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Chemical composition and nutritional effect of *Physalis peruviana* husk as hepato-renal protective agent

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Abstract

This work is designed to investigate the potential of *Physalis peruviana* as hepato-renal protective agent against fibrosis. Two compounds (15-desacetylphysabubenolide and Betuline) were isolated and their structures were elucidated. The biological evaluation was conducted on different rats groups; control, control treated with the methanolic husk extract, carbon tetrachloride (CCl_4), CCl_4 treated with husk extract and CCl_4 treated with silymarin. The evaluation was done through measuring oxidative stress markers; malondialdehyde (MDA), superoxide dismutase (SOD) and nitric oxide (NO). Liver function indices; aspartate and alanine aminotransferases (AST & ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), bilitubin and total hepatic protein were estimated. Kidney disorder biomarkers; creatinine, urea and serum protein were evaluated. The results revealed improvement of all the investigated parameters. Liver and kidney histopathologyical analysis confirmed our results. In conclusion, *Physalis peruviana* succeeded to protect liver and kidney against fibrosis. Further studies are needed for its pharmacological and clinical uses.

Keywords: *Physallis peruviana*, hepatoprotective, renal injury, oxidative stress.

Introduction

Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals, but also the pharmaceutical industries and drug regulatory agencies [1]. The available synthetics drugs that treat liver disorders also cause further damage to the liver [2]. Hence, herbal drugs have become increasingly popular and their use is widespread [3].

Despite the significant popularity of several herbal medicines in general and for liver diseases in particular, they are still unacceptable treatment modality for liver diseases [4]. The limiting factors that contribute to this problem are lack of standardization of the herbal drug, lack of identification of active ingredients, lack of randomized controlled clinical trials and lack of toxicological evaluation [5].

Kidney is one of the most affected organs by xenobiotics. It possesses most of the common xenobiotic metabolizing enzymes, and is thus able to make an important contribution to the body's metabolism of drugs and foreign compounds [6]. Renal fibrosis is the principal process involved in the progression of chronic kidney disease [7]. The presence of kidney fibrosis seems mostly to be viewed as an endpoint or marker of tissue or organ failure and loss of function [8].

Extracts of medicinal plants are believed to contain different chemopreventive or chemotherapeutic compounds, which possess more than one mechanism of actions. *Physalis peruviana* L. (Solanaceae) has been widely used in folk medicine for treating cancer, leukemia, hepatitis, rheumatism and other diseases [9-11]. Despite the favorable ethnopharmacological properties of Physalis peruviana, its protective effect against liver fibrosis has not previously been explored and its role as diminished factor of fibrosis could be a marker of therapeutic benefit. The aim of the present study was to evaluate the methanolic extract of *Physalis peruviana* husk against CCl_4 induced hepato-renal fibrosis in rats. The evaluation was done through measuring certain antioxidant parameters, liver and kidney function indices, immunoglobulin-G and the histopathological analysis of liver and kidney sections.

Materials and methods

Plant material

The plant was cultivated at the Experimental Farm of Faculty of Agriculture, Cairo University, Giza, Egypt. At harvest time in October and November, the plant (husk) was randomly chosen.

Chemicals

All chemical used were of high analytical grade, product of Sigma-USA, Aldrich Chemie-Germany and Biomedicals-France. Solvents were purchased from El Nasr Pharmaceutical Chemicals Co., Egypt.

Plant extraction

The air dried powdered husk (500g) was extracted with 99% methanol at room temperature, then with 50% methanol. The combined extracts were evaporated till dryness and kept at -80^{O}C for further biological examinations.

Determination of some chemical active ingredients

Five grams of the dried methanolic husk extract was examined for the presence of alkaloids [12] and total free withanolide [13]. One gram of dried sample was tested for the presence of flavonoids [14].

Purification and identification of the isolated compounds

Ten grams of the combined methanolic extracts were dissolved in small amount of 50% MeOH then defatted with n-hexane. The defatted MeOH extract was evaporated and partitioned between chloroform and water. The combined chloroformic extracts were evaporated till dryness then, mixed with 10 g silica gel and putted on the top of silica gel column (6mm x 80 cm). The column was eluted with n-hexane: ethylacetate with increasing polarity from hexane 100% to ethylacetate 100%, then with ethylacetate till methanol 100%. 50 ml fractions were collected, monitored on TLC silica gel plates using elution systems of chloroform: methanol 95:5 or ethylacetate: methanol 95:5 and detected with p-anisaldehyde reagent [15]. Similar fractions were combined, evaporated and monitored on TLC plates.

Biological study

Animals

Adult male healthy Wister strain albino rats weighing 150-200 g were obtained from the Animal House, National Research Center, Dokki, Egypt. Animals were fed on standard diet and water ad libitum.

Acute toxicity

Fifteen rats of 200 ±10g were subdivided into three subgroups (5 rats each) and received one oral dose of 500, 1000 and 1500 mg of husk extract as mg/kg b.wt. Numbers of dead animals were counted along 15 days. Mortality rate and LC_{50} were monitored.

Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals not suffer at any stage of the experiment. (Approval no: 134, 2010).

Doses and route of administration

Administration regime was twice a week for six consecutive weeks. 0.5 ml CCl_4/kg body weight diluted to 1:9 (v/v) in olive oil was injected intraperitoneally [16]. Husk extract (500 mg/kg body weight) were administered orally [17]. Legalon drug (Silymarin); a reference herbal drug (100 mg/kg body weight) was administered orally [18].

Experimental design

Forty rats were divided into five groups of eight rats each. Group 1 served as normal healthy control rats. Groups 2 were normal healthy rats received husk extract. Group 3 was CCl_4 treated rats. Groups 4 were injected with CCl_4 and treated with extract at the same time and for the same duration. Group 5 was injected with CCl_4 and treated with Legalon drug (Silymarin).

Samples preparation

Serum sample: Animals were sacrificed under anesthesia and blood samples were withdrawn from the reto-orbital venous plexus in clean and dry test tubes. Blood left 10 min to clot and centrifuged at 3000 rpm for 20 minutes at 4ºC. The supernatant serum was collected and stored at -80 ºC for further determination of kidney function tests, IgG and total serum protein.

Liver homogenate: A portion of liver was weighed and homogenized with saline (0.9% NaCl) (1:9 w/v) using a glass homogenizer at 4 ºC. The homogenates were centrifuged at 3000 rpm for 10 minutes at 4 ºC and the clear supernatant was used for further determination of liver function enzymes, antioxidant parameters and hepatic total protein content.

Biochemical assays

Malondialdehyde as a product of polyunsaturated fatty acids oxidation was determined by the method of Buege and Aust [19]. Superoxide dismutase was estimated by method of Nishikimi et al. [20]. Hepatic aspartate and alanine amintransferases were measured by the method of Gella et al. [21]. Hepatic alkaline phosphatase was determined by the method of Rosalki et al. [22]. Hepatic GGT was estimated by the method of Szasz [23], where GGT enzyme reacted with L-γ - glutamyl-3-carboxy-p-nitroanilide and glycyl-glycine to give L-γ – glutamyl-glycyl-glycine and 5-amino-2-nitrobenzoate. The decrease in absorbance was read at 450 nm at 1 min intervals for 3 minutes. Total protein was assayed by the method of Bradford [24]. Serum urea was determined by the method of Tabacco et al. [25]. Serum creatinine was measured

by the method of Bartels et al. [26]. Serum IgG was determined by sandwich enzyme immunoassay ELISA kit; Life Diagnostics, Inc., Catalog Number: 5010-2, West Chester, NY.

2.6.8. Histopathological analysis

Representative slices of liver and kidney tissues were taken from the eviscerated animals and fixed in buffer formalin (10%). After fixation, the paraffin embedded sections in 4μm thick were stained by haematoxylin and eosin (H&E) and Masson's trichrom [27].

2.7. Statistical analysis and calculations

Analysis of data was carried out by one way analysis of variance (ANOVA), Costat Software Computer Program accompanied with least significance difference between group at $p<0.05$.

Results and discussion

Concerning with the active ingredients of *P. Peruviana* husk extract, the present results revealed the presence of alkaloids in *P. Peruviana* husk (2.35%). Free withanolids are represented by 30.31% and glycowithanolides recorded 37.58%. Flavonoids was also present by 365.88 mg/100g (Table 1).

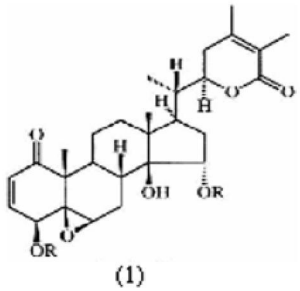
Table 1. Active ingredients of *P. Peruviana* husk extract.

Active ingredients (mg/100g)	Husk (calyx)
Alkaloids	2.35 ± 0.18
Free withanolides	30.31 ± 0.74
Glycowithanolides	37.58 ± 0.44
Flavonoids	365.88 ± 1.43

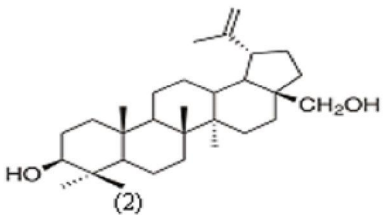
Data are means ±SD of three replicates.

Upon the identification of the isolated compounds, compound 1 was an amorphous powder obtained from a fraction of (n-hexane: ethylacetate 10:90). TLC investigation reveals the R_f value 0.57 using $CHCl_3$: MeOH (90:10) as solvent system. This compound gave the characteristic color of withanolide (pink) by spraying with p. anisldehyde. MS showed M^+ at m/z 486 for the molecular formula ($H_{28}H_{38}O_7$). Also it showed a peak ion at m/z 125 as a base peak. On the other hand, ^{1}H-NMR spectrum exhibited signals due to CH_3-21 at δ 0.95(s), CH_3-19 at 1.04 (s) and CH_3-10 at 1.37 (s) and signals at δ 1.34 (s) and 1.40 (s) for two vinyl methyls CH_3-27 and CH_3-28, respectively. Moreover, signals of H-4, H-15, H-22, H-2 and H-3 were observed at δ 3.63 (d), 4.19 (m), 6.15 (d) and 7.04 (dd), respectively. IR spectrum (KBr, cm^{-1}) showed the presence of OH (3422 cm^{-1}), CH2-CH2-CH3 (2923 cm^{-1}), α-β unsaturated δ lactone (1710 cm^{-1}), C=O (1653 cm^{-1}) and $C_6H_{11}OH$ (1088 cm^{-1}). Compound (1) was identified as 20S, 22R-5β, 6β-Epoxy-4β,14β,15α-trihydroxy-1-oxowith-2,24-dienolide (15-desacetylphy- sabubenolide) according to the above mentioned data which was confirmed by the results of [15] (Fig. 1).

Compound 2 was obtained from fraction (n-hexane: ethylacetate 10:90), which then was separated by preparative TLC using $CHCl_3$: MeOH (90:10) as running solvent and gave with R_f value of 0.40. The amorphous powder (6.7 mg) obtained gave characteristic color of triterpene lactone (Reddish violet) by spraying with p.anisldehyde and also, gave +ve preparative Libermann-Burchard test. Mass spectrum of this compound showed, M^+ at 442 for the molecular formula ($C_{30}H_{50}O_2$). The spectrum shows ion at m/z 188 which represent the base peak [28]. ^{1}H-NMR spectrum showed the main expected signals of betulin. The spectrum showed signals of five methyl groups from δ 0.89 to 0.99 (3H each,s). In addition, it showed isopropylene function at δ 1.75 and 3.40. While, carbinolic proton was appeared at δ 3.29 (dd) and the hydroxylmethylene group is showed at δ 4.20 and 4.54. IR spectrum (KBr, cm^{-1}) showed the main characteristic bands of betulin as follow, 3421 for (OH), 1645 for ($C=CH_2$), 1374 for (CH_3) and 1172 for (CH). Compound (2) was identified as betulin based on the above mentioned spectral data and its structure was as in (Fig.1).



(1)



(2)

Fig.1: Isolated compounds from *Physalis peruviana* extracts. (1): 15-desacetylphysabubenolide. (2): Betuline.

Table 2. Effect of *Physalis peruviana* on the antioxidant and IgG levels of CCl_4 intoxicated rats.

Parameters	Control	CCl_4	CCl_4 treated with husk extract	CCl_4 treated with silymarin
NO	40.19 ± 0.97d	140.12 ± 1.25a	69.05 ± 1.60c	109.08 ± 7.91b
µmol/mg protein	--	(248.64)	(71.82)	(171.41)
MDA	0.58 ± 0.02c	5.43 ± 0.35a	0.57 ± 0.05d	2.28 ± 0.20b
µmol/mg protein	--	(836.20)	(1.724)	(293.10)
SOD	111.67 ± 4.38a	12.87 ± 1.39d	84.28 ± 3.56b	14.69 ± 1.97c
µmol/mg protein	--	(-88.47)	(-24.52)	(-86.84)
IgG	112.02 ± 2.62d	214.86 ± 4.18a	131.11 ± 1.60c	143.81 ± 1.97b
ng/ml	--	(91.80)	(17.04)	(28.37)

Data are means ±SD of eight rats in each group. Statistical analysis is carried out by one way analysis of variance (ANOVA) using co-stat computer program Unshared letters between groups are the significant difference level at $P < 0.05$. Values between brackets are percentage change over control.

In vivo study the mortality rate of husk extract at concentration of 1500 mg/kg body weight reached 20%. The results revealed safety of extract at concentrations 500 and 1000 mg/kg body weight. The best recommended dose was 500 mg/kg body weight, as showed no mortality rate.

Rats treated with CCl_4 recorded significant increase in NO, MDA and IgG by 248.64, 836.20 and 91.80%, respectively. In opposite manner, SOD significantly decreased by 88.47% as compared to control group. Husk extract exhibited improvement in NO and MDA and SOD and IgG levels by 176.82, 837.93, 63.94 and 74.76%, respectively (Table 2). This was in accordance with Motawi et al. [29] who stated that various enzymatic and nonenzymatic systems have been developed by the cell to attenuate ROS. However, when a condition of oxidative stress establishes, the defense capacities against ROS becomes insufficient. Therefore, ROS affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH, decreases the activity of SOD and enhances lipid peroxidation [30]. Increased NO production was also found during chronic hepatic inflammation, suggesting a role for NO in the hepatic response to inflammatory stimuli [31]. In addition, the higher level of IgG in CCl_4 intoxicated group was in parallel with the results of [32] who study the level of serum IgG antibody as a possible indicator for hepatic fibrosis.

Table 3. Effect of *Physalis peruviana* extracts on liver function parameters in CCl_4 intoxicated rats.

Parameters	Control	CCl_4	CCl_4 treated with husk extract	CCl_4 treated with silymarin
AST	2.08 ± 0.68a	0.96 ± 0.019b	2.28 ± 0.37a	0.845 ± 0.24b
mg protein /g tissue	--	(-53.84)	(-1.44)	(-59.37)
ALT	7.31 ± 1.33a	2.88 ± 1.11b	7.185 ± 1.12a	3.67 ± 0.63b
mg protein /g tissue	--	(-60.60)	(-1.70)	(-49.79)
GGT	120.37±.89a	28.8 ± 4.18c	114.11 ± 11.95a	24.78 ± 6.97 b
µmol/min/mg protein		(-76.07)	(-5.20)	(-79.41)
ALP	130.54 ± 2.80c	304.13 ± 8.79 b	139.19 ± 4.13d	312.81 ± 12.85a
µmol/min/mg protein	--	(132.97)	(6.62)	(139.6)
Tissue protein	194.89 ± 17.59c	343.85 ± 11.85a	191.84 ± 17.52c	315.37 ± 20.76b
mg protein /g tissue	--	(76.43)	(-1.56)	(61.81)
Bilirubin	1.008 ± 0.05c	2.71 ± 0.18 b	1.008 ± 0.05d	2.88 ± 0.13a
mg/dl	--	(168.84)	(0.198)	(185.71)

Data are means ±SD of eight rats in each group. Statistical analysis is carried out by one way analysis of variance (ANOVA) using Co-stat Computer Program. Unshared letters between groups are the significant difference level at $P < 0.05$. Values between brackets are percentage change over control.

All liver function parameters revealed significant decrease by 53.84, 60.60 and 76.07% for AST, ALT and GGT, respectively, while ALP, serum total bilirubin and hepatic protein showed significant increase by 123.97, 168.84 and 76.43%, respectively as compared to control group (Table 3). Treatment with husk extract and silymarin improved AST by 63.46 and 5.52%, respectively. ALT recorded improvement by 58.89 and 10.80%, while GGT showed amelioration levels by 70.87 and 3.52%. ALP recorded enhancement levels reached to 126.35 and 6.64%, while total bilirubin revealed improvement by 168.8and 16.86%. Rats intoxicated with CCl_4 and treated with husk extract showed significant decrease in tissue protein level by 44.20% (Table 3).

These observations were in accordance with Al-Attar [33]. The inhibition of tissue ALT, AST and GGT activities could be regarded as an index of damage of the liver parenchymal cells [34]. Treatment with *Physalis peruviana* extracts normalized the levels of ALT, AST, GGT and ALP which may be an indication of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. Romero et al. [35] showed that CCl_4 intoxication induced changes in the process of protein synthesis. Hence, increase in total protein content in tissue can be deemed as a useful index of the severity of cellular dysfunction in liver diseases as clearly shown in our studies. Stimulation of protein synthesis has been advanced as a contributory self healing mechanism, which accelerates the regeneration process and the production of liver cells [36]. Attainment of normalcy in protein levels after treatment with Physalis extracts confirmed the hepatoprotective effect of the plant extract. In addition, the observed biochemical restorations of bilirubin may be due to the inhibitory effects on cytochrome P450 and/or promotion of its glucuronidation [37].

Regarding to kidney function indices, CCl_4 treated rats showed significant increase in creatinine and urea by 72.03 and 308.13%, respectively, while serum protein decreased significantly by 29.68%. CCl_4 treated rats with husk extract and silymarin recorded improvement in creatinine level by 55.78 and 28.75%. Urea showed improvement by 265.12 and 4.65%, while serum protein recorded enhancement by 21.43 and 0.45% (Table 4). Theses findings were in the line of Hamed et al. [38] and Ansari et al. [39] who concluded that theses alternation of these biochemical changes signify the potential damage of nephron structural integrity. In addition, the decreased level of serum protein in CCl_4 group confirmed the probable dysfunction of kidneys leading to proteinuria [40]. The corrective findings after treatment with Physalis extracts give an additional support that Physalis mops up free radicals generation by CCl_4, reduce inflammation, and induce healthy state of renal cells.

Table 4. Effect of *Physalis peruviana* on kidney function indices in CCl_4 intoxicated rats.

Parameters	Control	CCl_4	CCl_4 treated with husk extract	CCL_4 treated silymarin
Creatinine µmol/L	28.86 ±1.69 d --	49.65 ± 2.63a (72.03)	33.55 ± 2.06c (16.25)	41.35 ± 2.72 b (43.27)
Serum protein g/dL	6.67 ± 0.34a --	4.69 ± 0.45b (-29.68)	6.12 ± 0.28a (-8.24)	4.66 ± 0.52b (-30.13)
Urea µmol/L	0.86 ± 0.34c --	3.51 ± 0.45a (308.13)	1.23 ± 0.28b (43.02)	3.55 ± 0.51a (312.79)

Data are means ±SD of eight rats in each group.
Statistical analysis is carried out by one way analysis of variance (ANOVA) using co-stat computer program
Unshared letters between groups are the significant difference level at $P < 0.05$.
% Values between brackets are percentage change over control.

Normal liver histopathological analysis showed normal hepatic lobular architecture Fig. (2 a). Portal tracts were extended with no fibrous tissue or lymphocytes deposition (Fig. 2 b). Liver injured with CCl_4 revealed portal loss of hepatic lobular architecture and ballooning of hepatocytes. The hepatocytes showed marked degree of hydropic and steatotic changes. Portal tracts were extended with marked number of chronic inflammatory cells and fibrous tissue (Fig. 2 c, d). The same histopathological observations in CCl_4 injured liver were noticed by Motawi et al. [29]. Treatments of injured liver with husk extract showed well formed nucleated hepatocytes arranged in cord with obvious sinusoidal arrays. Mild inflammatory lymphocyte infiltration and mild fibrosis were seen (Fig. 2e, f). CCl_4 group treated with silymarin showed the worst appearance, where swelling and foamy appearance of hepatocytes were viewed. Hydropic and steatosis changes were also detected. Mild fibrotic tissue was still present (Fig. 2 g, h).

We compare the anti-fibrogenic effects of silymarin with extract and the results exhibited that husk extract had higher potency in inhibiting collagen deposition and fibrosis severity. Therefore,

silymarin, as a standard herbal hepatoprotective drug confirmed the potential effect of Physalis extracts as hepatic protective agent.

section of CCl4-treated rats (Fig. 3c) showed glomerular and tubular degenerations. Marked collagen deposition was recorded (Fig. 3d). Theses histopathological picture of injured kidney was in line with Hamed et al. [38]. CCl4 group treated with husk extract showed almost normal morphology and normal architecture of the kidney (Fig. 3e). Glomeruli and tubules appeared to be regenerated. Minimal collagen deposition was observed (Fig. 3f). Kidney section treated with silymarin showed swollen and deformed glomeruli with moderate dilatation or congestion of space of Bowman and interstitial inflammations (Fig. 3 g). Moderate collagen deposition in the interstitial space was detected (Fig. 3h). The corrective histopathological findings after treatment with Physalis extracts give an additional support that Physalis mops up free radicals generation by CCl_4, reduce inflammation, improve kidney function and induce healthy state of renal cells. This observation suggested the Physalis role as a renal protective agent.

To correlate between the effect of Physalis extract as hepato-renal protecting agent and its active ingredients, it was revealed the presence of the antioxidants; flavonoids, withanolide as free aglycon, withanolides glycosides and high content of alkaloids. This was in parallel with the previous reports of Ray and Gupta [41] and Ismail and Alam [42] that recorded secondary metabolites such as alkaloids, free and glycosilated withanolids and flavonoids in *P. Peruviana*.

In conclusion, although, all organisms possess antioxidant systems for protection from oxidative damage, these systems are insufficient to prevent all possible damage. That is why the interest towards the inclusion of non-toxic antioxidants in human diet has become greater. Physalis husk extract has the ability to down regulate free radicals elevation, improves liver and kidney functions, reduces collagen deposition and fibrosis severity and normalizes hepatic and kidney cells architecture. Physalis may be used as a new safe therapy that may enhance the antifibrotic mechanism, delay disease progression or reduce complications, which clearly seen in the histopathological analysis.

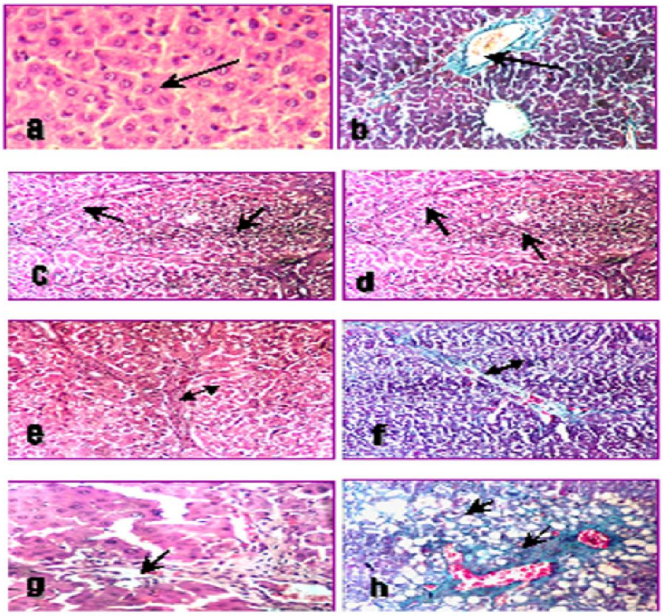


Fig.2: Photomicrograph of hematoxylin and eosin (H & E) and Masson's trichrom stained section (100x) of normal liver (a, b), CCl_4 (c, d), CCl_4 treated with husk extract (e, f), (g, h) and silymarin (k, l). Arrows indicate well arranged hepatocytes and normal level of collagen deposition. Small arrows indicate marked fibrosis, well developed lymphocytes inflammations and infiltrations and steatotic changes with foamy appearance of hepatocytes. Double head arrows indicate mild collagen deposition, more or less normal hepatocytes architecture.

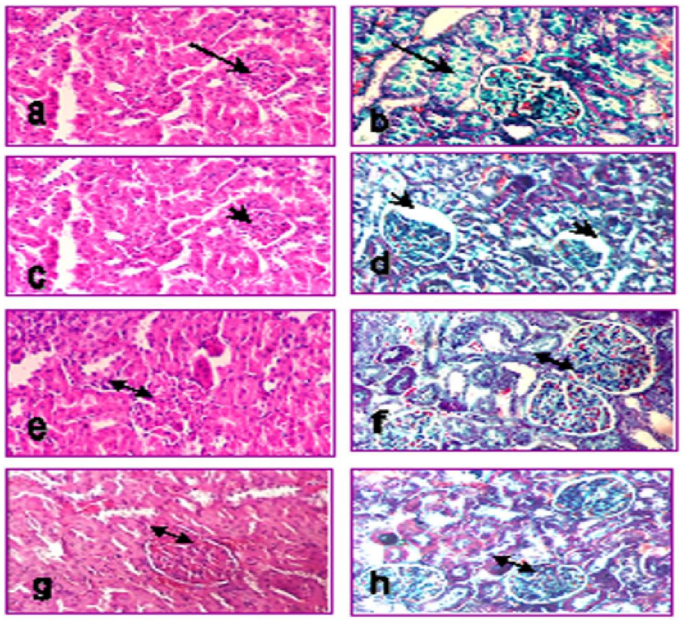


Fig.3: Photomicrography of hematoxylin and eosin (H & E) and Masson's trichrom stained section (100x) of normal kidney (a, b), CCl_4 (c, d), CCl_4 treated with husk extract (e, f), and silymarin (g, h). Arrows indicate normal glomeruli, tubules, tubulointerstitial cells and normal collagen deposition. Small arrows indicate dilated Bowman capsules with massive infiltrations and fibrosis in interstitial space. Double head arrows indicate mild collagen deposition and more or less normal glomeruli.

Kidney histopathological features of control (Fig. 3a) showed normal appearance of tubules, glomeruli and tubulointerstitial cells. Collagen deposition was of normal range in all control groups (Fig. 3b). Kidney

Competing interests

The authors declare no conflict of interest.

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