition in ATPase in ACR treated rats may be due to the high affinity site of hepatic ATPase was damaged by toxicity of ACR. The in situ functions of mitochondrial ATPase is ATP synthase [67] and the three ATPase catalytic sites are known to be attributed to the requirements of physiological energy [63]. In correlation with the present results, Vermeulen et al. [68] found that 35% reduction in

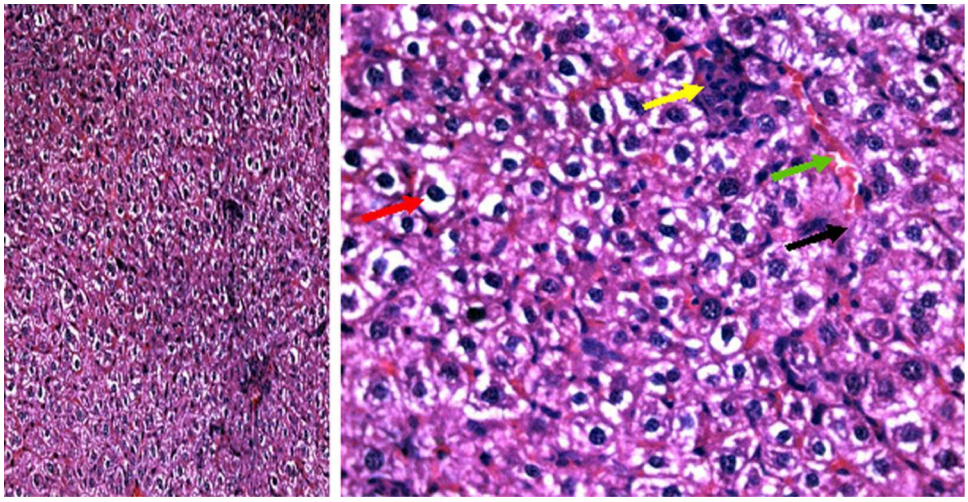

Photo 2. Hematoxylin and eosin stained section of liver of acrylamide intoxicated rats showed preserved (intact) lobular hepatic architecture and with mild lobular inflammation (yellow arrows), mild hydropic degeneration (red arrow) and thin fibrous tissue bands(black arrow) and congested blood vessels(green arrow) (H&E, x200).

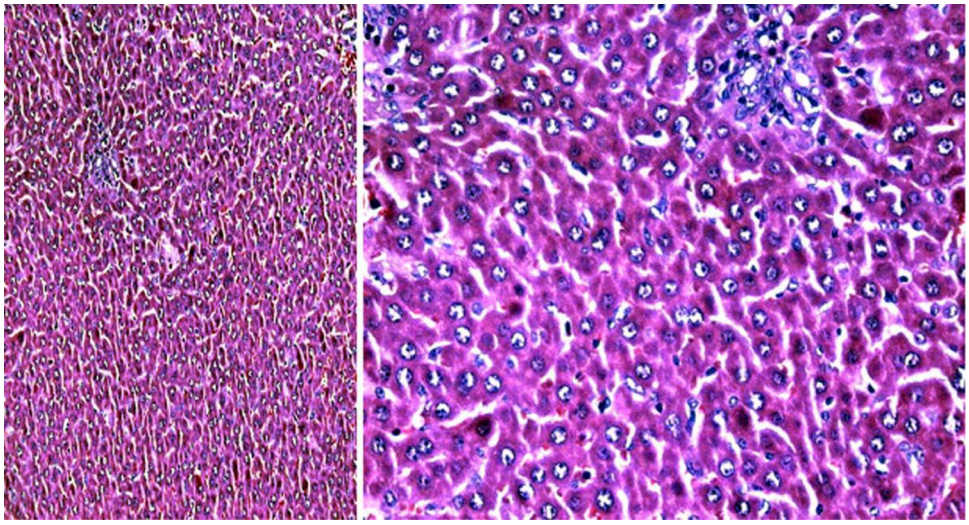

Photo 3. Hematoxylin and eosin stained section of liver of control rats treated with nanoparticles of SOAE showed liver with preserved (intact) lobular hepatic architecture and insignificant pathology changes(H&E, x200,x 400).

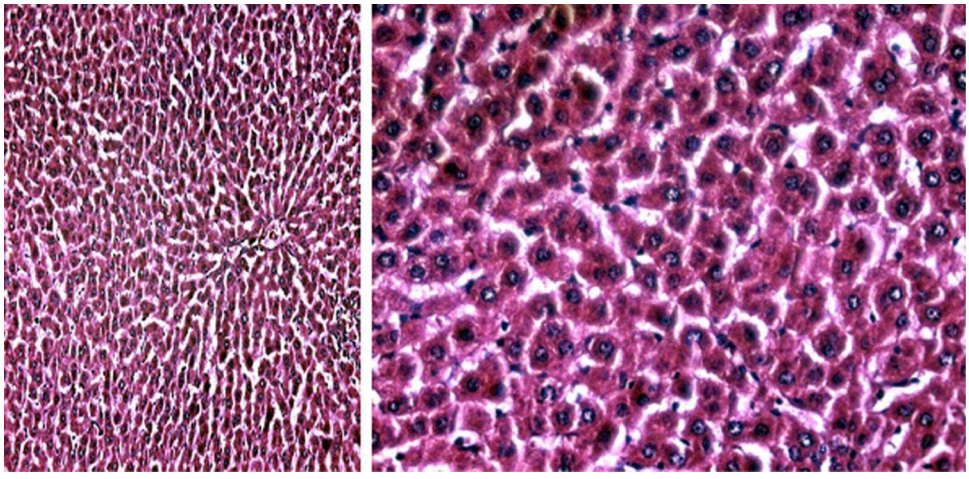

Photo 4. Hematoxylin and eosin stained section of liver of control rats treated with nanoparticles of naringenin showed liver with preserved (intact) lobular hepatic architecture and insignificant pathology changes(H&E, x200,x 400).

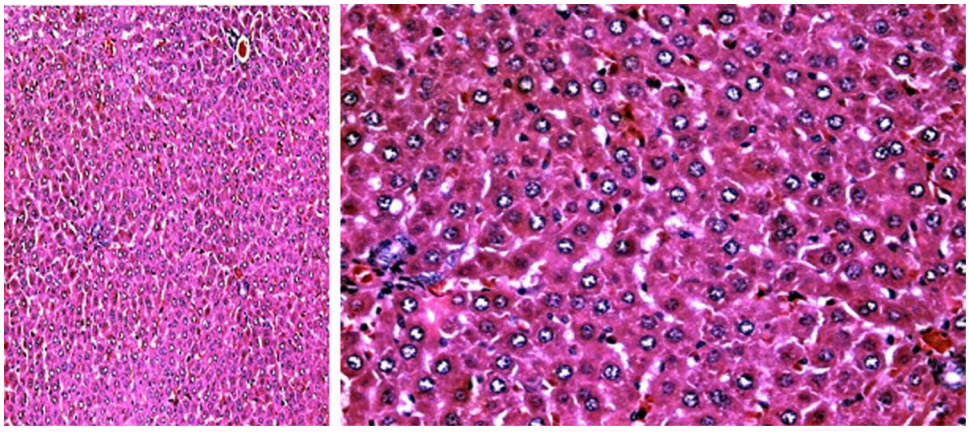

Photo 5. Hematoxylin and eosin stained section of liver of control rats treated with nanoparticles of hesperetin showed liver with preserved (intact) lobular hepatic architecture and insignificant pathology changes(H&E, x200,x 400).

mitochondrial ATPase activity occurred upon treated mitochondrial cells with ACR. The various mechanisms suggested that, ACR-induced hepatotoxicity. These include; change in lipid composition of membrane and/or content, high level of oxidative stress, alterations in homeostasis of calcium, oxidation and thiol groups alkylation of glutathione and proteins [68]. In addition, Frahat et al. [69] proposed that ACR- induced inhibition activity of ATPase by finding most of the mitochondrial phospholipids (except diphosphatidyl- glycerol)are

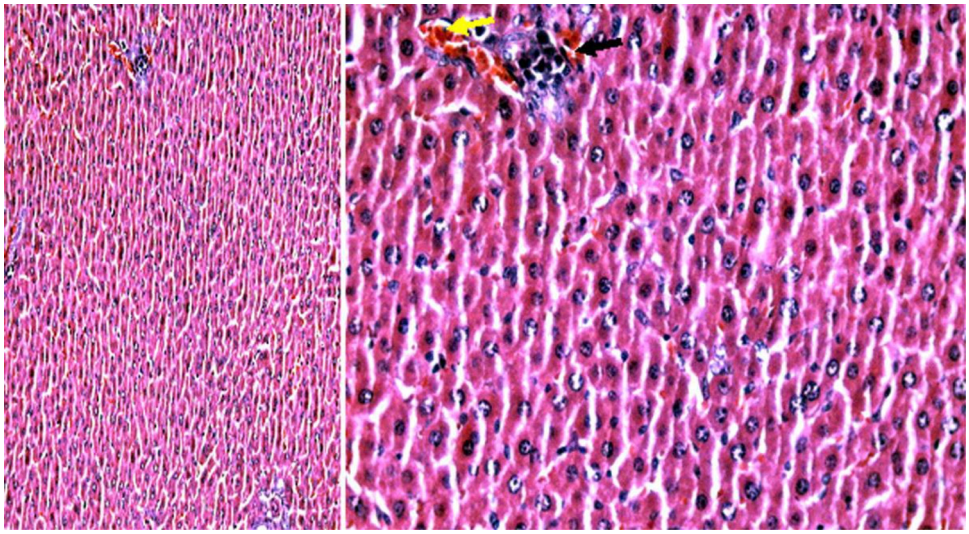

Photo 6. Hematoxylin and eosin stained section of liver of rats treated with nanoparticles of SOAE post-ACR administration showed liver with preserved (intact) lobular hepatic architecture and mild interlobular inflammation(black arrow)and blood congestion (yellow arrow), (H&E,x200,x400).

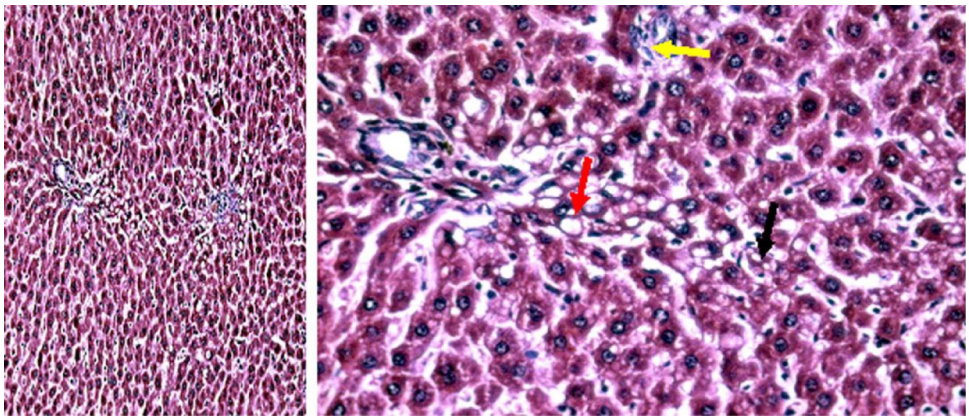

Photo 7. Hematoxylin and eosin stained section of liver of rats treated with nanoparticles of naringenin post-ACR administration showed liver with preserved (intact) lobular hepatic architecture and mild interlobular inflammation (black arrow) and sinusoidal congestion (red arrow), (H&E,x200,x400).

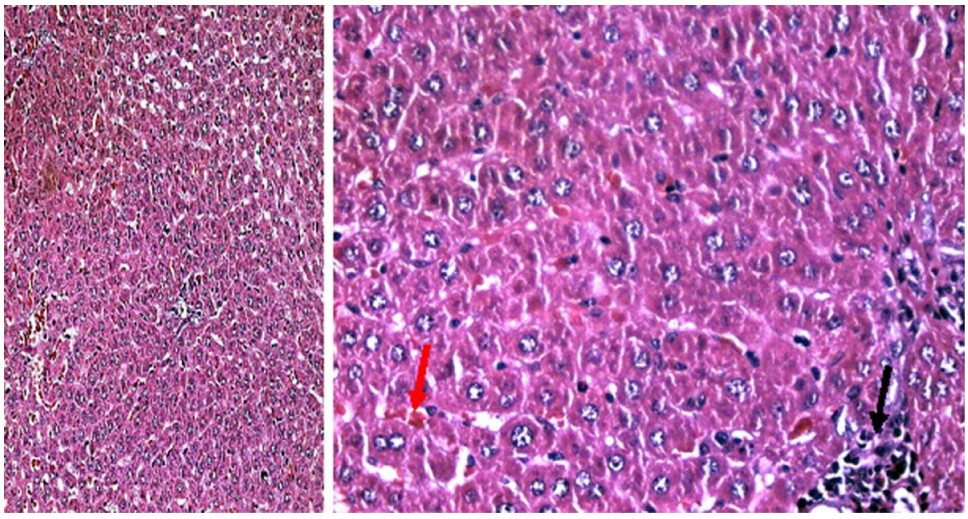

Photo 8. Hematoxylin and eosin stained section of liver of rats treated with nanoparticles of hesperetin post-ACR administration showed liver with preserved(intact)lobular hepatic architecture and mild interlobular inflammation (black arrow)and sinusoidal congestion (red arrow),(H&E,x200,x400**).**In addition, intoxicated rats treated with silymarin showed liver with preserved (intact)lobularhepatic architecture and insignificant pathological changes (Photo 9).

synthesized in the endoplasmic reticulum. Increased content of total phospholipid which noticed in mitochondria of animals treated with ACR could not be attributed to increased transport from the endoplasmic reticulum in the content of cholesterol really decreased. Treatment with ACR has been known to destruct cristae of mitochondrial membrane from which the leak out of enzymes are produced [7]. Hence, the total phospholipid content would deceptively look higher due to protein loss. Elevation in lipid peroxidation as a result of

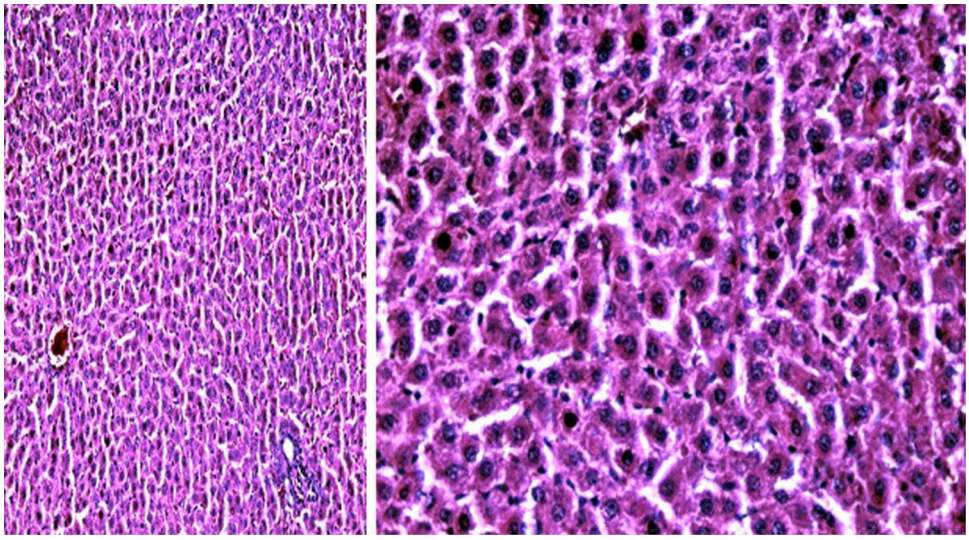

Photo 9. Hematoxylin and eosin stained section of liver of rats treated with silymarin post-ACR administration showed liver with preserved (intact) lobular hepatic architecture and insignificant pathology changes (H&E, x200,x 40).

oxidative stress induced by ACR is another possibility. However, sever illness of investigation have declared a marked dissociation between toxicity and lipid peroxidation [70,71]. Previously, ACR was shown to affect production of energy by glycolytic pathway inhibition in both in vitro and in vivo models [72,73]. Interestingly, we observed significant increase in reactive oxygen species, MDA and hepatic protein levels proposing the responsibility of ACR to initiate oxidative stress in hepatic tissues at high levels [74],the principal findings such decreased levels of GSH and diminished activity of SOD clearly illustrated that the hepatic tissue of rats are subjected to oxidative stress [75]. Therefore, it is thought that the ameliorative effects of three nanoparticles; sour orange albedo extract, hesperetin and naringenin against acrylamide induced oxidative damage of liver tissue may be attributed to the fact that these nanoparticles act as anti-inflammatory agents such as decreased levels of inflammatory cytokines. The beneficial effects of three nanoparticles against acrylamide induced hepatotoxic damage may be also associated with antioxidant properties. The current result also declared insignificant difference in all biomarkers under investigation in control rats supplemented with the three selected sample loaded silica NPs as compared to control rats. In these aspects Kim et al. declared that 50-nm silica-coated magnetic NPs cause no toxicity after intraperitoneal injection [76]. Nevertheless, because NPs of silica are principle nanomaterials in the field of biomedicine application, several steps must be more carefully evaluated in preclinical studies such as biocompatibility, long-term safety and clearance of well-characterized NPs of silica, before they could be used in clinical applications. Thus, we suggested that, SOAE, naringenin and hesperetin flavonoids, can prevent and protect against acrylamide toxicity in terms of liver damage and cytokine levels [73]. Hence, it could be concluded that, administration of, albedo extract, hesperetin and naringenin nanoparticles markedly ameliorate liver enzyme activities, oxidative damage, ATPase enzyme activity and liver biomarker enzymes(LDH and SDH) post-ACR administration. Beside normalization in hepatic architecture was examined.

5. Conclusion

Naringenin, hesperetin and sour orange albedo extract nanoparticles administrated to acrylamide intoxicated rats have the abilities to down regulate free radicals elevation, improve liver functions, ameliorate hepatic markers, and normalize hepatic cells architecture. The tested samples nanoparticles administered post–acrylamide treatment recorded fluctuating improving percent in the majority of these biomarkers. So, naringenin, hesperetin and albedoextract nanoparticles may be used as a new safe medication that may delay progression of hepatic damage and its complication

Transparency document

The Transparency document associated with this article can be found in the online version.

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