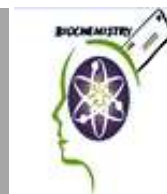




Scientific Research & Studies Center-Faculty of Science- Zagazig
University- Egypt

Biochemistry Letters

Journal home page:



The antifibrotic effect of policosanol in CCl₄-induced liver fibrosis in rats

Nabila zein¹, Shaza Magdy¹, Fathy Yassin²

¹Biochemistry Division, Chemistry Department, Faculty of science, Zagazig University, Zagazig, Egypt

²Organic chemistry Division, Chemistry Department, Faculty of science, Zagazig University, Zagazig, Egypt

ARTICLE INFO

Received : 6/6/2022

Accepted : 17/6/2022

Available online : 18/6/2022

Keywords:

Liver fibrosis, Liver enzymes,
Policosanol, silymarin

ABSTRACT

Background: Liver fibrosis characterized with extreme accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases. Progressive liver fibrosis consequences in portal hypertension, liver failure and cirrhosis which often requires liver transplantation. **Aim:** This study was carried out to evaluate the antifibrotic effect of policosanol in liver fibrosis induced in rats by CCl₄ administration. **Material and methods:** The rats were divided into four groups. Each group contains 10 Rats. The negative control group (NCG) received intra peritoneal (i.p.) injection of olive oil at dose of (0.1 ml/100 g BW) twice weekly for 8 weeks. The other three groups after induction of liver fibrosis by i.p. injection of 100% CCL₄/ Olive Oil at dose of 0.1 ml/ 100g BW twice weekly for 8 weeks were divided into positive fibrotic group (PFG), policosanol treated group (PTG) and silymarin treated group (PTG). The positive fibrotic group remains without any treatment. Additionally, the policosanol and silymarin treated groups were gavaged at dose of 100 mg/kg BW of either policosanol weekly twice dose for 8 weeks according to previous report of Silymarin dissolved in 0.5 percent CMC-Na as a daily dose for 8 weeks, Blood was drawn from the retro-orbital plexus through retro-orbital sinus puncture with a heparinized micro-hematocrit capillary tube, then centrifuged at 3000 rpm for 15 minutes to separate serum. Until biochemical analysis, they were kept at -20°C. **Results :** treatment of fibrotic rats with policosanol decrease level of ALT, AST and Albumin as compared with Positive fibrotic group. we noticed that serum level of TGF-beta showed elevation in positive fibrotic group as compared by Negative control group, while after treatment by policosanol or silymarin showed significant decrease, while serum levels of oxidative stress marker (SOD) showed significant reduction in positive fibrotic group as compared by Negative control group but treatment with either policosanol or silymarin had significantly increased those serum levels as compared by positive fibrotic group. **Conclusion:** Both silymarin and policosanol were found to have anti-fibrotic and antioxidant effects against CCL₄-induced hepatic fibrosis, with the latter having a stronger effect than the former. Furthermore, we

Corresponding author: Nabila zein, Biochemistry Division, Chemistry Department, Faculty of Science, Zagazig University, Egypt. Email: dr.nabila.zein@gmail.com.

propose that, in addition to these pathways, the reduction of TGF-beta and Albumin, as well as an increase in liver enzymes and SOD, particularly in the PTG, might be considered as another key mechanism for developing therapeutic.

Introduction:

Hepatic fibrosis occurs as the consequence of a sustained wound healing response of liver toxic, infectious or metabolic agents and is characterized by excessive accumulation of extra-cellular matrix (ECM) leading to ultimate liver dysfunction and irreversible cirrhosis [1]. Its main causative factors in developing countries are diseases with hepatitis and C viruses and parasitic infection with *Schistosoma mansoni*, while it can be excessive alcohol consumption in developed Countries [2].

Medicinal plants have been used from ancient times for the treatment of a large variety of diseases as well as for hepatotoxicity. Policosanol supplementation was well known to significantly improve blood pressure among patients [3], However there are few reports about the therapeutic effect of policosanol on liver fibrosis [4], as it is being rich in various compounds such as triglycerides, flavonoids, and polyphenols [5]. In the present study we aimed to evaluate the anti-fibrosis effect of policosanol in comparison with silymarin which is known by its antioxidant effects in liver diseases [6].

Materials and methods

Materials

Policosanol was sourced from NOW Foods. Cairo, Egypt. Elarayf of Food Industries Company, Cairo, Egypt, provided gum acacia for dissolving policosanol. The pharmaceutical company Sedico provided Silymarin.. El-Gomhouria business For Drugs & Medical Supplies Cairo, Egypt provided CMC, Na, and CCl₄. Dr olive Company in Cairo, Egypt, provided the olive oil.

Experimental Animals

Forty male albino rats aged 8 weeks (180-200 g) were purchased from the laboratory animal house of the National research center Cairo Egypt. They were kept individually in stainless steel wire bottomed cages at room temperature. Animal experimentation was approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of science, Zagazig University (approval No.ZU-1Acuc/1/F/113/2019).

Methods

Experimental design

The rats were divided into four groups. Each group contains 10 Rats. The negative control group (NCG) received intra peritoneal (i.p.) injection of olive oil at dose of (0.1 ml/100 g BW) twice weekly for 8 weeks. The other three groups after induction of liver fibrosis by i.p. injection of 100% CCl₄/ Olive Oil at dose of 0.1 ml/ 100g BW twice weekly for 8 weeks [7] were divided into positive fibrotic group (PFG), policosanol treated group (PTG) and silymarin treated group (PTG). The positive fibrotic group remains without any treatment. Additionally, the policosanol and silymarin treated groups were gavaged at dose of 100 mg/kg BW of either policosanol weekly twice dose for 8 weeks according to previous report of [8] Silymarin dissolved in 0.5 percent CMC-Na as a daily dose for 8 weeks, as directed by [9].

Sampling of blood

Blood was drawn from the retro-orbital plexus through retro-orbital sinus puncture with a heparinized micro-hematocrit capillary tube, then centrifuged at 3000 rpm for 15 minutes to separate serum. Until biochemical analysis, they were kept at -20°C.

Biochemical analysis:

Analysis of serum liver enzymes (ALT, AST) by (the Reitman-Frankel colorimetric procedure) [10], serum albumin level by [11], serum oxidative stress markers SOD, MDA by [11], NO by [12], TGF-beta level by [13].

Statistical analysis

For multiple comparisons between all analysed groups, a one-way ANOVA was used, followed by Bonferroni's test. The data were given as mean standard error (SE), with P values less than 0.05 considered significant

Results**Effect of policosanol and silymarin on ALT, AST and Alb levels:**

ALT, AST, and Albumin levels in the blood are commonly used as indications of hepatocellular injury. Due to fibrotic effect of CCL4 injection a significant increase in the levels of ALT and AST in positive fibrotic group was detected as compared by negative control group. On the other hand by treatment by policosanol or silymarin, we noticed significant decrease in these levels while level of albumin showed significant decrease in positive fibrotic group as compared by negative control group and by treatment with either policosanol or silymarin, we noticed a significant decrease was showed after treatment.

Effect of policosanol on Tumor growth factor –beta and SOD as an antioxidant enzymes, MDA and NO as oxidative stress markers

In the present study, we noticed that serum level of TGF-beta showed elevation in positive fibrotic group as compared by Negative control group, while after treatment by policosanol or silymarin showed significant decrease, while serum levels of oxidative stress marker (SOD) showed significant reduction in positive fibrotic group as compared by Negative control group but treatment with either policosanol or

silymarin had significantly increased those serum levels as compared by positive fibrotic group. In contrast (MDA, NO) levels showed marked increase in positive fibrotic group as compared by Negative control group and after treatment by policosanol or silymarin showed significant decrease in these levels.

Discussion

Liver fibrosis is one of the most common health disorders in the world, and it's linked to chronic liver diseases like cirrhosis, hepatitis, and hepatocellular carcinoma. It is considered as the main reason for death of ~1.4 million patients per year. Notably, Liver fibrosis is one of the most common health problems in impoverished nations, and it is linked to a variety of disorders such as parasite infection with *Schistosoma mansoni* and hepatitis B and C viruses.[14]. Interestingly, CCL4 has previously been shown to produce liver fibrosis in rats in an experimental setting. Up To Date, there are no synthetic drug could be used for curing liver fibrosis; but only tissue transplantation can be used [1]. The positive fibrotic group in the present investigation, showed significant elevation of serum ALT, AST, than that of the negative control group. While significant decrease in serum albumin had been associated in positive fibrotic group and the level of albumin shifted to be the normal level by treatment with policosanol. Similar effect for CCL4 was previously documented [15]. Interestingly, Previous studies have shown that CCL4 is metabolised by cytochrome p450 in hepatocytes producing trichloromethyl $CCl_3\cdot$ and $Cl_3COO\cdot$, which then bind covalently to cell components and cause lipid peroxidation as well as an increase in blood AST, ALT, and GGT. [16].

TGF- β has various profibrogenic and also anti-inflammatory, immunosuppressive effects. The constancy of these actions is necessary for keeping tissue homeostasis and an atypical expression of TGF-beta is participated in a series of disease processes in the liver, TGF- is also a potent inducer of apoptosis and a negative regulator of proliferation. [17]. The comprehensive pathophysiologic significance of TGF-beta suggests its measurement in blood as a diagnostic tool. Abnormal expression of TGF- β is accompanied by a number of diseases processes including fibrosis and cancer [18]. We noticed that liver fibrosis is associated by increase in the level of TGF- β as compared by Negative control group and by treatment by policosanol as well as silymarin showed reduction in its level and this is in agreement of [19].

MDA was revealed to be substantially greater in the current research. in positive fibrotic group where hepatic fibrosis was found to be associated with lipid peroxidation [20].

Our results showed that policosanolas well as silymarin decreases lipid peroxidation level due to its possible antioxidant effect. Our results are in agreement with [21] who reported that the increase in malondialdehyde (MDA) levels in liver fibrosis suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals in positive fibrotic group in rats. Treatment with policosanol significantly reversed these changes, and it may be due to its possible antioxidant effect.

Excessive nitric oxide (NO) synthesis in the cell may be caused by viral or bacterial infections, and it can aid pathogenesis by inducing oxidative stress, tissue injury, and even cancer.

[22]. In the current investigation, intake of CCl₄ resulted in a considerable rise in NO, implying that CCl₄ affects macrophage functions. Our findings revealed that policosanol inhibits NO production significantly, which could be owing to its anti-inflammatory properties. When nitric oxide combines with free radicals to form peroxynitrite, it can directly oxidise LDL, causing irreversible cell membrane damage. As an antioxidant, policosanol can scavenge free radicals, preventing them from reacting with nitric oxide, resulting in reduced damage.

Conclusion

Both silymarin and policosanol were found to have anti-fibrotic and antioxidant effects against CCL₄-induced hepatic fibrosis, with the latter having a stronger effect than the former. Furthermore, we propose that, in addition to these pathways, the reduction of TGF-beta and Albumin, as well as an increase in liver enzymes and SOD, particularly in the PTG, might be considered as another key mechanism for developing therapeutic options for liver fibrosis. Furthermore, policosanol therapy enhanced liver function serum ALT, AST, and TGF-beta.

Conflict of interest

The authors declare no conflict of interest.

References

- [1] Sharma, A., Nagalli, S(2021):Chronic Liver Disease, StatPearls, StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC., Treasure Island (FL).
- [2] Schaefer, T.J., John, S. (2021): Acute Hepatitis, StatPearls, StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC., Treasure Island (FL).
- [3] Askarpour, M., Ghaedi, E., Roshanravan, N., Hadi, A., Mohammadi, H., Symonds, M.E.,

- Miraghajani, M. (2019): Policosanol supplementation significantly improves blood pressure among adults: A systematic review and meta-analysis of randomized controlled trials. *Complement Ther Med.*, 45: 89-97.
- [4] Fayez, A.M., Zakaria, S., Moustafa, D. (2018): Alpha lipoic acid exerts antioxidant effect via Nrf2/HO-1 pathway activation and suppresses hepatic stellate cells activation induced by methotrexate in rats. *Biomedicine & Pharmacotherapy.*, 105: 428-433.
- [5] Bayram, H.M., Majoo, F.M., Ozturkcan, A. (2021): Polyphenols in the prevention and treatment of non-alcoholic fatty liver disease: An update of preclinical and clinical studies. *Clinical Nutrition ESPEN.*, 44: 1-14.
- [6] Gillessen, A., Schmidt, H.H.J. (2020): Silymarin as Supportive Treatment in Liver Diseases: A Narrative Review. *Advances in therapy.*, 37(4): 1279-1301.
- [7] Sayed, H.E.S.A.E., Morsy, L.E.S., Emara, T.M.A., Galhom, R.A. (2019): Effect of Carbon Tetrachloride (CCL₄) on Liver in Adult Albino Rats: Histological study. *The Egyptian Journal of Hospital Medicine.*, 76 (6): 4254-4261.
- [8] Cho, K.-H., Yadav, D., Kim, S.-J., Kim J.-R., (2019): Blood Pressure Lowering Effect of Cuban Policosanol Is Accompanied by Improvement of Hepatic Inflammation, Lipoprotein Profile, and HDL Quality in Spontaneously Hypertensive Rats. *Molecules.*(Cho, K.H., et al.2018, 23, 1080, Correction), 24(1) 194.
- [9] Clichici, S., Olteanu, D., Nagy, A.L., Oros, A., Filip, A., Mircea, P.A. (2015): Silymarin inhibits the progression of fibrosis in the early stages of liver injury in CCl₄-treated rats, *J Med Food.*, 18(3) 290-8.
- [10] Crowley L.V. (1967):The Reitman-Frankel colorimetric transaminase procedure in suspected myocardial infarction. *Clinical chemistry.*, 13(6): 482-7.
- [11] Satoh K. (1978): Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica chimica acta; international journal of clinical chemistry.*, 90(1): 37-43.
- [12] Montgomery, H., Dymock, J.F.J.A. (1961): Determination of nitrite in water, *Analyst.*, 86: 414-416.
- [13] Fabregat, I., Moreno-Càceres, J., Sánchez, A., Dooley, S., Dewidar, B., Giannelli, G., Ten Dijke, P., (2016): TGF- β signalling and liver disease. *The FEBS journal.*, 283(12): 2219-32.
- [14] Zhao, J., Qi, Y.-F., Yu, Y.-R. (2021):STAT3: A key regulator in liver fibrosis, *Annals of Hepatology.*, 21: 100224.
- [15] El-Toumy, S.A., Salib, J.Y., El-Kashak, W.A., Marty, C., Bedoux G., Bourgougnon, N. (2018):Antiviral effect of polyphenol rich plant extracts on herpes simplex virus type 1. *Food Science and Human Wellness.*, 7(1): 91-101.
- [16] Wang, M., Zhang, X.J., Feng, R., Jiang, Y., Zhang, D.Y., He, C., Li, P., Wan, J.B. (2017):Hepatoprotective properties of *Penthorum chinense* Pursh against carbon tetrachloride-induced acute liver injury in mice. *Chinese medicine.*, 12: 32.
- [17] Baba, A.B., Rah, B., Bhat, G.R., Mushtaq, I., Parveen, S., Hassan, R., Hameed Zargar M., Afroze, D.(2022):Transforming Growth Factor-Beta (TGF- β) Signaling in Cancer-A Betrayal Within. *Front Pharmacol.*, 13: 791272.
- [18] Fabregat I., Caballero-Díaz D. (2018): Transforming Growth Factor- β -Induced Cell Plasticity in Liver Fibrosis and Hepatocarcinogenesis. *Frontiers in oncology.*, 8:357.
- [19] Xu, X., Hong, P., Wang, Z., Tang, Z., Li, K. (2021):MicroRNAs in Transforming Growth Factor-Beta Signaling Pathway Associated With

Fibrosis Involving Different Systems of the Human Body. *Front. Mol. Biosci.*, 8: 707461.

[20] MacDonald, G.A., Bridle, K.R., Ward, P.J., Walker, N.I., Houghlum, K., George, D.K., Smith, J.L., Powell, L.W., Crawford, D.H., Ramm, G.A. (2001): Lipid peroxidation in hepatic steatosis in humans is associated with hepatic fibrosis and occurs predominately in acinar zone 3. *J Gastroenterol Hepatol.*, 16(6): 599-606.

[21] Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P.V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess Y., Beyrouthy, M.E., Polito L., Iriti M., Martins N., Martorell M., Docea A.O., Setzer W.N., Calina D., Cho W.C., Sharifi-Rad, J. (2020): Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front Physiol.*, 11: 694.

[22] Ramos-Tovar, E., Muriel, P. (2020): Molecular Mechanisms That Link Oxidative Stress, Inflammation, and Fibrosis in the Liver. *Antioxidants (Basel)*, 9(12): 1279.

Table (1): ALT, AST level and Albumin serum level in NCG,P FG, PTG and STG.

	NCG	PFG	PTG	STG	P-value
ALT(U/ml)	42.33±4.16 ^a	221.66±37.75	75.4±0.4 ^b	105±9.3 ^b	<0.001
AST(U/ml)	99.5±9.7 ^a	264.9±37	128.3±6.4 ^b	132.83±9.37 ^b	<0.001
Albumin(U/ml)	4.32±0.07 ^a	2.63±0.1	3.56±0.12 ^b	3.0±0.1 ^b	<0.001

One-way For multiple comparisons between groups, ANOVA followed by Bonferroni's test (P 0.001). Significant differences between NCG and P FG are indicated by different letter (a), while significant differences between PTG, STG, and P FG are indicated by different letter (b).

Table (2): TGF-beta and SOD levels in NC, FG, PTG and STG

	NCG	PFG	PTG	STG	P-value
SOD(U/gp)	6.5±0.64 ^a	4.5±0.18	5.8±0.19 ^b	5.4±0.14 ^b	<0.001
MDA (nmol/g)	31.4±14.5 ^a	126.8±13.5	49.4±4.8 ^b	61.9±4.9 ^b	<0.001
NO (umol/g)	41.9±9.4 ^a	79.2±4.5	49.8±4.2 ^b	60.9±1.8 ^b	<0.001
TGF-beta (ng/ml)	28.46±1.59 ^a	171.1±6.7	45.85±3.0 ^b	52± 4.2 ^b	<0.001

For comparisons between groups, one-way ANOVA followed by Bonferroni's test (P 0.001). Significant differences between NCG and P FG are indicated by letter (a), while significant differences between PTG, STG, and P FG are indicated by letter (b)