AUCTORES
Globalize your Research
International Journal of Clinical Nephrology
E. Ismail *
Open Access
Research Article

# Effect of Peritoneal Solution on Peritoneal MB and Therapeutic Issues: Experimental Study

**E. Ismail ***

Service de Néphrologie, CHU Sahloul, Sousse, Tunisie

***Corresponding Author:** E. Ismail, Service de néphrologie, CHU Sahloul, Sousse, Tunisie

**Received Date:** April 11, 2023; **Accepted Date:** April 21, 2023; **Published Date:** April 29, 2023

**Citation:** E. Ismail (2023), Effect of Peritoneal Solution on Peritoneal MB and Therapeutic Issues: Experimental Study, *International Journal of Clinical Nephrology*. 5(1); **DOI:10.37579/2834-5142/052**

**Copyright:** © 2023, E. Ismail. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Abstract

Nephrotic syndrome (NS) is a condition characterized by proteinuria, hypoalbuminemia, oedema, and hyperlipidaemia. It is caused by damage to the glomerular filtration barrier, resulting in the loss of protein in the urine. NS can be classified into primary and secondary forms, with the former being idiopathic and the latter resulting from an underlying disease or condition.

**Keywords:** nephrotic Syndrome; hypoalbuminemia; oedema; hyperlipidaemia

## Introduction

Peritoneal dialysis is increasingly used as a replacement therapy for chronic end-stage renal disease.
Conventional solutions used in peritoneal dialysis are usually not very biocompatible due to a high content of glucose and its degradation products as well as an acidic pH, factors of aggression of the peritoneal membrane.
Indeed, the progressive development of morphological and functional alterations of the peritoneum remains a formidable complication that reduces the survival of the technique.
The use of pharmacological agents would be an alternative for the prevention of the peritoneal membrane.
The objective of this work is to evaluate the oxidative stress status, genotoxicity and morphological alterations of the peritoneal membrane upon contact with PD solutions in the presence or absence of therapeutic intervention.

## Materials And Methods

### Animals

We established a rat model of peritoneal inflammation and fibrosis.
The study was performed on male Wistar rats (n=30) (Rattus norvegicus), 2 –3 months of age and weighed ranging 250 ±20 g that were bred and kept at the Biologically Compatible Substances Research Laboratory of the university of Monastir,Tunisia and the experiments were conducted in accordance with animal care guidelines.
They were housed in 12 h light/dark cycle and 60 ± 5% humidity; the temperature was at 25 to 30 °C. Food and water were given ad libitum throughout the experimental period.

### Peritoneal solutions

We used 2 types of peritoneal solution: dextrose 3.86 % and incodextrin.

***Chemicals***

**Silymarin :** the dominant flavonoid in *Silybum marianum* (milk thistle) seed extract

### Glycosaminoglycann: Sulodexide

***Experimental procedure***

*The rats were randomly assigned to 5 groups of 6 rats each:
group I : negative control (C)
group II: positive control: dextrose
group III: icodextrin,
group IV: dextrose-silymarin
group V: dextrose-sulodexide
Peritoneal inflammation was established in rats in the groups II–V by daily intraperitoneal injection of 10 ml of 3.85% glucose dialysate (Baxter Healthcare,).
Food and water were given ad libitum to all the groups.
Rats in groups III received 10 ml of icodextrin
Rats in group IV received 200 mg/kg/day of silymarin.
Rats in groupe V received 15 mg/Kg/day of sulodexide .

These medications were dissolved in drinking water immediately before administration by gastric gavage once daily in the morning.

**Sample collection and biochemical assays**

Rats from all the treatment groups were euthanized on day 30, and blood samples were obtained. Peritoneum, tissue samples were obtained from the abdominal wall away from the injection site. The tissue samples were stored at 70 °C. Liver and kidney were also analyzed to evaluate the effect of solutions and treatment in these organs

**Laboratory measurement:**

Rat blood samples were collected into biochemistry tubes centrifuged at 4000 rpm for 10 minutes at +4 °C

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate deshdrogenase (LDH), urea and creatinine levels were measured by spectrophotometric methods with an auto-analyzer.

***oxidative stress assessment***

**1.protein extraction**: After sacrificing the animals, the peritoneum is ground in 800 µl of cold Tris-Hcl . The determination of total protein was performed by the Bradford method (Bradford 1974).ref

**2. Measurement of carbonyl protein:**

The determination of carbonylated proteins was performed according to the method described by Mercier et al (2004). ref

**3 Measurement of MDA:**

peroxidation, was spectrophotometrically measured by using the thiobarbituric acid assay (Ohkawa et al. 1979). MDA formed a colored complex in the presence of thiobarbituric acid, which was detectable by measurement.

**3.3. Measurement of catalase activity** Catalases are enzymes that intervene in the defense of the cell against oxidative stress by eliminating oxygenated species ($H_2O_2$). their activity was measured according to the technique described by Clairborne in 1985

**3.4. Measurement of superoxyde dismutase activity (SOD)**

The activity of SOD is determined according to the method of MARKLUND (1985) whose principle is based on the capacity of the inhibition of the autoxidation of pyrogallol by SOD.

**Genotoxicity : Evaluation of DNA damage with Comet assay**

Cells are embedded on agarose –coated slide and lysed, after electrophoresis and fluorescent staining, the damaged DNA is separated from the intact DNA (the “head”) and generates a comet “ tail”. A visual count of the comets is performed per slide according to the intensity of the fluorescence of the tail obtained following the fragmentation of the DNA and then classified in 5 classes; from class 0 (intact nucleoli) to class 4 (totally damaged nucleoli) (Collins et al 1996).

**Histological Tissue Analysis**

Tissue samples were then immersed in 10% formalin for 24 h and embedded in paraffin. Sections (2 mm) were stained with hematoxylin and eosin (HE). The inflammation, neovascularisation and the thickness of the connective tissue between the mesothelium and the abdominal wall was evaluated by the same anathomopathologist with a blind evaluation.

## Statistical analysis

All obtained numerical data are expressed as the mean One-way analysis of variance (ANOVA).

All statistical calculations were processed using the SPSS 11.0. values of less than 0.05 were considered statically significant.

## Results:

**Oxidative stress**

In our study, we started by demonstrating the effect of peritoneal solution on peritoneal membranes. we treated a batch with dextrose and we compared results of the measurement of stress oxidative markers with the control group.

The treatment with dextrose for 4 weeks generates oxidative stress compared with control group.The Carbonylated protein, a marker of oxidative stress, was higher in the dextrose group (1.62 mmol/mg protein) compared to the control group (1.12) with a statistically

significant difference. The results of this study showed significantly higher MDA levels ($p<0.05$) in the dextrose-treated batch compared to the control group (17.79 mmol/mg vs. 8.54 mmol /mg). The catalase and SOD activity were also higher after treatment with dextrose as showen in the Table 1. Thus, dextrose significantly increased all stress oxidative markers.

In order to compare the effect of peritoneal dialysis solutions on the peritoneum, rats of batch 3 received icodextrin on a daily basis and were then sacrificed. The results obtained were compared to those of the control groups: negative control (batch1) and positive control (batch2) Icodextrine induced an increase in the level of catalase activity from 114.75 to 252,26 compared to the control group, but this increase was significantly less important than that induced by dextrose (341.6)

This glucose polymer induced a higher superoxide dismutase activity compared to the control group but the enzymatic activity was lower than that induced by dextrose with

$p= 0.011$. The **Table 1** shows that the increase of these oxidative stress markers by icodextrin is significantly less important than dextrose.

| | Control | Dextrose | icodextrin |
|---|---|---|---|
| PC (mmol/mg de protéines) | 1,12 | 1,62 | 1,42* |
| MDA (mmol/mg de protéines) | 8,54 | 17,79 | 13,62* |
| catalase (µmol/min/mg de protéines) | 114,75 | 341,6 | 252,26* |
| SOD (UI/mg) | 49,71 | 85,34 | 69,36* |

PC : carbonyl protein , MDA : lipoperoxydation
SOD : superoxyde dismutase, * P<0.05

**Table 1:** Effect of dextrose and icodextrin on oxydative stress.

In our study we tested two therapeutics to decrease this effect of peritoneal solution on peritoneum. Results showed that silymarin had a significant effect on decreasing oxidative stress induced by dextrose. In fact, silymarin reduced carbonyl protein from 1.62 to 1.26 compared to the group treated by dextrose only (p=0,002). The superoxide dismutase activity (SOD) and the rate of malondialdehyde (MDA) decreased significantly with silymarin. the catalase had a higher activity in the batch treated with dextrose only 341.6 vs 150.89.**(table 2)**

| | Dextrose | Silymarine + Dextrose |
|---|---|---|
| PC (mmol/mg of protein) | 1,62 | 1,26* |
| MDA (mmol/mg) | 17,79 | 11,58* |
| Catalase activity (µmol/min/mg) | 341,6 | 150,89* |
| SOD activity (UI/mg) | 85,34 | 59,34* |

**Table 2: Protective effect of silymarin**

The sulodexide proved also its efficiency on reducing oxidative stress markers .The MDA test performed for the batches treated with sulodexide, showed that the treatment with these glycosaminoglycans significantly decreases the lipoperoxidation induced by dextrose (p=0.006).the SOD and catalase activity were also significantly reduced by sulodexide.

The administration of sulodexide with dextrose decreased carbonyl protein but this
reduction was not significant. **(Table 3)**

| | dextrose | sulodexide | sylimarine |
|---|---|---|---|
| **PC** | **1.62** | **1.42** | **1.26*** |
| **Catalase activity** | **341.6** | **189.8*** | **150.89*** |
| **MDA** | **17.79** | **14.1*** | **11.58*** |
| **SOD activity** | **85.34** | **67.33*** | **59.94*** |

**Table 3:** Comparison of the effect of sulodexide and sylimarine

The comparison of the two treatment tested in our study, showed that Silymarin was significantly superior to sulodexide in lowering the level of carbonylated proteins, a marker of oxidative stress with p<0.05.

Comparison of the effects of silymarin and sulodexide on the rate of lipoperoxidation in the presence of dextrose showed that the difference

between the two molecules was not significant (p= 0.13), thus not showing a superiority of one treatment over the other.

There was no superiority of one molecule over the other in reducing catalase or SOD activity.

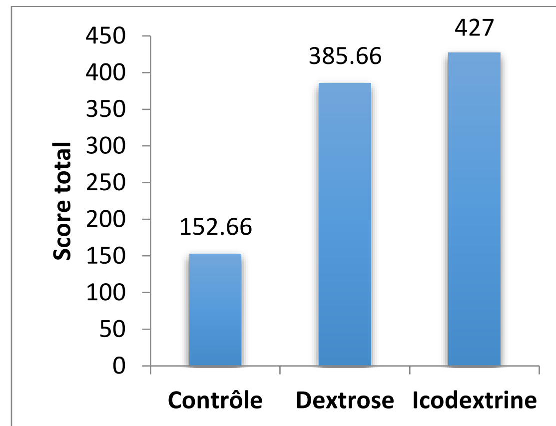
Therapeutic intervention permits then to decrease the oxidative stress induced by dextrose with a superiority of sylimarine.

### Genotoxicity

In order to evaluate the cellular damage induced by dextrose and the capacity of sylimarine and sulodexide in preventing the DNA damage we used the comet assay.

For the batches that received the dextrose and icodextrin peritoneal dialysis solutions, the results represented in graphic 1, show that compared to the control group, these rats had a higher comet score (respective total score of 385, 427 and 152 respectively), and thus a higher DNA fragmentation with a statistically significant difference (p<0.05) for both batches.

The comet score for icodextrin was higher compared to dextrose (427 vs 385) but this difference was not statistically significant (p=0.28).




**Graphic 1: Effect of dextrose and icodextrine on DNA fragmentation**

The results of this study showed that silymarin has a protective effect on DNA fragmentation of mesothelial cells with a comet score at 249.33 after administration of this treatment and 385.66 with dextrose alone with a statistically significant difference (p<0.05). (Table 3)

| | Dextrose | Sylimarine+dextrose |
|---|---|---|
| **Comete score** | 385.66* | 249.33* |

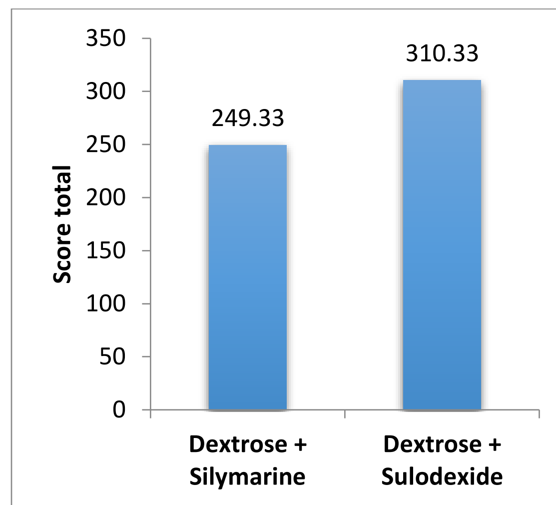
**Table 4: Protective effect of silymarin on DNA damage**

Sulodexide decreased dextrose-induced cellular DNA fragmentation with a significantly lower comet score (p=0.004). **(Table 4).**

| | Dextrose | sulodexide+dextrose |
|---|---|---|
| **Comete score** | 385.66* | 310.33* |

**Table 5: Protective effect of sulodexide on DNA damage**

Comparison of the effect of silymarin and sulodexide on DNA fragmentation of peritoneal cells by calculating the comet score showed superiority of silymarin with p at 0.002




**Graphic 2:** Comparison of the protective effect of silymarin and sulodexide on DNA damage

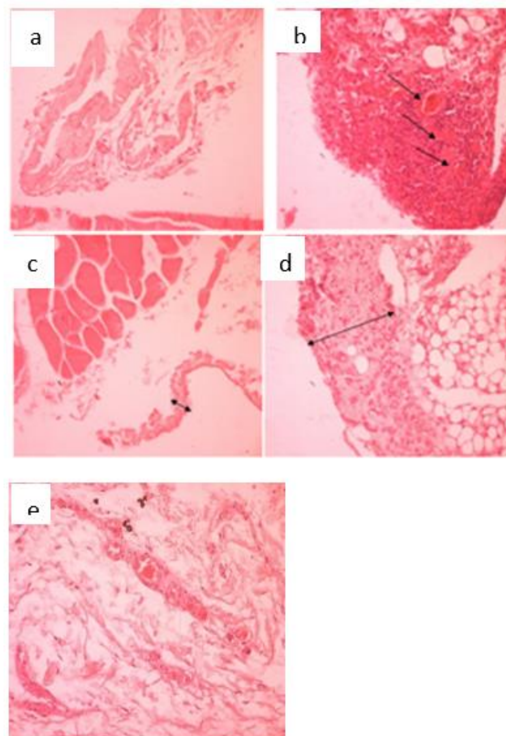
## Histological analysis

After the rats were sacrificed, the parietal peritoneum was removed for each of the 5 batches and a histological study was performed to evaluate the changes in the peritoneal membrane induced by the different therapies tested.

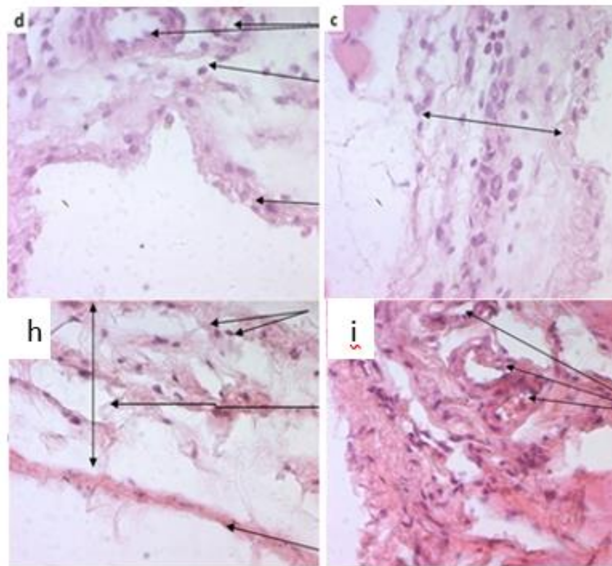
Inflammation, fibrosis and neovascularization of the peritoneal membrane were evaluated for each batch.

The histological examination showed Morphological changes of the peritoneum were evaluated by HE and Masson's trichrome. As shown in picture a, normal rat peritoneum was thin, consisting of a linear layer of mesothelial cells, and there were few inflammatory cells.

Histological study of the peritoneum of PD group showed an increase in the number of vessels with vascular congestion, (indicated by the arrow) with the presence of some inflammatory cells and a thickening of the submesothelial zone in comparison with the control lot. No signs of fibrosis were observed.




Light microscopy of the peritoneum of the icodextrin-treated rats showed an increase in the number of vessels but this was less pronounced than in the dextrose group and no inflammatory infiltrate or fibrosis was observed (picture e).




Treatment with silymarin reduced the histological damage to the peritoneum induced by dextrose, with fewer inflammatory cells, a slight submesothelial thickening and some vessels without fibrosis as showen in the picture f and g.

Histological study of the peritoneum of sulodexide-treated rats showed rare inflammatory cells with slight submesothelial thickening. These peritoneal changes were less pronounced compared to the dextrose group (picture h,i)

Sylimarine and sulodexide permits then to prevent the changes in the peritoneal membrane induced by the exposure to dextrose.

## Discussion

Long-term contact between the peritoneal membrane and glucose-based PD solutions profoundly alters the mesothelial cells, which form the majority of the exchange surface [1,2]

Indeed, it is currently well established that exposure of the peritoneal membrane to PD solutions leads to functional and structural alterations that cause failure of the technique with denudation of the mesothelial cells, neovascularization, vasculopathy and peritoneal fibrosis [3-5].

The causal link between alteration of the peritoneal membrane and the role of hypertonic solutions rich in glucose has been proved in several studies [6-9]

The glucose contained in these solutions contributes to these peritoneal alterations and this by a direct effect but also indirectly by increasing the level of PDGs released during the sterilization processes [10,11].

In our study, the PD solution with a high concentration of glucose led to an increase in the various markers of oxidative stress. Indeed, dextrose induced an increase in the levels of carbonylated proteins, lipoperoxidation as well as catalase and superoxide dismutase enzymatic activities.

This high glucose content also induced a marked genotoxicity with elevation of the comet score reflecting DNA fragmentation of mesothelial cells.

Our results also showed that dextrose caused marked histological lesions in the peritoneal membrane, with an increase in the number of vessels, submesothelial thickening and even signs of local inflammation.

Similar results with thickening of the submesothelial zone and increased cellularity were observed in a study of rats treated with glucose-rich PD solutions [12].

Whether in in vitro, ex vivo or in vivo studies, icodextrin has been shown to be potentially less toxic, leading to fewer membrane alterations than glucose-rich solutions [13-12].

Unlike glucose, icodextrin does not diffuse through the peritoneal membrane, but is absorbed by lymphatic convection [15]

This molecule is considered biocompatible for the peritoneal membrane due to its iso-osmotic character.

In our study, the results were consistent with those of the literature, in fact, we found with icodextrin the same findings observed with dextrose but to a lesser degree. Indeed, the results obtained showed that this glucose polymer causes oxidative stress with an increase in the levels of carbonylated proteins and MDA. icodextrin also induced an increase in catalase and superoxide dismutase enzymatic activities. In terms of genotoxicity, DNA fragmentation of mesothelial cells was observed with a high comet score.

In our experimental study, histological sections of the peritoneal membrane showed that after exposure to icodextrin, the vascularization becomes more pronounced with neoangiogenesis and this in comparison with the control group. However, the thickening of the submesothelial zone observed with dextrose was not found.

Recent human studies have examined the role and effectiveness of antioxidants in the prevention and treatment of certain diseases [16].

Indeed, antioxidants prevent oxidative stress by counterbalancing the effect of free radicals and therefore prevent their deleterious effects and thus prevent and treat pathologies related to this stress [16].

Among these treatments we find milk thistle or Silymarin which has proven its effectiveness as a cytoprotector thanks to its antioxidant property. Silymarin (silybum marianum) also has an antioxidant, membrane stabilizing effect [17]. The mechanism of action is blocking and adjusting cellular transporters, estrogen, glycoproteins and nuclear receptors. Silymarin is mainly used as a hepatoprotector, it has shown anticancer effects and has also proven its effectiveness in some renal pathologies (diabetic nephropathy).

By analogy to its effects on liver and kidney, and thanks to its antioxidant and anti-inflammatory effect, this study tried to evaluate the effect of silymarin in the prevention of peritoneal membrane alterations in rats exposed to PD solutions.

In our study, rats were given silymarin by gavage for 4 weeks at a dose of 200mg/kg/d. This dose was chosen on the basis of previous clinical trials [18,19] and animal studies [20,21].

Silymarin has been shown to protect the peritoneal membrane by decreasing oxidative stress at the peritoneal level and decreasing morphological alterations; indeed, the assay of oxidative stress markers, such as carbonylated proteins, catalase and superoxide dismutase activity and the lipoperoxidation assay were found to be significantly lower with silymarin

In the literature review, no studies were found evaluating both the parameters of oxidative stress and genotoxicity of mesothelial cells, hence the interest of our work.

The results of our experiment were confirmed by the histological study of the parietal peritoneum, which showed less inflammation compared to the group receiving dextrose alone, as well as less pronounced fibrosis. The number of vessels was significantly higher in the absence of silymarin.

Glycosaminoglycans (GAGs) are the most abundant group of heteroploysaccharides in the body and, thanks to their negatively charged properties; they play a key role in various biological processes

The use of GAGs in the clinic is very frequent, mainly in the treatment of thrombotic pathologies.Sulodexid is a GAG that has been approved for use in humans and its use was mainly in cases of proteinuria or cardiovascular disease in diabetics [22,23]. Recently anti-inflammatory effects have been described as well as endothelial protective effects(24).

Indeed, sulodexide has been shown to be beneficial to the peritoneal membrane, improving its function by increasing the D/P urea ratio and decreasing peritoneal protein loss [25]. This molecule has also been shown to improve histological changes in the peritoneal membrane in rats with sclerosing peritonitis (26).

In our study, sulodexide was administered orally to rats for 4 weeks at a dose of 15mg/kg/d. The choice of this dose was based on previous clinical trials (27), (28) and animal studies (29), (26).

Sulodexide has been shown to protect the peritoneal membrane by decreasing oxidative stress at the peritoneal membrane level and decreasing morphological alterations.Indeed, the level of oxidative stress markers, such as catalase activity, superoxide dismutase and lipoperoxidation assay were found to be significantly lower with sulodexide.The level of carbonylated proteins decreased in the presence of sulodexide but this decrease was not significant.

The DNA of peritoneal cells, studied by the comet assay, was significantly less fragmented and altered in the presence of sulodexide.

Our results were supported by the histological study of the parietal peritoneum, which showed less inflammation compared to the group receiving dextrose alone. As well as a less pronounced fibrosis. The number of vessels was significantly higher in the absence of sulodexide.

The comparison of the 2 molecules tested in our study showed a superiority of the silymarine in decreasing oxidative stress markers and preventing DNA damage.

## Conclusion

It was demonstrated through this study that dextrose leads to an alteration of the peritoneal membrane with induction of oxidative stress, genotoxicity and histological abnormalities.Icodextrin was less deleterious.

The treatments tested showed a protective effect on these morphological alterations with a superiority of silymarine to sulodexide.

These treatments can be applied in our peritoneal dialysis patients, given their beneficial effects and their innocuousness, in order to avoid functional and morphological alterations and thus increase the survival of the technique.

## References


1. Krediet RT, Zweers MM, van der Wal AC, (2000).Struijk DG.Neoangiogenesis in the peritoneal membrane. PeritDial Int; 20(Suppl 2):S19–25.
2. De Vriese AS, Mortier S, Lameire NH. (2001).What happens to the peritoneal membrane in long-term peritoneal dialysis? Perit Dial Int.;21 Suppl 3:S9-18.
3. Mortier S, Faict D, Schalkwijk CG, Lameire NH, De Vriese AS. Long-term exposure to new peritoneal dialysis solutions: Effects on the peritoneal membrane. Long-term exposure to new peritoneal dialysis solutions: Effects on the peritoneal membrane.
4. Nilsson-Thorell CB, Muscalu N, Andrén AH, Kjellstrand PT, Wieslander AP. (1993) Heat sterilization of fluids for peritoneal dialysis gives rise to aldehydes. Perit Dial Int.;13(3):208-213.
5. Inagi R, Miyata T, Yamamoto T, et al: (1999). Glucose degradation product methylglyoxal enhances the production of vascular endothelial growth factor in peritoneal cells: Role in the functional and morphological alterations of peritoneal membranes in peritoneal dialysis. FEBS Lett 463:260–264,
6. De Vriese AS, Tilton RG, Stephan CC et al. (2001). Vascular endothelial growth factor is essential for hyperglycemia-induced structural andfunctional alterations of the peritoneal membrane. J Am Soc Nephrol; 12: 1734–1741.
7. Higuchi C, Tanihata Y, Nishimura H, Naito T, (2005) Sanaka T.Effects of glucose and plasminogen activator inhibitor-1 oncollagen metabolism in the peritoneum. TherApher Dial;9:173–181.
8. Nakao A. Nakao K. Takatori Y. Kojo S. Inoue J. Akagi S. Makino H. (2009). Effects of icodextrin peritoneal dialysis solution on the peritoneal membrane in the STZ-induced diabetic rat model with partial nephrectomy. Nephrology Dialysis Transplantation 25(5) 1479–1488.
9. Hirahara I, Kusano E, Yanagiba S, Miyata Y, Ando Y, Muto S, (2006 ) Asano Y. Peritoneal injury by methylglyoxal in peritoneal dialysis. Perit Dial Int. May-Jun;26(3):380-392.
10. Plum J, Hermann S, Fusshöller A, Schoenicke G, Donner A, Röhrborn A, (2001)et al. Peritoneal sclerosis in peritoneal dialysis patients related to dialysis settings and peritoneal transport properties. Kidney Int Suppl.;78:S42-47.
11. Di Paolo N, Sacchi G. (2000).Atlas of peritoneal histology. Perit Dial Int.;20 (Suppl 3):S5-96.
12. Davies SJ. (2006).Exploring new evidence of the clinical benefits of icodextrin solutions. Nephrol Dial Transplant. Jul;21 Suppl 2:ii47-50.
13. Liberek T, Topley N, Mistry CD et al. (1993). Cell function and viability inglucose polymer peritoneal dialysis fluids. Perit Dial Int; 13:104–111.
14. Parikova A, Zweers MM, Struijk DG et al. (2003) .Peritoneal effluent markersof inflammation in patients treated with

icodextrin-based and glucosebaseddialysis solutions. AdvPerit Dial; 19: 186–190.

15. Lin A. Qian J. Li X. Yu X. Liu W. Sun Y. (2009). Randomized Controlled Trial of Icodextrin versus Glucose Containing Peritoneal Dialysis Fluid. Clinical Journal of the American Society of Nephrology 4(11) 1799–1804.
16. Kaur G. Athar M. &Alam M. S. (2009). Dietary supplementation of silymarin protects against chemically induced nephrotoxicity inflammation and renal tumor promotion response. Investigational New Drugs 28(5) 703–713.
17. Soto C, Recoba R, Barrón H, Alvarez C, Favari L. (2003) Silymarin increases antioxidant enzymes in alloxan-induced diabetes in rat pancreas. Comp Biochem Physiol C Toxicol Pharmacol. Nov;136(3):205-12.
18. Ehrlich J. & Stivala S. S. (1973). Chemistry and Pharmacology of Heparin. Journal of pharmaceutical Sciences 62(4) 517–544.
19. Jorpes J. E. (1962). Heparin its chemistry pharmacology and clinical use. The American Journal of Medicine 33(5) 692–702
20. He Q. (2004). Silymarin Protects Against Liver Damage in BALB/c Mice Exposed to Fumonisin B1 Despite Increasing Accumulation of Free Sphingoid Bases. Toxicological Sciences 80(2) 335–342.
21. Noti C. & Seeberger P. H. (2005). Chemical Approaches to Define the Structure-Activity Relationship of Heparin-like Glycosaminoglycans. Chemistry & Biology 12(7) 731–756.
22. Pletinck A. Van Landschoot M. Steppan S. Laukens D. Passlick-Deetjen J. Vanholder R. & Van Biesen W. (2011). Oral supplementation with sulodexide inhibits neo-angiogenesis in a rat model of peritoneal perfusion. Nephrology Dialysis Transplantation 27(2) 548–556.
23. Suminska-Jasinska K, Polubinska A, Ciszewicz M, Mikstacki A, Antoniewicz A, Breborowicz A. (2011) Sulodexide reduces senescence-related changes in human endothelial cells. Med Sci Monit. Apr;17(4):CR222-226.
24. Vrtovsnik F. Coester A. M. Lopes-Barreto D. de Waart D. R. Van der Wal A. C. Struijk D. G. Zweers M. M. (2010). Induction of chronic kidney failure in a long-term peritoneal exposure model in the rat: effects on functional and structural peritoneal alterations. Peritoneal Dialysis International 30(5) 558–569.
25. Pawlaczyk K, Kuzlan-Pawlaczyk M, Anderstam B, Heimbürger O, Bergström J, Waniewski J, Breborowicz A, Lindholm B. (2001) .Effects of intraperitoneal heparin on peritoneal transport in a chronic animal model of peritoneal dialysis. Nephrology Dialysis Transplantation 16(3) 669–671.
26. Weigert C. Brodbeck K. Häring H. U. Gambaro G. & Schleicher E. D. (2001). Low-molecular-weight heparin prevents high glucose- and phorbol ester-induced TGF-β;1 gene activation. Kidney International 60(3) 935–943.
27. Brenner B. M. Cooper M. E. de Zeeuw D. Keane W. F. Mitch W. E. Parving H.-H. … Shahinfar S. (2001). Effects of Losartan on Renal and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Nephropathy. New England Journal of Medicine 345(12) 861–869.
28. Fern RJ, Yesko CM, Thornhill BA, Kim HS, Smithies O, Chevalier RL. (1999 ).Reduced angiotensinogen expression attenuates renal interstitial fibrosis in obstructive nephropathy in mice. J Clin Invest. Jan;103(1):39-46.
29. Kagami S. Border W. A. Miller D. E. & Noble N. A. (1994). Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. Journal of Clinical Investigation 93(6) 2431–2437.

This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here: **Submit Manuscript**

**DOI:**10.37579/2834-5142/052

**Ready to submit your research? Choose Auctores and benefit from:**

- fast, convenient online submission
- rigorous peer review by experienced research in your field
- rapid publication on acceptance
- authors retain copyrights
- unique DOI for all articles
- immediate, unrestricted online access

At Auctores, research is always in progress.

Learn more https://auctoresonline.org/journals/international-journal-of-clinical-nephrology