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Evaluation of Therapeutic Effect of Rice Husk Silica Combined with Platelets Derived Growth Factor in Hepatic Veno-Occlusive Disease

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Abstract

Veno-occlusive disease is an important pattern of hepatotoxicity associated with antineoplastic drugs. The study aimed to investigate the possible therapeutic impact of rice husk silica (RHS) nanoparticles combined with a platelet-derived growth factor (PDGF) on the Veno-occlusive disease in liver (VOD) elicited by dactinomycin (DAC) in rats. In this work, nano silica (SiO₂) was successfully prepared from rice husk and its physicochemical characteristics were investigated using energy dispersive X-ray (EDX), X-ray diffraction (XRD), N₂ adsorption-desorption isotherm, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques.

Forty-eight male Sprague-Dawely rats were classified into six groups, 8 rats each. The first group served as control, the second, animals were infused by intraperitoneal injection with DAC (0.015 mg/kg; 1-3 days IP). The third group, rats were injected IP with DAC and then at 24 h followed the last dose of DAC received nano RHS incorporated with PDGF twice a week for four weeks. The fourth group, normal animals were injected with RHS. The fifth group normal rats were received with PDGF, and the sixth group normal rats were received nano RHS incorporated with PDGF. The prepared nano silica showed type II adsorption isotherm characteristic for mesoporous materials with specific surface area of 236 m²/g. TEM imaging proves the production of nanoparticles via the followed preparation procedure. Radical scavenging potential for nano RHS was determined using two different in vitro assays: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals. The results of this work showed that administration of nano RHS joined with PDGF significantly reversed the oxidative stress effects of DAC that was evidenced by decreasing in liver function. It could be concluded that the nano RHS combined with PDGF is
useful in preventing of oxidative stress and hepatic Veno-occlusive induced by receiving chemotherapy such as DAC.

Keywords: Veno-occlusive, rice husk silica, nanoparticles, dactinomycin, platelet growth factor, scanning electron microscopy, transmission electron microscopy.

Introduction

The reasons for venous-occlusive sickness is still vague, be that as it may, a blend of pre-transplant danger components and transplant-related conditions are accepted to trigger a fundamental hepatic sinusoidal harm. This can rapidly stretch out to a hepatocytic and panvasculitic ailment, which is trailed by multiorgan disappointment that is connected with generous mortality (Cesaro et al. 2005). The starting pathophysiological occasions have incited this type of liver illness be renamed sinusoidal obstacle disorder (SOS) (Corbacioglu et al. 2006). This can rapidly reach out to a hepatocytic and panvasculitic infection, which is trailed by multiorgan disappointment that is connected with considerable mortality. The starting pathophysiological occasions have incited a pattern of liver malady be renamed sinusoidal hindrance disorder (Miyata et al. 2011). These are hepatomegaly, ascites and/or weight increment, jaundice caused by hepatocyte necrosis and damage to the venous endothelium and intrahepatic sinuses.

It has been shown that the vanishing of the typical filtration boundary in cirrhotic livers results in a hindered bidirectional trade between the sinusoidal blood and parenchymal cells (DeLeve, 2011). Accordingly, capillarization of the sinusoidal endothelium might be a noteworthy giver to hepatic disappointment in patients suffer from cirrhosis. Rice husk is an agricultural waste from the rice milling process. Getting rid of this waste either by land filling or burning can cause
harmful impacts on both environment and human health, so it is recommended to utilize this waste in both ecofriendly and economic manners (Cheng et al. 2012; Totlani et al. 2012). When optimum conditions are achieved, pyrolysis of rice husk in an oxygen atmosphere can produce white rice husk ash; the latter mostly consists of pure silica (Mariana et al. 2013). Silica (SiO$_2$) obtained from rice husk had proven their suitability in different applications such as wastewater treatment (Adam et al. 2012 and 2013; Yener & Helvaci, 2015), cement and concrete industry (Antiohos et al. 2014; Carreno et al. 2015) and thermal insulation (Sembiring et al. 2016).

PDGF as the second imperative cytokines required in angiogenesis (Gotink & Verheul, 2010), encourage the enrollment of pericytes and smooth muscle cells and are vital for the sprouting and the capacity of the vasculature (Jain, 2003).

This growth factor has been recognized as the strongest polypeptide growth factor able to reinforce the proliferation of culture-activated HSC. Moreover, PDGF isoforms are disulphide-reinforced dimers of either AA, AB, or BB polypeptide chains. Their impacts on target cells are interceded by dimeric transmembrane receptors made out of and/or subunits with inherent protein-tyrosine kinase activity (Lindblom et al. 2003).

The subunit links both the A and the B- chain of PDGF with high affinity, though the subunit binds the B- chain only (Lindblom et al. 2003). PDGF is accepted to assume a vital part in the cell reaction to tissue damage, both as a stimulant of mesodermal cell development and activity and as a chemoattractant to different cells required in the repair process (Eppley et al. 2004).

In this part, PDGF seems to cooperate with Transforming Growth Factor-Beta-1 (TGFβ1), which is discharged by degranulating platelets at the wellspring of the harmed tissue (Eppley et al. 2004). The sources of PDGF amid wound repair incorporate platelets (prevalently PDGF-AB),
smooth muscles (PDGF-AA) (Eppley et al. 2004) monocyte-derived macrophages (PDGF-BB), and endothelial cells (PDGF-BB) (Floege et al. 1993; Roscioli et al. 2006).

Because defenestration and cellar layer arrangement result in a scattered exchange between the sinusoidal blood and hepatocytes, it is important to reestablish the ability of liver sinusoidal cells keeping in mind the end goal to switch cirrhosis. PDGF provides the perfect means to achieve this, on account of it advances fenestration and penetrability (Roscioli et al. 2006).

Moreover, the neovascular response stimulated by the BB homodimeric form of recombinant PDGF-BB was evaluated for its capacity to protect tissue from necrosis (Brown et al. 1995). On account of these ideas, we studied the prospective effectiveness of the silica nanoparticles prepared from the rice husk joined with PDGF in the curing of VOD in rats.

Materials and Methods

Ethics Statement

The study was a convoy correspondingly to the ethical guidelines set by the European Economic Community (EEC) regulations (Revised Directive 86/609/EEC) and approved by the Ethical Committee at National Center for Radiation Research, Egyptian Atomic Energy Authority, Cairo, Egypt (NCRR-EAEA).

Animals

Forty-eight male Sprague-Dawely rats (180-200 g) were procured from the animal farmstead of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Rats were housed in a healthy environment, at a temperature of 25°C with alternatively 12 h light and dim cycles.
Animals were acclimated for one week before experimentation. They were preserved on a standard diet and water *ad libitum*.

**Experimental Design**

After one week of adjustment, the animals were split into six groups. Eight rats were utilized as a control group without treatment (GI). Sixteen underwent VOD, these animals were injected with Dactinomycin (0.015 mg/kg 1-3days IP); it corresponds with induction chemotherapy scheme applied in children with nephroblastoma *(Borowska et al. 2003)*, eight of these animals left without treatment throughout the period of the trial (GII) and after 24 h of the last dose of DAC, the other eight of these animals were injected with nano RHS (100 mg/kg BW) incorporated with PDGF (17 units/100g), twice a week for four weeks (GIII). Whereas, the fourth group normal rats were injected with nano RHS (100 mg/kg BW), two times a week for four weeks (GIV). Fifth group, normal rats were injected with PDGF (17 units/100 g), twice a week for four weeks (GV). The sixth group (GVI) normal rats were injected with nano RHS combined with PDGF with the same manner of GIII.

**Preparation of nanosilica from rice husk**

Rice husks were collected from an Egyptian farm. Hydrochloric acid 34% was supplied by El Nasr for pharmaceutical chemicals, Abu Zaabal, Egypt.

The rice husk agricultural waste was first grinded repeatedly by a 150 watt, stainless steel blade grinder, then refluxed with a hydrochloric acid solution (17%) with a ratio of 1:10 w/v for 1 hour. Then the treated rice husks were filtered and washed several times with distilled water,
followed by drying overnight. Afterwards, the dried rice husks were calcined at 650°C for 2 hours and were subjected to sudden cooling.

Characterization of nano rice husk silica

The resulted white silica powder was characterized by energy dispersive x-ray technique using JOEL JSM-6510LA, as well as x-ray diffraction technique using the PHILIPS® MPD X'PERT diffractometer which has the Bragg-Brentano geometry. The X-ray tube used was a Copper-tube operating at 40 KV and 30 mA, the divergence slit angle was 0.5°, the receiving slit was 0.1°, step scan size 0.03 and the scan step time was 2 seconds.

Adsorption–desorption isotherm of purified N₂ at 77 K was carried out using Nova 2000, Quanta Chrome (commercial BET unit) that allowed prior outgassing to a residual pressure of 10⁻⁵ Torr at 150°C overnight to remove all moisture adsorbed on silica surface and pores. The calculation of pore size distribution was carried out using Barrett-Joyner-Halenda (BJH) method.

The external morphology of rice husk silica was observed using scanning electron microscope (SEM) FEI Quanta 250 FEG while its particle shape and size were investigated by transmission electron microscopy (TEM) using a JEOL JEM-1230 microscope.

2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity for nano RHS

In order to evaluate the in vitro free radical scavenging activity of nano RHS, modified DPPH and ABTS assay were used (Serpen et al. 2007). In its radical form, DPPH has an absorption band at 517 nm, which disappears upon reduction by an antioxidant nano particles or a radical.
species. For the photometric assay, different volumes of the nano RHS were taken in different test tubes. The volume was adjusted to 100 L with methanol, 5 mL of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Results were compared with the activity of ascorbic acid. The capacity to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging activity (inhibition %) = (Ac-As/Ac) x 100

Where, Ac: the absorbance of the control reaction; As: the absorbance in the presence of the sample.

2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Assay for nano RHS

ABTS was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. Then, 2 mL of the diluted ABTS solution was added to the sample varying concentrations of nano RHS. The blank contained water in place of nano RHS. After 30 min of incubation at room temperature, the absorbance was recorded at 734 nm and compared with standard ascorbic acid. The highest antioxidant capacity of the sample exhibited a smaller production of free radicals.

Inhibition (%) = (A₀-A₁/A₀) x 100

Where, A₀ is ABTS absorbance of the control reaction; A₁ is the ABTS absorbance in the presence of the sample.

Pharmacological study acute toxicity (LD₅₀) testing for nano rice husk silica
Determination of acute toxicity ($LD_{50}$) was accomplished using the method of (Lorke, 1983). Fourteen rats were used for this testing for the determination of $LD_{50}$ in two phases. In the initial one, the rats were split into three categories of 5 rats each. They were administered 10, 100 and 1000 mg/kg of the nano rice husk silica IP. The rats were observed for 48 hours for any mortality. In the other phase, the rats were coordinated into three of five rats each and treated with the extract at varying dosages (1600, 2900 and 5000 mg/kg). The animals were noticed as the first phase, and the final $LD_{50}$ value was determined from the minimum concentration (full death) and maximum concentration (no death) of the dose.

$$LD_{50} = \frac{M_0 + M_1}{2}$$

Where, $M_0$ = Highest dose of test substance that gave no mortality,

$M_1$ = Lowest dose of test substance that gave mortality.

**Collection of blood and liver tissue samples**

At the termination of 4th week, blood specimens were collected from all groups by heart puncture into heparin-coated and dry tubes. These samples were centrifuged at 3000 RPM for 30 min for obtaining plasma and sera.

**Liver homogenization**

The liver tissue was excised and suspended in a physiological saline which contained heparin 0.16 mg/ml to remove blood clots. Liver tissue (0.5g) was added to 4.50 ml of the homogenizing buffer 20 mM Tris, 1ml M EDTA, HCL pH 7.4 and homogenized using a glass Homogenizer. The homogenate of liver was centrifuged at 6000 RPM at 4°C for 15 min. The supernatant was stored as aliquot at -70°C for hepatic analysis.
Recombinant, Human Platelet-Derived Growth Factor (rhPDGF)

Lyophilized of rh-PDGF purchased from Sigma Aldrich as from a 0.2 µm filtered solution in phosphate buffered saline containing 0.5 mg bovine serum albumin, MDL number (MFCD01324253 Product No. P 5208).

The animals were received an intraperitoneal injection (IP) of PDGF (17 units/100 g weight) (Reed et al. 1987), twice a week for four weeks.

Befittingly diluted conditioned media were measured for tumor necrosis factor-α (TNF-α) using a commercial rat enzyme-linked immunosorbent assay (ELISA) kit.

Serum biomarkers of liver damage

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Reitman & Frankel, 1957) and bilirubin (TB) were performed spectrophotometrically utilizing an automatic biochemical analyzer (Instrumental Laboratory, Holliston, MA, USA). ALP, total protein, albumin and GGT (Roy, 1970; Bradford, 1976; Doumas et al. 1971; Tietz, 1994) respectively, were measured in serum.

Determination of serum VEGF levels

Plasma levels of VEGF were determined by a commercially available ELISA kit (R&D Systems), according to the manufacturer's instructions (Werther et al. 2002).

Plasma NO according to (Miranda et al. 2001), Thyroid hormones (T₃ & T₄) were determined by using the solid phase radioimmunoassay (RIA).

Determination of Lipid Peroxidation
The lipid peroxidation was estimated by measuring the amount of thiobarbituric acid-reactive substances (TBARs). In Brief, 0.5 g of liver tissue obligingly minced in 4.5 ml of 0.25 M sucrose. The minced tissues gently homogenized afterward, centrifuged at 2000 RPM for 30 min. Then, 0.1 ml of the supernatant was taken and treated with a buffer, including 0.75 ml of thiobarbituric acid (TBA; 0.8%, w/v), 0.75 ml of 20% acetic acid with pH = 3.5, and 0.1 ml of sodium dodecyl sulfate (8.1%, w/v). The solution was mingled up with 2 ml of distilled H2O and heated in a boiling water bath for 60 min. Then, the absorbance was determined at 532 nm by Unicam UV-Visible Spectrometry Helios, United Kingdom (Onkawa et al. 1979).

GSH Determination

The procedure of Ellman (1959) was used to detect the reduced glutathione. In brief, 100 mg of hepatic tissues was homogenized within a buffer, including EDTA (0.2 M) to get 4% (w/v) whole homogenate. Then, 1.5 ml of the suspension was mixed with a buffer, including 2.5 ml distilled H2O and 0.5 ml of 50% TCA. Then, the blend was centrifuged at 3000 RPM for 15 min and 1 ml of the supernatant mixed with 1 ml of Tris buffer (0.4 M, pH = 8.9) and 0.1 ml of DTNB (0.01 M). The absorbance was measured after 5 min at 412 nm using Unicam UV-Visible Spectrometry Helios, United Kingdom.

Determination of superoxide dismutase and glutathione S-transferase (SOD and GST) activities in liver tissues

The Cytosol portion of the liver was used in this assay as formerly described in Kakkar et al. (1984). This portion (0.05 ml) was added with sodium pyrophosphate buffer (0.052 M, pH 8.3, 1.2 ml), phenazine methosulphate (0.186 mM, 0.1 ml), nitro blue tetrazolium chloride (0.3 mM, 11
0.3 mL), and NADH (0.78 mM, 0.2 ml). The reaction was ceased after 90 second with glacial acetic acid. The color intensity of the chromogen was extracted in butanol solution (2.0 ml) and shake strenuously. The resulted combination was centrifuged at 3000 RPM for 10 min and the supernatant was measured at 560 nm while the absorbance of the GST was recorded at 340 nm according to Habig et al (1974).

**Determination of Catalase (CAT) Activities in liver tissues**

The catalase enzyme activity in tissues was assayed following the procedure of Sinha (1972). Homogenate (0.1 ml) was incubated with H2O2 (0.2 M, 0.5 ml), in the existence of 0.01 M phosphate buffer (pH 7.4). Afterwards, 5% dichromate solution was added, the reaction was stopped. After that, samples were incubated in boiling water for 15 min. The absorbance of upper layer of the mixture was read at 570 nm.

**Detection of collagen and NF\(_{\kappa}\)B gene expression using real time PCR (RT–PCR)**

**RNA extraction**

Total RNA was detached from cardiac tissue homogenates utilizing RNeasy purification reagent (Qiagen, Valencia, CA) consistent with manufacturer’s instruction. The pureness (\(A_{260}/A_{280}\) ratio) and also the concentration of RNA were obtained utilizing spectrophotometry (Gene Quant 1300, Uppsala, Sweden). RNA quality was affirmed by gel electrophoresis.

**Synthesis of cDNA**

First-strand cDNA was synthesized from 4 \(\mu\)g of total RNA using an Oligo(dT)12-18 primer and Superscript\(^{\text{TM}}\) II RNase Reverse Transcriptase, this mixture was incubated at 42\(^{\circ}\)C for 1 h, the kit was provided by (Life Technologies, Breda, the Netherlands).
Real-time quantitative polymerase chain reaction (RT-PCR)

RT-PCR amplification was meted out victimization 10 µL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA USA), equal to 8 ng of reverse-transcribed ribonucleic acid and 300 nM primers, Table 2 shows the sequences of DNA primer pairs used for each gene. Reactions were performed by an ABI PRISM 7900 HT detection system (Applied Biosystems) PCR reactions consisting of 95°C for 10 min (1 cycle), 94°C for 15 s, and 60°C for 1 min (40 cycles), data were resolved with the ABI Prism sequence detection system software and quantified using the v1.7 Sequence Detection Software from PE Biosystems (Foster City, CA). We calculated the relative expression of genes utilizing the comparative threshold cycle method. Moreover, all values were normalized to the glyceraldehyde-3-phosphate dehydrogenase (GADPH) (Livak & Schmittgen, 2001). The sequences of primers are described in Table 2.

Western blot analysis for TGFβ-1

The dismembered liver tissues were homogenized in fluid nitrogen with a denaturing lysis support containing 20 mM Tris (pH 7.4), 2% SDS supplemented with 1% phosphatase repression, Cocktail II (Sigma-Aldrich, USA) and protease inhibitor blend (Roche, Indianapolis, IN, USA). After a short period of incubation (5 min at 95°C), the lysates were quickly sonicated and centrifuged (15,000 g at 4°C for 15 min) to expel insoluble materials. We determined the protein focus in the supernatant utilizing the BCA protein examine Kit (Rockford, USA). Equivalent amounts of protein (15 µg per sample for TGFβ-1 were separated on 12% SDS-polyacrylamide and exchanged to polyvinylidene difluoride transfer membranes (Millipore Co., USA). The membranes were blocked with 4% non-fat dry milk in Tris-buffered saline containing...
Tween-20 (TTBS) and incubated with the primary Ab (rabbit anti-TGFβ1 pAb, 1:10 000 at 4°C overnight. After three washing steps with TTBS, the membranes were incubated with horseradish peroxidase-conjugated anti-rabbit secondary Ab (1:3000, Novocastra UN) for one hour at room temperature. The signal was imagined utilizing the upgraded chemiluminescence (ECL) detection system and ECL hyperfilm (Amersham Biosciences). All layers were stained with Ponceau S (Sigma-Aldrich) as well as, to check break even with protein stacking. TGFβ-1 bands were scanned and analyzed using the quantification software (Quantity one, Bio-Rad laboratories, USA). Protein magnitudes were expressed as arbitrary units. We used β-Actin as a stacking and the obtain bands were scanned.

Histopathological studies

Liver tissues were removed from the animals and fixation was done in 10% formalin for about 24 hours. Then, they paraffin sections were cut into 4-µm thick. The sections then were stained with Hematoxylin-Eosin dye and examined by light microscopy (Bancroft & Stevens 1996).

Statistical Analysis

The parameter values were all expressed as the mean ± SEM. Significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using SPSS 22.0 software package program. The significant results were reported if the p value is $P< 0.05$.

Results

In vitro results
Figure 1 exhibits the XRD pattern in addition to the EDX spectrum (inset) of the prepared silica. Fig 2 represents the N₂ adsorption-desorption isotherm of the prepared silica as well as its pore size distribution (inset). Other textural parameters are presented in Table 2. The morphology and the size and shape of prepared silica particles were examined using SEM and TEM images shown in Fig 3.

**DPPH scavenging activity for nano RHS**
The antioxidant activities were determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical. The DPPH scavenging activity of nano RHS was determined by its absorbance at 570 nm, which is due to the presence of antioxidants. The percentage of DPPH radical scavenging activity of nano RHS were expressed in (Fig. 4A). This shows the maximum DPPH scavenging activity of nano RHS were 80.12 % at 100 µg/ml whereas ascorbic acid (standard) was found to be at 84.35 % at 100 µg/ml.

**ABTS Scavenging activity nano RHS**
The percentage of ABTS radical scavenging activity of nano RHS was expressed in (Fig. 4B). nano RHS displayed a maximum ABTS scavenging activity of 75.24 % at 100 µg/ml, whereas for ascorbic acid (standard) it was found to be 82.20 % at 100 µg/ml.

**In vivo results**

**Mortality and adverse effects in the nano rice husk silica combined with PDGF treated and control each other and sham- rats**

None of the nano rice husk silica combined with PDGF-treated or controls each other rats died during the experimental period. From the acute toxicity study of nano rice husk silica, according
to Table 3, it would be observed that the LD50 was 1600 mg/kg, which implies that at this concentration; fifty percent of the animal population can be killed.

Table 4 shows the body weight, baseline liver weight and after induction VOD, liver biochemistry of the nano rice husk silica + PDGF-treated and control sham- rats. DAC induced significant increase of TBARS, NO, NF-κB expression, weight of the body and liver as compared to the normal animals. On the opposite side, the treatment of nano RHS+ PDGF decreased of TBARS, NO, NF-κB expression and the body weight and liver weight significantly when compared to VOD group. The current results elucidated significant ($p < 0.05$) increase in serum ALP, AST, ALT, GGT and total bilirubin in DAC administered rats and significantly decreased total protein levels as compared to the normal rats (Fig 5). Treatment of DAC administered rats with the nano rice husk silica + PDGF induced significant ($P < 0.05$) decrease of serum ALP, ALT, AST, GGT and total bilirubin and increased significantly ($p < 0.05$) of total protein levels (Figs 5A & B). Similarity was reported regarding the weight of the body and the liver between the nano rice husk silica + PDGF and control groups. The antioxidant levels (GSH-PX, CAT, GST, SOD and GSH) in liver were significantly lower in the DAC-treated group ($P < 0.05$) compared to normal, while group treated with nano rice husk silica + PDGF after DAC-induced VOD had improved trend by returning these parameters to control level (Table 5).

Similarly, the present data showed that the DAC- induced a significant increase of liver TGFβ1 and collagen-1 (Figure 6 A& B), plasma TNFα, IL6 and T3 as compared to normal rats. Rats treated with nano rice husk silica + PDGF showed an improved significant level of TNFα, IL6 and T3 as compared to untreated rats) (Table 6). On the contrary, DAC caused a significant decrease of T4 and VEGF but the injection of nano RHS combined with PDGF revealed a rise in the incidence of T4 and VEGF (Table 6).
Histopathological Findings

Under H&E staining, there was no histological difference in the liver tissues in the control group (GI) (Fig7A). In VOD group (0.015mg/kg 1-3 days IP) on the 4th week, the hepatic capsule (Glissons Capsule) showed thickening with fibrosis and inflammatory (Fig 7B & C), associated with deposition of the fat in a focal manner all over the capsule (lipidosis) (Fig7 D & E). There was congestion in the central veins and sinusoids (Figure7 F) Severe infiltration by the inflammatory cells was seen in the portal area mainly surrounding the bile ducts (Fig7 G).

Congestion was noticed in the central veins associated with degeneration in the hepatocytes of GIII (Fig7H & I). There was mild dilatation of the central vein and surrounding sinusoids of GIV (Fig7J). In addition, there was mild congestion in the central veins and sinusoids of GV (Fig7 K). Dilatation was reported in the central vein with degeneration in the adjacent hepatocytes in the surrounding area in G VI (Fig7 L & M).

Discussion

Our study investigated and evaluated the influence of curing with nano RHS combined with PDGF on sinusoidal obstruction syndrome. The EDX spectrum exhibits only two peaks corresponding to silicon and oxygen confirming that the produced material is pure silica. The crystallinity of the silica extracted from rice husks depends mainly on the treatment through which extraction takes place. For example, several researchers (Yalcin & Sevinc 2001; Liou 2004; Wang et al. 2012) derived amorphous silica from rice husks while others (Jang et al. 2009; Atta et al. 2012; Hassan et al. 2014) obtained crystalline ones. In the present article, our experimental route for silica extraction provides one with both amorphous and crystalline phases as confirmed by the XRD pattern of the prepared silica as indicated by featureless diffract-grams...
with diffuse maxima around $\theta=22.2^0$ and a characteristic crystalline peak at $\theta=26.7^0$,
respectively. This result is in accordance with that obtained earlier by Bui et al. 2012. The
adsorption isotherm is of type II according to the classification of Brunauer–Deming–Deming–
Teller (Gregg & Sing 1982) exhibiting an H3 hysteresis loop (Gregg & Sing 1982; Tanev &
Vlaev 1993) indicating the existence of mesoporosity. This type of hysteresis arises from
aggregates (assemblage of particles which are loosely coherent) of plate like form giving rise to
slit shaped pores. Previous researches on silica prepared from rice husk had introduced materials
with various specific surface areas ranging from ca. 21 to 1024 $\text{m}^2/\text{g}$ (Jang et al. 2009; Adam
& Thankappan, 2010; Tadjarodi et al. 2012; Ugheoke & Mamat 2012; Adam et al. 2013;
Davarpanah & Kiasat 2015). For our sample, the specific surface area was found to be 236
$\text{m}^2/\text{g}$. From SEM image of the prepared silica one can observe that the particles are
agglomerated in a flake-like structure while the TEM image reveals that the silica particles are
nano sized ranging from 5 to 35 nm with spheroid shape. This indicates that the followed
preparation procedure is successful in producing pure nano silica particles from rice husks.
DPPH radical is considered to be a model of lipophilic radical. In this mode, scavenging activity
is attributed to the hydrogen donating ability of antioxidants (Philips et al. 2010). Although Si-
NPs possess good DPPH scavenging activity, it was evident that the Si-NPs could serve as free
radical inhibitors or scavengers. ABTS assay is an excellent tool for determining the
antioxidant activity of the hydrogen ion donating antioxidants and of chain-breaking
antioxidants (Leong & Shui 2002).
Numerous procedures have been attempted for the therapy of VOD, but none has been uniformly
effective. Platelet growth factors are utilized directly to the wound surface to augment the growth
of skin, flexible tissue, and blood vessels. In the present study, nano RHS joined with PDGF
exerts a liver protective effect in a rat model of VOD provoked by an injection of dactinomycin. A liver sinusoidal hindrance syndrome is a well-recognized problem after high-dosage chemotherapy in the setting of HSC transplantation (Coppell et al., 2010). Hepatic VOD after traditional chemotherapy is uncommon and is mainly confined to pediatric patients receiving dactinomycin-based chemotherapy for Wilms tumor (Jang et al. 2009). The curing effect of the nano RHS incorporated with PDGF was demonstrated by evaluating the liver function parameters such as AST, ALT and bilirubin as well as, ALP, total protein and albumin. In the current study, a considerable increase in these parameters in animals of the DAC alone group was observed. The increment of hepatic activity may be contributing to that direct damage by DAC. However, histological findings of liver tissue may be difficult to implement as a result of ascites and coagulopathy. A regression model proposed by Bearman et al. (2001) suggests that patients who develop hyperbilirubinemia and significant fluid retention earlier and worsen faster are at high risk of severe VOD. Research also finds that the intensification of clinical features is accompanied by the extent of histological changes rather than the occlusive of the small hepatic venues”.

Our study suggests that the hepatic TGFβ-1 elevated synthesis after administration of DAC this confirmed that activation of this growth factor is a possible response to chemotherapy in patients who eventually have hepatic veno-occlusive disease (HVOD) (Green et al. 1998). Hepatocyte harm inspires an incendiary reaction through enactment of tissue macrophage Kuepfer cells. These enacted cells discharge a variety of cytokines, including tumor rot component TNFα, changing development element β, platelet-inferred development element, and the different variables that follow up on hepatic stellate cells that add to fibrogenesis (Kumar et al. 2003). It must be dissociated from this complex to come to be biologically forcible concentrated, if
measured after induction chemotherapy, robustly reciprocal with the progress of HVOD. It is possible that this activation process is augmented in patients suffer with hepatic veno-occlusive ailment or pulmonary fibrosis develops, and accordingly more mature TGFβ would be coincident in the plasma. The presence of this form of liver damage requires liver biopsy, and readiness to this possibility in examining the biopsy tissue. The presence of perivenular necrosis, endophlebitis and fibrotic changes of terminal and sub-lobular hepatic veins are congruous with VOD (Schwabe & Brenner, 2006).

The role of VEGF as an angiogenic element is less manifest. Iguchi et al found increased serum levels of VEGF for the duration of development of SOS inpatients (Iguchi et al. 2001), leaving open the question of whether VEGF-induced increase in the rate of vasopermeability, neovascularization, and/or expression of coagulopathy tissue elements of circulating mononuclear cells can also act a role in SOS pathogenesis (Ballermann et al. 2007).

Key pathogenic elements in VOD, include glutathione depletion, nitric oxide depletion, increased expression of VEGF, and activation of clotting factors (Xu et al. 2008). Foremost, drugs, leading to VOD are metabolized completely through the hepatocellular cytochrome P450 systems, for which glutathione is an antioxidant recovery mechanism. VEGF is an important regulator of angiogenesis and the coefficient of vascular permeability to the blood-brain barrier endothelial and permeability of the fenestrated glomerular endothelium (McCuskey, 2000; DeLeve et al. 2003). VEGF can reinforce fenestration and permeability of liver sinusoidal endothelial cells; therefore, it can improve the exchange between hepatic sinusoidal blood and hepatocytes, which argues for the development of VEGF gene therapy for cirrhosis. PDGFs also exert indirect effects on angiogenesis and vasculogenesis by increasing the expression of
angiogenic molecules such as VEGF. Buchberger et al. (2011), assessed the efficacy of growth factors, alone or united with different technologies in the remediation of diabetic foot ulcers.

Oxidative stress occurs when there is an imbalance between prooxidants and the ability of the antioxidants to scavenge excess reactive oxygen species (Buchberger et al. 2011). Furthermore, recent studies demonstrated that PDGF-AA and -BB protected cultured neurons against oxidative stress and suppressed H₂O₂-induced caspase-3 activation through PDGFR-α or -β expressed on these cells (Poli et al. 2004). Also they reported that the oxidative stress-induced Ca²⁺ overload in cultured neurons was markedly suppressed by PDGF. Moreover, PDGF induced Na+/H+ exchange activity is linked to the activation of PI3-K and is blocked by preincubation with PI3-K inhibitors of downstream signaling events essential for growth factor-mediated cytoskeletal (Zheng et al. 2013).

In the present work, the exposure to DAC leads to depletion of antioxidants such as GSH, GSH-Pₓ, SOD, GST and CAT. Severe depletion of the antioxidant levels in VOD renders them susceptible to cell death; prophylactic infusion of glutathione or these antioxidants prevent the progress of SOS in the monocrotaline-treated rat model (Fan & Crawford, 2014) nano RHS combined PDGF potentiates these antioxidants and lead to alleviate from SOS.

NF-κB is a transcription factor which regulates genes involving in inflammation. It is activated by endotoxin, cytokines, and oxidative stress (Pahl, 1999). Activation of NFκB increased expression of proinflammatory cytokines and chemokines that were key factors in ethanol-induced liver injury rats (Yuan et al. 2006). In the present study, NF-κB activation was confirmed induction in DAC treated group. The present study showed that nano RHS+ PDGF
improved ethanol-induced liver injury by reduction of oxidative stress and inhibition of NF-κB activation. It is well known that TNF- induced NF-κB is important in inflammation. Moreover, NF-Kb itself can transcriptionally induce TNF-a thereby further amplifies TNF/ TNFR signaling pathways (Sedger & McDermott, 2014).

Moreover, nano RHS+ PDGF induced significant decrease of TNF- α, since cytokine activation may be contributed in the pathogenesis of VOD, inhibitors of cytokines may be useful. This treatment is promising as it has a vigorous inhibitor effect on cytokine production. In a report, PDGF, a modulator of TNF-a which inhibits the transcription of TNF messenger RNA, was reported to have a protective effect against VOD with no significant adverse effects.

It has been reported that PDGF enhances the tissue repair after acute liver injury (Lou et al. 2004). In chronic liver diseases, however, the presence of reiterative tissue damage associated with a persistent inflammatory state may cause a sustained release of PDGF involved in the deposition of extracellular matrix. The management of PDGF activity by antagonists may prevent aggressive liver fibrosis and improve prognosis of hepatitis B. The effect may be related to alleviating lipid peroxidation and inhibiting the expression of NFκB (Wei et al. 2013).

Moreover, it has anti-inflammatory effects which may relate its ability to inhibit NFκB, which contributes to the production of proinflammatory mediators such as and IL-6, TNF-α. The results of the present work revealed that DAC induced highly significant elevation (p< 0.05) of NFκB hepatic mRNA expression, Co-administration of nanosilica with PDGF significantly reduced serum TNF-α, IL6 and NFκB activity at p< 0.05 compared with DAC treated group.

In this study, PDGF had a similar effect to RHS on TNF-α and IL-6 in the liver. But PDGF in combination with RHS did not show a satisfying synergistic action on NFκB, thus we cannot
exclude the possibility that PDGF and RHS affect in different ways, and further works are demanded.

Endothelial harm seems to be the commencing juvenile in the cascade of activities causing the hepatic alterations and clinical manifestation of VOD. A rat model of liver VOD has been described that has participated much to our comprehension of events that lead to the presence of the histological drastic changes. In this model, the hepatic injury is initiated by treatment with a DAC and leads to manifestations analogous to those seen clinically, including hyperbilirubinemia, ascites, and hepatomegaly. In this model, the earliest changes after DAC injection appear in the sinusoids. This leads to the privation of the endothelial cell fenestrations and the occurrence of gaps in the lining, which is accompanied by effusion of red cells in the space of Disse. Sinusoidal endothelial cells are injured extensively, resulting in prevalent disrobing of the sinusoidal lining (Schoepfer et al. 2007). In the early stages of VOD, the histological findings, display thickening of the subintimal zone of the central and sublobular venues because of edema. The reduced venous outflow leads to excessive hepatic congestion and sinusoidal dilatation, appreciated on the histological findings, and portal hypertension characteristic of VOD. These changes within the vascular bed is proof of hepatocyte damage and death, and these changes look like on the whole localized to the centrilobular region of the liver. The low-flow state prompted via the sinusoidal obstruction effects in enormous heterogeneity in the sinusoidal blood flow and redistribution of hepatic microcirculation (Helmy, 2006). These modifications can bring about focal ischemia and progressive microvascular, parenchymal, and Kupffer cell phagocytic derangements in the liver.

Mediators such as 5-hydroxytryptamine (5-HT), prostaglandins (PGs), leukotrienes, and free radicals released by platelets, Kupffer cells, leukocytes, and mast cells might also play a role in
the endothelial damage and the downstream sequence of occasions, leading to hepatocellular ischemia and injury (Helmy, 2006). DeLeve et al. (2002) correlated the histological findings in VOD, together with the frequency of hepatic venular occlusion, degree of occlusion, eccentric luminal narrowing, hepatocyte sinusoidal fibrosis, and necrosis. Moreover, they reported that the involvement of the hepatic venules was not an important feature of the disease, consistent with the concept that the primary obstruction occurs in the sinusoids, and that more intense disease was recorded in patients with fibrosis of both the sinusoids and the venules.

Conclusions

Mesoporous silica nanoparticles were successfully prepared from rice husks. This is the first study to demonstrate the ability of nano RHS+ rh-PDGF to decrease DAC-induced VOD in rats. The protective mechanisms of nano RHS+ rh-PDGF may be attributed to its free radical scavenging activity and protective effect on antioxidant system damage. On the basis of the results obtained, nano RHS+ rh-PDGF can be used for a variety of beneficial chemo-preventive effects. Further studies on the specific components of nano RHS+ rh-PDGF and in vivo studies are in progress to understand the detailed mechanism by which nano RHS+ rh-PDGF exerts its curing effect. Moreover, and to conclude, this research validates the nano RHS+ rh-PDGF significant therapeutic potential, and represents a rich source for future scientific discovery and the development of unconventional compounds of medical value.

Abbreviations

RHS: Rice husk silica
DAC: Dactinomycin
XRD: X-ray diffraction

SEM: Scanning electron microscopy

Si-NPs: Silica nanoparticles

TEM: Transmission electron microscopy.

PDGF: Platelet-derived growth factor

TGFβ1: Transforming growth factor beta one

TNFα: Tumor necrosis factor alpha

GSH-Px: Glutathione peroxidase

GST: Glutathione-S-transferase

SOD: Superoxide dismutase

CAT: Catalase

TBARS: Thiobarbituric acid-reactive substances

NO: nitric oxide

IL6: Interleukin 6

T3: Triiodothyronine

T4: Thyroxin

VEGF: Vascular endothelial growth factor
SOS: Sinusoidal obstacle disorder

BW: Body weight

IP: Intraperitoneal

EDTA: Ethylene diamine tetra acetic acid

Rh-PDGF: Recombinant, Human Platelet-Derived Growth Factor

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

ALP: Alkaline phosphatase

cDNA: Complementary DNA

RT-PCR: Real-time quantitative polymerase chain reaction

GADPH: Glyceraldehyde-3-phosphate dehydrogenase

HSC: Hematopoietic stem cells

TTBS: Tris-buffered saline containing Tween-20

NADH: Nicotinamide adenine dinucleotide hydrate

DPPH: 2,2-diphenyl-1-picrylhydrazyl

ABTS: 2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)

Competing interests
The authors declare that they have no competing interests.

**Authors’ contributions**

All authors participated in the design of the study and revised it critically; Amal I. Hassan & Eithar K performed the study and carried out the biochemical analyses, analyzed and interpreted the data and drafted the manuscript. Ahmed Shebl and M. M. Hazem performed the study, prepared the nano silica from rice husk and recognized its physicochemical characteristics using energy dispersive X-ray (EDX), X-ray diffraction (XRD), N$_2$ adsorption-desorption isotherm, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques, and drafted the manuscript. All authors read and approved the final manuscript.

**Acknowledgments**

The authors are grateful to Dr. Adel M. Bakeer Kholoussy, Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt for his help in the examination of the histopathological slides and for his valuable comments. Also the authors are thankful to Dr. Mohamed Abdelhay Ahmed, Chemistry Department, Faculty of Science, Ain Shams University, Egypt for his valuable comments on the physicochemical characterization of the nano RHS.

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(II) on RHA and carbon embedded silica obtained from RHA. Chemical Engineering J. 181–
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Legend of figures

Figure 1. XRD and EDX (inset) of the prepared silica.

Figure 2. N\textsubscript{2} Adsorption-desorption isotherm and pore size distribution (inset) of the prepared silica.

Figure 3. a) SEM and b) TEM images of the prepared silica.

Figure 4. a) DPPH and b) ABTS of nano silica

Figure 5. Liver function after induction VOD baseline, liver biochemistry of the nano rice husk silica + PDGF -treated and control sham- rats.

Figure 6. TGFβ1 and collagen -1 after induction VOD baseline, liver biochemistry of the nano rice husk silica + PDGF -treated and control sham- rats.

Figure 7. Histopathological results of Liver after induction VOD baseline, liver biochemistry of the nano rice husk silica + PDGF -treated and control sham- rats.
### Table 1. Primer sequences used for RT-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
</table>
| Collagen-w1 | FORWARD 5' TGCCG TGAC CTCAAGATGTG-3'  
REVERSE 5' CACAA GCCTGCTGTAGGTGA -3' according to gene bank accession number NM_053304.1 |
| NFkB | Forward 5' CATGAAGAGAAGACACTGACCATGGAAA3'  
Reverse 5' TGGATAGAGGCTAAGTGT AGACACG3' according to gene bank accession number NM_010902.3 |
| GAPDH | Forward: 5'- CTCCCATTCTTCCACCTTTG-3'  
Reverse: 5'- CTTGCTCTCAGTATCCTTGC-3' according to gene bank accession number XR_145951.1 |

### Table 2. Textural parameters from N₂ adsorption-desorption analysis of the prepared nano silica.

<table>
<thead>
<tr>
<th>BET surface area (m²/g)</th>
<th>Average pore diameter (nm)</th>
<th>External surface area (m²/g)</th>
<th>Micropore area (m²/g)</th>
<th>Total pore volume (cc/g)</th>
<th>Micropore volume (cc/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>236</td>
<td>5.48</td>
<td>223.9</td>
<td>12.1</td>
<td>0.323</td>
<td>0.005</td>
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</table>
Table 3: Pharmacological Study Acute Toxicity (LD) Testing for Rice Husk Silica

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>The result of the first phase (mortality) n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0/5</td>
</tr>
<tr>
<td>100</td>
<td>0/5</td>
</tr>
<tr>
<td>1000</td>
<td>0/5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>The result of 2nd phase (mortality) n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1600</td>
<td>0/5</td>
</tr>
<tr>
<td>2900</td>
<td>1/5</td>
</tr>
<tr>
<td>5000</td>
<td>2/5</td>
</tr>
</tbody>
</table>

LD50 = 1600 mg/kg
Table 4: Effect of rice husk silica and/or PDGF on lipid peroxidation, NO, BW and liver weight in normal liver and VOD in experimental modal

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TBARS (nmol/ml)</th>
<th>NO (µmol/l)</th>
<th>BW (g)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (G1)</td>
<td>0.52± 0.06b</td>
<td>68.55± 5.34b</td>
<td>192.00±4.26b</td>
<td>5.91±0.07b</td>
</tr>
<tr>
<td>VOD (GII)</td>
<td>1.03± 0.22a</td>
<td>94.11± 7.85a</td>
<td>213.25±10.33a</td>
<td>8.61±0.78a</td>
</tr>
<tr>
<td>VOD+ RHS-PDGF (GIII)</td>
<td>0.56± 0.05b</td>
<td>63.97± 4.89b</td>
<td>198.75±7.80ab</td>
<td>6.07±0.55b</td>
</tr>
<tr>
<td>RHS (GIV)</td>
<td>0.42± 0.07b</td>
<td>62.59± 5.26b</td>
<td>193.00±4.97b</td>
<td>5.69±0.52b</td>
</tr>
<tr>
<td>PDGF (GV)</td>
<td>0.58± 0.03b</td>
<td>63.65± 5.92b</td>
<td>191.25±8.81b</td>
<td>5.83±0.34b</td>
</tr>
<tr>
<td>RHS- PDGF (GVI)</td>
<td>0.61± 0.06b</td>
<td>68.73± 3.79b</td>
<td>189.00±8.98b</td>
<td>5.86±0.56b</td>
</tr>
<tr>
<td>F- value P</td>
<td>16.81*</td>
<td>31.24*</td>
<td>4.50*</td>
<td>21.20*</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
<td>0.004</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SE. Small letters: Statistically significant from the control or experimental groups at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. * F value (P<0.05).
Table 5: Effect of rice husk silica and/or PDGF on antioxidants in normal liver and VOD in experimental modal

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>GST (µ/g)</th>
<th>GSH-PX (µ/g)</th>
<th>GSH (µmol/g)</th>
<th>SOD (µ/g)</th>
<th>CAT (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (GI)</td>
<td>5.29±0.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.16±3.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.17±1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.10±8.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.46±2.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>VOD (GII)</td>
<td>3.12±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.0±1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.40±0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.40±6.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.23±0.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>VOD+ RHS-PDGF (GIII)</td>
<td>5.01±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.37±4.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.05±1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129.96±10.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.63±1.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RHS (GIV)</td>
<td>5.93±0.56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>39.50±2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.97±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.55±12.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.50±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PDGF (GV)</td>
<td>5.58±0.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.50±2.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.17±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.00±9.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.67±2.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RHS-PDGF (GVI)</td>
<td>5.08±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.40±3.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.99±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129.85±7.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28±1.35&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

F-value: 21.12*<sup>P</sup> 34.77*<sup>P</sup> 14.70*<sup>P</sup> 40.29*<sup>P</sup> 22.19*<sup>P</sup>

Data expressed as mean ± SE. Small letters: Statistically significant from the control or experimental groups at P<0.05 using one-way ANOVA followed by Tukey as a post-hoc test. *F value (P<0.05).
Table 6: Effect of rice husk silica and/or PDGF on Thyroid hormones, IL6, TNF and VEGF in normal liver and VOD in experimental modal

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>T3 (ng/dl)</th>
<th>T4 (µg/dl)</th>
<th>IL6 (pg/ml)</th>
<th>TNFα (pg/ml)</th>
<th>VEGF (pg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control (GI)</td>
<td>95.83±10.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.84±0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.13±1.45&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>173.38±7.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>VOD (GII)</td>
<td>101.55±12.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.77±3.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>264.32±12.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>137.75±8.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>VOD+ RHS-PDG (GIII)</td>
<td>78.24±8.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.04±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.06±1.92&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>105.03±8.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.22±10.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RHS (GIV)</td>
<td>73.70±6.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.74±0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.88±1.81&lt;sup&gt;i&lt;/sup&gt;</td>
<td>100.03±4.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>177.71±8.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PDGF (GV)</td>
<td>77.50±7.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.05±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.23±1.64&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>100.53±6.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>177.25±9.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RHS- PDGF (GVI)</td>
<td>82.52±6.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.42±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.05±2.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>111.70±8.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>168.20±6.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F- value</td>
<td>13.70&lt;sup&gt;*&lt;/sup&gt;</td>
<td>18.0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>38.11&lt;sup&gt;*&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

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Fig 1: XRD and EDX (inset) of the prepared silica.

359x150mm (300 x 300 DPI)
Figure 2. N2 Adsorption-desorption isotherm and pore size distribution (inset) of the prepared silica.
Figure 3. a) SEM and b) TEM images of the prepared silica.

359x164mm (300 x 300 DPI)
Figure 4. a) DPPH and b) ABTS of nano silica

115x47mm (300 x 300 DPI)
Figure 5. Liver function after induction VOD baseline, liver biochemistry of the nano rice husk silica + PDGF - treated and control sham- rats.

81x48mm (300 x 300 DPI)
Figure 6. TGFβ1 and collagen -1 after induction VOD baseline, liver biochemistry of the nano rice husk silica + PDGF -treated and control sham- rats.
Figure 7. Histopathological results of Liver after induction VOD baseline, liver biochemistry of the nano rice husk silica + PDGF -treated and control sham- rats.

337x218mm (300 x 300 DPI)