Presence of gallic acid and rutin improve the hepatoprotective strength of *Withania coagulans*

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Abstract Carbon tetrachloride (CCl_4) is one of the chemicals used in industry reported to accelerate the risk of liver diseases in workers especially in developing countries, if it is not handled carefully. Therefore, the present study conducted to evaluate the liver protective and oxidative stress reducing activities of methanolic (MFEt) and aqueous methanolic fruits (AqMFEt) extracts of *Withania coagulans* against CCl_4-induced liver damage in rats. These fruits extracts in oral doses of 800 mg/kg were found effective in their respective test groups in decreasing weight loss, maintaining hepatic membrane integrity, biosynthetic and conjugative abilities by improving liver and bile duct specific enzymes (alanine and aspartate transferases, alkaline phosphatase, γ-glutamyltranstransferase), total protein and bilirubin profiles, uric acid levels plus uplifting the efficacy of hepatic antioxidant enzymes and protein by minimizing lipid peroxidation. All these beneficial effects confirmed by observing normal anatomical features of liver tissues in test groups. Total phenolic compounds were found high in AqMFEt. Interestingly, for the first time, gallic acid and rutin are identified and quantified in these extracts and thought to improve hepatoprotective potential of *W. coagulans.*

Keywords: Carbon tetrachloride, Gallic acid, Hepatoprotective, Rutin, *Withania coagulans.*

INTRODUCTION

Liver is involved in regulating important physiological functions of the body, though it is sensitive to many heterogeneous factors *viz.*, genetic, biological and acquired like environmental and work place poisonous substances, metabolic intermediates and xenobiotics (Shamsi-Baghbanan *et al*., 2014). However, detoxifying mechanism and rapid regenerating potential keep hepatocytes safe from xenobiotic- and toxin-induced injuries (Luedde *et al*., 2014). Unfortunately, the sustained exposure of these acquired factors produced harmful effects on liver starting from acute inflammation, if it lasts for a long period of time then may lead to an array of chronic problems including steatosis, steatohepatitis, fibrosis, cirrhosis, and to hepatocellular carcinoma in severe cases (Reyes-Gordillo *et al*., 2017). Symptoms management and prevention of complications has been the prime target for the medical treatment of chronic liver diseases for many years. On the other hand, liver transplantation becomes the only proven treatment for end-stage liver disease regardless of its etiology (Poordad, 2015). Interestingly, most of the medicines either derived from herbs (silymarin) or mixture of various herbs (Liv 52) are available in market for providing relief from liver problems (Vergas-Mendoza *et al.*, 2014, Choijamts *et al.*, 2018). Therefore, investigation on the natural products is necessary to find out better hepatoprotective agent or which could be the source of new active principle for future liver saving medicine.

Withania coagulans Dunal (Family: *Solanaceae*), a plant of various medicinal and ethno-pharmacological uses, is widely spread in South Asia region (Gupta, 2012). Different fruits extracts of same plant have been reported as digestant, anti-flatulent, sedative, antihyperglycemic, antihyperlipidemic, antimicrobial, anti-inflammatory, antitumor, hepatoprotective, cardioprotective, immunosuppressive, free radical scavenging and central nervous system depressant agents (Maurya, 2010; Lateef and Qureshi, 2014 and 2016; Mudassir *et al.*, 2018). The well-famous historical use of these fruits is in milk coagulation (Pandey and Nama, 2015). Number of withanolides has been isolated from *W. coagulans* so far (Khodaei *et al*., 2012), of which 3β–hydroxyl-2,3-dihydrowithanolide F was reported in aqueous fruit extract of same plant and found effective in reversing carbon tetrachloride (CCl_4) induced liver damage (Budhiraja *et al*., 1986). Therefore, the current study aimed to investigate the liver protective potential of methanolic and aqueous methanolic fruits extracts of *W. coagulans* by focusing the presence of total phenolic compounds in them.

MATERIALS AND METHODS

Experimental animals

Albino female *Wistar* rats weighing between 170-220 g purchased from authentic breeding house of Dow

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University of Health Science (DUHS) and kept in conventional animal house of Biochemistry Department, University of Karachi with free access to water and standard laboratory food for one week before starting the experiment to acclimatize them with environment (Qureshi *et al.,* 2016). The complete procedure of the study followed the internationally accepted guidelines (Nadari *et al.,* 2012) and approved by Board of Advanced Study and Research (BASR) in 2015 of same university.

Preparation of fruits extracts

Dry fruits of *W. coagulans* purchased from local market, Sadar Karachi and authenticated by taxonomist in Department of Botany, University of Karachi (voucher No.KU/BCH/SAQ/06). The fruits were coarsely powdered and 40 gm of which soaked in 1 L methanol for overnight, filtered twice, then subjected filtrate to rotary vacuum evaporator to obtain brown residue, called as MFEt (Azmi and Qureshi, 2012). Whereas 80% aqueous methanolic fruits extract (AqMFEt) was prepared by extracting 10 gm of same fruits powder with 100mL of 80% aqueous methanol thrice, filtered, pooled and concentrated over water bath at 90°C to get gummy brown residue (Azmi and Qureshi, 2014; Azmi *et al.*, 2018).

Quantitative analysis of total phenolic compounds

i. Spectrophotometric method

Folin–Ciocalteu method used to estimate total phenolic compounds in fruits extracts and their concentration were expressed in term of mg of total phenols in gm of starting material by using standard curve of gallic acid (Singleton and Rossi, 1965).

ii. High performance liquid chromatography (HPLC)

HPLC performed on Shimadzu system equipped with a pump of LC-10AC type, UV-Vis spectrophotometer (SPD-6AV) detector and a column C18 (size: 4.6 x 250 mm; particle size: 5µm) of Agilent Technologies. The mobile phase was consisting of water and methanol (50:50) with a flow-rate of 0.7mL/min. The injected volume of sample was set to 20µL. The detection performed at a wavelength of 260 nm at room temperature. The retention times and the peak areas noted on HPLC chromatograms of MFEt and AqMFEt by using gallic acid and rutin as standards, these were purchased from Merck, Germany.

Experimental protocol

After acclimatization a period of one week, the rats randomly divided into five groups, each consisting of six and served as:

Group I and II (Normal and Hepatotoxic Controls): each treated with distilled water (1mL/kg).

Group III (Positive Control): treated with silymarin (100 mg/kg).

Group IV (Test Group): treated with MFEt (800mg/kg).

Group V (Test Group): treated with AqMFEt (800mg/kg).

The rats belonged to group II–V were intraperitoneally injected with carbon tetrachloride (CCl_4; 3mL/kg) in 1:1 diluted with olive oil on 3rd and 5th day of experiment and after 24 hrs of last dose of this hepatotoxin, rats were decapitated to collect serum while liver tissues were dissected out carefully. The length of animal trial was 5 days and treatments were done orally. Silymarin (Siliver 200mg/kg; Abbott Laboratories, Pak. Pvt. Ltd) was used as reference drug whereas dimethyl sulphoxide (DMSO 0.05%; Fisher Scientific, UK) used as dissolving medium for preparing doses of fruits extracts. Acute toxicity of extracts has already been published where these were found non-toxic from 10 to 2000mg /kg (Lateef and Qureshi, 2014), therefore 800mg/kg dose of each of extract was used in present study without any hesitation.

Evaluation of percent change in body weight and liver index

The percent change in body weight of each group was calculated by using following formula (Azmi and Qureshi, 2013), after measuring weights of each rat on initial and final day of trial.

$$\text{Percent body weight change} = \left[\frac{\text{Final day weight} - \text{Initial day weight}}{\text{Initial day weight}}\right] \times 100$$

Liver index was calculated with the formula (Huo *et al*., 2011),

$$\text{Liver Index (\%)} = \left(\frac{\text{Liver weight}}{\text{Final day body weight}}\right) \times 100$$

Evaluation of serum biochemical parameters

Liver-specific biochemical parameters including alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), total protein (TP), albumin (ALB), and uric acid (UA) were estimated by using commercially available reagent kits (Randox, UK). The percent protection in ALT and AST and percent gain/loss in other biochemical parameters were calculated by formulae (Qureshi *et al*., 2016), Where, A_t= average values of treated including test or positive control group, A_x= average value of hepatotoxic control group and A_o= average value of normal control group

$$\text{Percent Protection} = 1 - \left(\frac{\text{At} - \text{Ao}}{\text{Ax} - \text{Ao}}\right) \times 100$$

$$\text{Percent gain/loss} = \left(\frac{\text{At} - \text{Ax}}{\text{Ax}}\right) \times 100$$

Assessment of antioxidant parameters

Percent inhibition of catalase (CAT), super oxide dismutase (SOD), reduced glutathione (GSH) and lipid per oxidation (LPO) were estimated by using standard manual methods (Lateef and Qureshi, 2013).

Histopathological study of liver tissues

Dissected out liver tissues from each group by immersing in 10% formalin in separate bottle and sent to Dr. Essa's Diagnostic Laboratory, Abul-Hassan Isphahani Road, Karachi, Pakistan for histological studies (Alturkistani *et al.,* 2016) while slides of this study photographed by using system microscope (Olympus BX51) and digital camera (Olympus DP72).

STATISTICAL ANALYSIS

Results are expressed as mean ± standard deviation (SD). To determine the treatment effects, one-way analysis of variance (ANOVA) carried out by using statistical package for social sciences (SPSS, Illinois, United States, version 17.0), where level of significance among the various treatments calculated by least significance difference (LSD) test at $p<0.05$, 0.01 and 0.0001.

RESULTS

Total phenolic compounds in MFEt and AqMFEt

The amount of total phenolic compounds in MFEt and AqMFEt of *W. coagulans* were found as 125.9 and 196.9 mg/gm respectively.

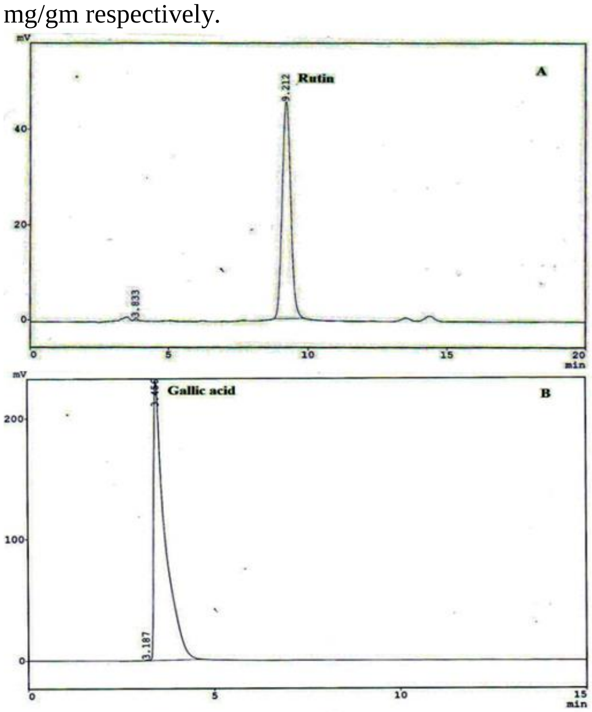

Fig. 1: High performance liquid chromatograms representing peaks of standards rutin (A) and gallic acid (B) at 260nm

HPLC analysis of MFEt and AqMFEt

The peaks of gallic acid and rutin with retention times of 3.439 & 9.393 and 3.459 & 9.478 min respectively were appeared in chromatograms of MFEt and AqMFEt and identified by comparing their peaks on chromatograms of these standards. However, the high amount of rutin (2.34 mg) found in AqMFEt while the same amount (39 mg) of gallic acid was found in both extracts (table 1; figs. 1 and 2).

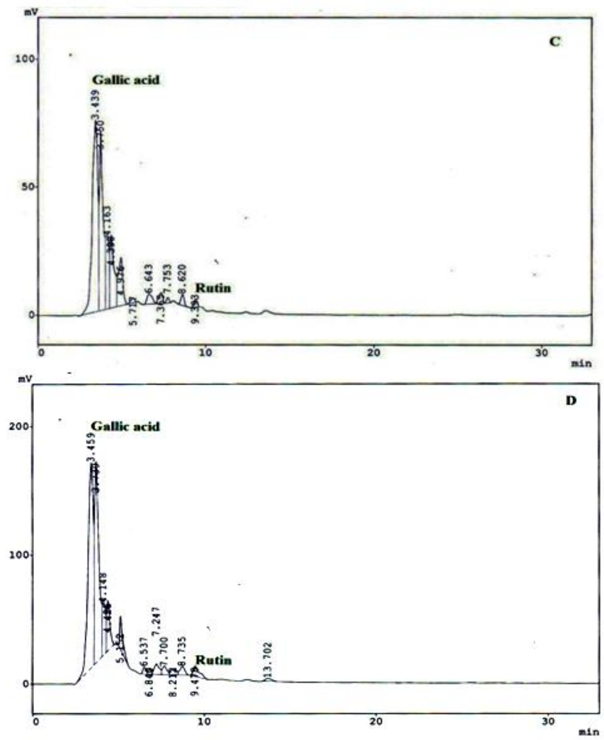

Fig. 2: Identification of standards rutin and gallic acid in high performance liquid chromatograms of MFEt (C) and AqMFEt (D) at 260nm

Effect of MFEt and AqMFEt on percent body weight change and liver index

A drastic reduction (-12.08%) in body weight was observed in rats of group II which were only treated with CCl_4 whereas prominent decrease in body weight reduction was observed in rats belonging to group III-V ($p<0.01$ & 0.0001) especially in last group treated with AqMFEt of *W. cogulans* (table 2). Again, AqMFEt found effective in reducing liver index in group V and brought it almost same that was found in group I (normal control). However, MFEt and silymarin also produced significant effects ($p<0.0001$) on this same parameter in their respective groups III and IV as compared to group II (table 2).

Effect of MFEt and AqMFEt on liver specific enzymes

Group II showed elevated levels of ALT, AST and ALP (U/L) whereas their levels became very much decreased in groups III-V especially in test groups IV and V treated with MFEt and AqMFEt in dose of 800 mg/kg respectively with percent protection from 80 to 105% was found in aminotransferases and phosphatase levels ($p<0.05$ & 0.0001). On the other hand, GGT level was found low in all treated groups including group II in comparison to group I (table 2).

Table 1: Identification and quantification of gallic acid and rutin in MFEt and AqMFEt at 260nm through HPLC analysis

	Standard	MEFEt	AqMFEt
Gallic Acid			
Retention time (min)	3.456	3.439	3.459
Peake area (mAU/min)	4751289	1746084	3364765
Peak height (mAU)	230688	75066	159098
Concentration (mg)	99.9219	39.7648	39.6424
Rutin			
Retention time (min)	9.212	9.393	9.478
Peake area (mAU/min)	988198	41651	198352
Peak height (mAU)	45543	3125	8047
Concentration (mg)	99.6084	0.9486	2.3369

Table 2: Effect of MFEt and AqMFEt on Percent Body weight, Liver Index and Liver-Specific Enzymes

S.N	Groups/Treatments	Percent		Liver-specific Enzymes (U/L)			
		Body Weight change	Liver index	ALT	AST	ALP	GGT
1	I (Normal control)	1.55±1.83	5.26±0.24^d	46.00±0.9^d	173.50±102.86^d	100.01±12.0^d	10.00±0.00
2	II (hepatotoxic control; CCl_4 3ml/kg)	-12.08±2.21	9.58±1.18	1869.00±84.99	2413.00±262.0	186.0±41.0	8.16 ±6.30
3	III (Silymarin 100mg/kg + CCl_4 3ml/kg)	-8.76±1.24^b	6.50±0.68^d	194.0±14.0^d (91.0%)	590.0±134.4^d (82%)	155.5±21.4^a (35.57%)	0.50 ±0.54^d
4	IV (MFEt 800mg/kg + CCl_4 3ml/kg)	-2.32±1.62^d	7.38±0.45^d	117.02±26.26^d (96.2%)	589.0±183.0^d (81.5%)	100.02±10.0	1.50 ±0.54^d
5	V (AqMFEt 800mg/kg + CCl_4 3ml/kg	-1.86±1.67^d	5.75±0.47^d	110.33±19.01^d (96.46%)	378.0±131.2^d (90.9%)	95.16±9.7^d (105.19%)	1.83 ±0.40^d

Results are represented as mean ±SD (n=6). Where a, b & d are representing the p<0.05, 0.01 & 0.0001 respectively when compared to group II. Figures in brackets indicate the percent protection in enzymes.

Table 3: Effect of MFEt and AqMFEt on Total Bilirubin & Protein Profiles and Uric Acid Level

S. No.	Groups	Parameters					
		TB	DB	IB	TP	ALB	UA
1	I	0.1 ±0.0	0.10 ±0.0	0.00 ±0.0^d	5.49 ±0.3^d	4.47 ±0.23^d	1.81± 0.5^d
2	II	0.43 ±0.2	0.28 ±0.2	0.15 ±0.1	4.13 ±0.40	2.75 ±0.16	10.13±0.2
3	III	0.20 ±0.0^d (-53.5%)	0.10 ±0.0^d (-64.3%)	0.10 ±0.0^b (-33.3%)	6.19 ±0.2^d (49.8%)	4.21 ±0.2^d (53.09%)	9.76 ± 0.25^a (-3.65%)
4	IV	0.23 ±0.0^d (-46.5%)	0.13 ±0.0^b (-53.6%)	0.10 ±0.0^b (-33.3%)	5.35 ±0.5^d (29.5%)	4.45 ±0.14^d (61.8%)	1.58 ± 0.3^d (-84.40%)
5	V	0.30 ±0.0^a (-30.2%)	0.20 ±0.0^a (-28.6)	0.10 ±0.0^b (-33.3%)	5.77 ±0.1^d (39.7%)	4.57 ±0.3^d (66.2%)	1.40 ± 0.08^d (-86.17%)

Results reflected the mean ±SD (n=6). a, b & d are representing the p<0.05, 0.01 & 0.0001 respectively when compared to group II. Values in brackets indicate the percent gain / loss in each parameter. The values of TB, DB, IB and UA are in mg/dl whereas TP and ALB in gm/dL.

Table 4: Effect of MFEt and AqMFEt on Antioxidant Parameters

S. No	Groups	Percent Inhibition			
		CAT	SOD	GSH	LPO
1	I	18.8 ± 3.3	34.0 ± 1.3	23.0 ± 3.0	42.1 ± 3.0
2	II	47.0 ± 7.35	64.9 ± 7.4	50 ± 9.0	8 ± 2.3
3	III	18.0 ± 3.5^d	29.1 ± 2.2^d	28.33 ±5.7^d	24.3 ±0.7^d
4	IV	17.0 ± 1.85^d	28.0 ± 6.6^d	16.0 ± 3.0^d	36.3 ± 3.2^d
5	V	16.34 ± 2.0^d	23.2 ± 5.6^d	16.5 ± 1.3^d	46.2 ± 1.7^d

Results expressed as mean ± SD (n=6). d = p < 0.0001 when compared with group II

Effect of MFEt and AqMFEt on total bilirubin & protein profiles and uric acid level

CCl_4 treatment caused a significant increase in total bilirubin levels (mg/dL) in group II especially in indirect one where indirect bilirubin was significantly decreased up to -33% ($p<0.01$) in positive control and test groups (table 3). Similarly, uric acid level (mg/dL) was also reduced more than 80% ($p<0.01$) in test groups IV and V while silymarin was not found effective in this regard in group III (table 3). On contrary, protein profile found significantly improved in groups III-V by showing percent gain ($p<0.0001$) from 53 to 66% in ALB levels (gm/dL) and in total protein (gm/dL) from 29 to 49% as compared to group II (table 3).

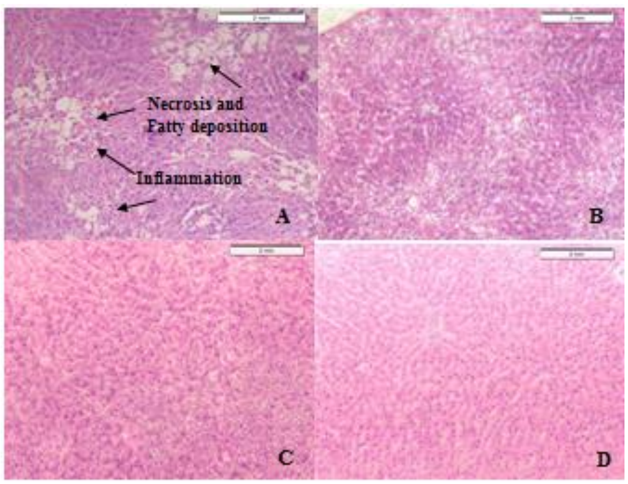

Fig. 3: Histology of liver tissues: CCl_4 treated hepatotoxic group (A), Silymarin treated positive control group (B), MFEt and AqMFEt treated test groups (C) and (D) respectively.

Effect of MFEt and AqMFEt on antioxidant status

Silymarin and both extracts (MFEt and AqMFEt) were found to lowering ($p<0.0001$) the percent inhibition of CAT, SOD and GSH in their respective groups III to V when matched with group II. Whereas the percent inhibition of LPO was much higher in test groups (table 4).

Liver histological study

The histology of hepatotoxic group II showed fatty deposition, inflammation and ruptured hepatocytes (necrosis) whereas all these toxic effects of CCl_4 were completed removed in liver tissue slides of test groups treated with MFEt and AqMFEt in dose of 800mg/kg. However, few of the CCl_4 intoxication impacts still seen in silymarin treated positive control group III (fig. 3).

DISCUSSION

The risk of liver diseases is accelerating day by day because of acquired factors especially chemicals used in work place (Malaguarnera *et al*., 2012). Among these, CCl_4 is used as a grease-removing agent in leather industries (Huang *et al*., 2014). No doubt, it is beneficial in one way but it is highly toxic to human tissues especially liver in other way if people are not taking precaution in its handling. As its continuous inhalation, produce membrane macromolecular reactive metabolites including trichloromethyl ($\bullet CCl_3$) and peroxytrichloromethyl ($\bullet OOCCl_3$) after passing through hepatic cytochrome P_{450} (CYP2E1 isoform) that induce lipid peroxidation, oxidative damage to nucleic acids, alter the cell membrane integrity of mitochondria and endoplasmic reticulum (Xiang *et al*., 2017). This accelerate the production of more free radicals, disturb calcium homeostasis, activating membrane degrading enzymes and activating tumor necrosis factor alpha (TNF-α), all these constitute oxidative stress in body which leads to inflammation, necrosis, and fatty deposition in liver (Mahmoodzadeh *et al*., 2017).

Alteration in hepatic and bile duct cells' membrane permeability triggers the release of their specific enzymes (Ortega-Alonso *et al*., 2016) including alanine & aspartate aminotransferases (ALT & AST), γ-glutamyltranspeptidase (GGT) and alkaline phosphates (ALP). The same was observed in present study where CCl_4 treated heptotoxic control group II showed elevated levels of aminotransferases and phosphates in serum. On contrary, test groups treated with MFEt and AqMFEt in a dose of 800mg/kg showed much lower levels ($p<0.05$ & 0.0001) of these enzymes with percent protection from 81 to 105% in aminotransferases and phosphatase levels that indicate the cell protective activity of these fruits extracts. In addition, much better activities of these enzymes were also observed in well-known hepatoprotective medicine silymarin treated group III (table 1). However, bile duct specific enzyme GGT found low in hepatotoxic control group II and very low in test and positive control groups. GGT is involved in the degradation of antioxidant protein glutathione (Turgut and Tandogan, 2011) but in case of CCl_4-induced oxidative stress more glutathaione is likely to require to subside free radicals therefore this could be the reason of having its suppressed activity observed in test and positive control groups to make glutathione more available to combat the oxidative stress (Koenig and Seneff, 2015).

Liver is the chief site of metabolism and helps in digestion (Guerra *et al*., 2016). Hence, disturbance in liver function also decreases food intake and induce weight loss (Zehra and Abdurrahman, 2017). The same was true in present study that showed a drastic reduction (-12.08%) in body weights of hepatotoxic control group II whereas a prominent decrease in body weight reduction was observed in rats belonged to group III-V ($p<0.01$ & 0.0001) especially in last group treated with AqMFEt of *W. coagulans*, this finding also supports the liver improving ability of *W. coagulans* fruits extracts.

CCl_4-induced liver damage also affects the biosynthetic function of the same tissue. Similar observation was verified in group II by showing decrease concentration of total protein and albumin (g/dL). Reverse of these parameters by showing percent gain ($p<0.0001$) from 53 to 66% in albumin levels and in total protein from 29 to 49% in test (IV and V) and positive control (III) groups was observed as compared to group II that clearly reflects the improvement in liver biosynthetic capacity and this may be due to the stabilization of hepatic structure. In addition, enlargement in liver size and weight was observed in many conditions including deficiency of total protein concentration and CCl_4-induced hepatotoxicity and it may continue till the normal level of total protein is resumed (Palmes and Spiegel, 2004). Both possibilities were present in hepatotoxic control group II of this study which demonstrated increased liver index. Interestingly, hepatoprotective activity of *W. coagulans* fruits extracts fortified by observing prominent decrease in liver index of test groups accompanied by normal level of total protein and liver weights in these groups. Again, AqMFEt found more effective in reducing the liver index in group V and brought it almost as same as it was found in group I (normal control). However, MFEt and silymarin were also produced significant effects ($p<0.0001$) on this same parameter in their respective groups III and IV as compared to group II.

Betterment in liver function also confirmed by monitoring normal levels of total bilirubin, direct bilirubin and decreased level of indirect bilirubin in test and positive control groups and the decrease in indirect bilirubin was found up to -33% in positive control and test groups. Indirect bilirubin is lipophilic and it has to convert in conjugative or direct one after conjugating with two molecules of glucuronides through conjugation reaction that takes place in liver in presence of glucuronyltransferase enzyme but it is only possible when liver structure is intact and works properly (Sticova and Jirsa, 2013). Another beneficial effect of fruits extracts of *W. coagulans* was established by obtaining decrease in uric acid (mg/dl) more than 80% ($p<0.01$) in extracts treated test groups IV and V while silymarin was not found effective in this regard in group III. CCl_4 encourages oxidative damage to nucleic acid that accelerates the catabolism of purine and pyramidine. Uric acid is the end product of purine catabolism, its increase concentration in serum indicates number of pathological conditions including the cell lysis (El Ridi and Tallima, 2017). Increased concentration of uric acid also reflects the increased activity of xanthine oxidase, the rate-limiting enzyme of uric acid synthesis, which is also one of the enzymes involved in generation of hydrogen peroxide (H_2O_2). Large amount of this free radical in turn contribute oxidative stress and cell lysis (Ghaffari *et al*., 2012). Fruits extracts treatment in test groups overturn this condition by protecting the architecture of hepatocytes, thereby inhibiting the release of uric acid in serum. CCl_4-induced oxidative stress clearly observed in hepatotoxic group II by showing high percent inhibition of antioxidant markers including CAT, SOD, GSH and low percent inhibition of oxidative stress marker *viz.*, LPO whereas MFEt, AqMFEt and silymarin treated groups showed vice versa situation. This finding supports the radical scavenging activity of fruits extracts of *W. coagulans* that also previously found in high fat induced hyperlipidemic animal model (Lateef and Qureshi, 2014 and 2016).

All beneficial effects of MFEt and AqMFEt of *W. coagulans* in improving liver functions evidenced by histopathological study of liver tissues like necrosis, inflammation and ballooning were observed in liver tissues of CCl_4-induced hepatotoxic control rats, to some extend these were also appeared in silymarin treated group. Whereas all these destructive effects of CCl_4 were completely disappeared in liver tissues of test groups treated with MFEt and AqMFEt.

A previous study claimed Withanolide F as the active liver protective compound found in aqueous fruits extract of *W. coagulans* (Budhiraja *et al*., 1986). Whereas, the present study first time describes the presence and possible amount of gallic acid (non-flavonoid) and rutin (flavonoid), the well-known phenolic compounds in MFEt and AqMFEt of *W.coagulans* through HPLC. However, the high amount of rutin (2.34 mg) was found in AqMFEt whereas gallic acid was present in same quantity (39 mg) in both extracts. Similarly, when total phenolic compounds in term of gallic acid (mg/g of starting material) were estimated through spectrophotometric method in start of this study, it was also found that AqMFEt possessed more phenolic compounds than MFEt. Both gallic acid and rutin are well-reported compounds involved in hepatoprotective and antioxidant activities and isolated from other natural sources like *Alnus nitida, Fagonia olivieri, Rhodiola imbricate,* etc (Senthilkumar *et al*, 2014; Sajid *et al*., 2016; Rashid *et al*., 2016). In short, total phenolic compounds may ameliorate the hepatoprotective and antioxidant activities of fruits extracts of *W. coagulans*.

CONCLUSION

The results support and confirm the hepatoprotective and antioxidative potential of fruit extracts of *W. coagulans* and assumed that the presence of total phenolic compounds especially gallic acid and rutin in these extracts improve their strength in this regard.

REFERENCES

Hani A Alturkistani, Faris M Tashkandi and Zuhair M Mohammedsaleh (2016). Histological Stains: A

Literature Review and Case Study. *Glob. J. Health Sci.*, **8**: 72-79.

Azmi MB and Qureshi SA (2012). Methanolic root extract of *Rauwolfia serpentina* Benth improves the glycemic, antiatherogenic and cardioprotective indices in alloxan-induced diabetic mice. *Adv. Pharmacol. Sci.*, 11 pages (online publication).

Azmi MB and Qureshi SA (2013). *Rauwolfia serpentina* ameliorates hyperglycemic, haematinic and antioxidant status in alloxan-induced diabetic mice. *J. Appl. Pharmceut. Sci.*, **3**: 136-141.

Azmi MB and Qureshi SA (2014). Glucose lowering potential of hydromethanolic extract of Rauwolfia. *World J. Pharmaceut. Sci.*, **2**: 219-223.

Azmi MB, Qureshi SA, Ahmed SDH, Mudassir HA, Imtiaz F, Rais S, Khan AA and Ahsan M (2018). Ameliorative effect of hydro-methanolic extract from roots of *Rauwolfia serpentina* on some biochemical parameters of type 1 diabetic mice. *Pak. J. Pharmaceut. Sci.*, **31**(Suppl): 663-668

Budhiraja RD, Sudhir S, Garg KN and Arora B (1986). Protective effect of 3 beta-hydroxy-2,3 dihydrowithanolide F against CCl_4 induced hepatotoxicity. *Planta Med.*, **52**: 28-29.

Choijamts G, Batsyren Ch, Orkhon B, Otgontyr, Narantstsetseg D, Oyunerdene X, Zagirjav T, Erdenesuvd, Enkhbayar, Pyrevbazar, Oyundelger, Tungalag, Erdenetsetseg D, Enkhtsetseg, Bilegmaa M, Sarantuya S and Palaniyamma D (2018). Role of Liv.52 DS tablets as a hepatoprotective agent in tuberculosis patients receiving antitubercular drugs: A double blind placebo controlled study. *J. Liver Clinical Res.*, **5**: 1042-1050.

El Ridi R and Tallima H (2017). Physiological functions and pathogenic potential of uric acid: A review. *J. Adv. Res.*, **8**: 487-493.

Ghaffari H, Ghassam BJ and Prakash HS (2012). Hepatoprotective and cytoprotective properties of *Hyptis suaveolens* against oxidative stress-induced damage by CCl_4 and H_2O_2. *Asian Pacific J. Trop. Med.*, **5**: 868-874.

Guerra S, Mamede AC, Carvalho MJ, Laranjo M, Tralhao JG, Abrantes AM, Maia CJ, and Botelho MF (2016). Liver diseases: What is known so far about the therapy with human amniotic membrane? *Cell Tissue Bank*, **17**: 653-663.

Gupta PC (2012). *Withania coagulans* Dunal: An overview. *Int. J. Pharmaceut. Sci. Rev. Res.*, **12**: 68-71.

Huang B, Lei C, Wei C, and Zeng G (2014). Chlorinated volatile organic compounds (Cl-VOCs) in environment-Sources, potential human health impacts, and current remediation technologies. *Environ. Int.*, **71**: 118-138.

Huo HZ, Wang B, Liang YK, Bao YY and Gu Y (2011). Hepatoprotective and antioxidant effects of Licorice extract against CCl_4-induced oxidative damage in rats. *Int. J. Mol. Sci.*, **12**: 6529-6543.

Khodaei M, Jafari M and Noori M (2012). Remedial use of withanolides from *Withania coagulans* (Stocks) Dunal. *Adv. Life Sci.*, **2**: 6-19.

Koenig G and Seneff S (2015). Gamma-Glutamyltransferase: A predictive biomarker of cellular antioxidant inadequacy and disease risk. *Disease Markers*, 2015: 18 pages (online publication).

Lateef T and Qureshi SA (2013). *Centratherum anthelminticum* ameliorates antiatherogenic index in hyperlipidemic rabbits. *Int. J. Pharm.*, **3**: 698-704.

Lateef T and Qureshi SA (2014). Ameliorative effect of *Withania coagulans* on experimentally-induced hyperlipidemia in rabbits. *J. Nat. Remedies*, **14**: 83-88.

Lateef T and Qureshi SA (2016). *Centratherum anthelminticum* and *Withania coagulans* improves lipid profile and oxidative stress in triton X-100 induced hyperlipidemic rabbits. *Int. J. Pharmacog. Phytother. Res.*, **8**: 933-940.

Luedde T, Kaplowitz N, and Schwabe RF (2014). Cell death and cell death responses in liver disease: Mechanisms and clinical relevance. *Gastroenterol.*, **147**: 765-783.

Mahmoodzadeh Y, Mazani M, and Rezagholizadeh L (2017). Hepatoprotective effect of methanolic *Tanacetum parthenium* extract on CCl_4-induced liver damage in rats. *Toxicol. Rep.*, **4**: 455-462.

Malaguarnera G, Cataudella E, Giordano M, Nunnari G, Chisari G and Malaguarnera M (2012). Toxic hepatitis in occupational exposure to solvents. *World J. Gastroenterol.*, **18**: 2756-2766.

Maurya R (2010). Chemistry and pharmacology of *Withania coagulans*: An ayurvedic remedy. *J. Pharm. Pharmacol.*, **62**: 153-160.

Mudassir HA, Nazim K, Khan UA and Qureshi SA (2018). Comparative evaluation of hypoglycemic activity, trace minerals and phytochemical contents of some potential medicinal plant extracts. *Int. J. Biol. Biotechnol.*, **15**: 55-62.

Naderi MM, Sarvari A, Milanifar A, Boroujeni SB and Akhondi MM (2012). Regulations and ethical considerations in animal experiments: International laws and Islamic perspectives. *Avicenna J. Med. Biotechnol.*, **4**: 114-120.

Ortega-Alonso A, Stephens C, Lucena MI and Andrade RJ (2016). Case characterization, clinical features and risk factors in drug-induced liver injury. *Int. J. Mol. Sci.*, **17**: 714.

Palmes D and Spiegel HU (2004). Animal models of liver regeneration. *Biomaterials*, **25**: 1601-1611.

Pandey I and Nama KS (2015). *Withania coagulans* (Stocks) Dunal A rare ethnomedicinal plant of the Western Rajasthan Desert. *Int. J. Pharmaceut. Biomed. Res.*, **2**: 34-40.

Poordad FF (2015). Presentation and complications associated with cirrhosis of the liver. *Curr. Med. Res. Opin.*, **31**: 925-937.

Qureshi SA, Rais S, Usmani R, Zaidi SS, Jehan M, Lateef T and Azmi MB (2016). *Centratherum anthelminticum* seeds reverse the carbon tetrachloride-induced hepatotoxicity in rats. *African J. Pharm. Pharmacol.,* **10**: 533-539.

Rashid U, Khan MR and Sajid M (2016). Hepatoprotective potential of *Fagonia olivieri* DC. against acetaminophen induced toxicity in rat. *BMC Complement. Altern. Med.,* **16**: 449.

Reyes-Gordillo K, Shah R and Muriel P (2017). Oxidative stress and inflammation in hepatic diseases: Current and future therapy. *Oxid. Med. Cell. Longev.,* 3140673.

Sajid M, Khan MR, Shah NA, Shah SA, Ismail H, Younis T and Zahra Z (2016). Phytochemical, antioxidant and hepatoprotective effects of *Alnus nitida* bark in carbon tetrachloride challenged Sprague Dawley rats. *BMC Complement. Altern. Med.,* **16**: 268.

Senthilkumar R, Chandran R and Parimelazhagan T (2014). Hepatoprotective effect of *Rhodiola imbricata* rhizome against paracetamol-induced liver toxicity in rats. *Saudi J. Biol. Sci.,* **21**: 409-416.

Shamsi-Baghbanan H, Sharifian A, Esmaeili S and Minaei B (2014). Hepatoprotective herbs, Avicenna viewpoint. *Iranian Red Crescent Med. J.,* **16**: e12313.

Singleton VL and Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.,* **16**: 144-158.

Sticova E and Jirsa M (2013). New insights in bilirubin metabolism and their clinical implications. *World J. Gastroenterol.,* **19**: 6398-6407.

Turgut O and Tandogan I (2011). Gamma-glutamyltransferase to determine cardiovascular risk: Shifting the paradigm forward. *J. Atheroscler. Thromb.,* **18**: 177-181.

Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, Esquivel-Soto J, Esquivel-Chirino C, García-Luna y González-Rubio M, Gayosso-de-Lucio JA and Morales-González JA (2014). Hepatoprotective effect of silymarin. *World J. Hepatol.,* **6**: 144-149.

Xiang H, Han Y, Zhang Y, Yan W, Xu B, Chu F, Xie T, Jia M, Yan M, Zhao R, Wang P and Lei H (2017). A new oleanolic acid derivative against CCl_4-induced hepatic fibrosis in rats. *Int. J. Mol. Sci.,* **18**: 553.

Zehra KF and Abdurrahman K (2017). Review: Nutrition on chronic liver diseases. *SL Gastroenterol.,* **1**: 111.