

The Protective Effect of Annona Extracts on Renal Histopathology Induced by Ethylene Glycol in Male Rabbits

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Abstract

Annona is one of the many plant extracts that have been explored owing to their anti-inflammatory and anticancer effects. Male locale rabbit were separated into five groups, Group I consider as control, The 4th other groups were treated orally with 0.75% ethylene glycol (EG) to induce renal damage this treatment done till 30th days. Group III to V animals were served as curative regimen and received flavonoids, glycosides and alkaloids extract of the plant of Annona at a dose of 100 mg/kg body weight from 15th day to 30th day. The extracts was administered twice daily by oral route. significant kidney injury in EG animals as demonstrated by a significant elevation (P= 0.05) in serum activities urea, creatinine, total protein and calculated globulin levels. Meanwhile there was significant drop (P=0.05) in albumin, and A/G ratio compared to the control and treated group , The levels of lipid peroxidation were significantly higher in the Serum of the groups of animals treated with EG , while the levels of SOD, CAT and GPx were significantly lower than animals were treatment of EG group with Annona significantly elevated the antioxidant mechanisms differ with that before treatment. Renal Histopathological changes includes deposition of oxalates in the renal tubules lumen, degeneration and necrosis of epithelium of renal tubules , congestion of blood cells , infiltration of inflammatory cells and atrophy in glomerular tuft leading to expansion of bowmans spaces. While Histopathological changes in animals group treated with Annona extract and ethylene glycol were limited dilatation of renal tubules with vascular changes with blood vessels congestion and interstitial hemorrhage , reduced renal damage revealed by less degenerative and necrotic lesion in tubular epithelium and no inflammatory reaction and the glomeruli appeared normal compared with ethylene glycol sections.

Keyword: Anonna extract , Ethylene glycol, Antioxidant , Kidney, Histopathology

Introduction

Ethylene glycol is a colorless , odorless and sweet testing synthetic organic chemical substance , which is used in different consumer and industrial application (Staples et al., 2001) , further more ethylene glycol used anti freezer , poly ester fiber and poly ester resins , heat transfer agent and as solvent, manufactured of asphalt emulsion paints (song et al., 2017). As time progresses, we become more aware of what nature contains as beneficial to our health, and with it our desire to learn more about these elements, One of these ancient elements in its existence, which has been talked about in recent research is the fruit of the cream (Annona), where research on the components of this fruit and on its health

benefits (Asma et al., 2020 (. Annona is one of the many fruit extracts that have been explored due to their anti-inflammatory and anticancer properties. Extensive phytochemical evaluations on different parts of the Annona plant have shown that the presence of different compounds and phytoconstituents which include alkaloids, megastigmanes , flavonol, triglycosides, phenolics , cyclopeptides and essential oils (Coria-Téllez et al., 2018). The barks, seeds , leaves and fruit which taken from Annona have been used for various ethnomedicinal purposes by traditional healers to treat several diseases including cancer, inflammation, diabetes, liver diseases, and abscesses , Also It is well known that fruits are rich in various antioxidants, including ascorbic

acid, carotenoids, and phenolics(Yajid et al., 2018). Antioxidants are substances present at low concentration that protect cells components from damage caused by unstable molecules known reactive oxygen species. (Gyesi eal.,2019 ; Manochai et al., 2018).

Material and Methods

Annona was purchased from the local market. It was classified according to plant classification references related to medicinal plants (10). Also a vouchers specimens of the plant was identified and authenticated at the herbariums of the College of Education, University of Mosul.

- Preparation of extracts

Preparation of flavonoids, glycosides and alkaloids extracts of Annona were done according to the method described by(11).

- Animal grouping

Male locale rabbit's weightings between 700-800 gm were used , rabbits were separated into 5 groups, each containing 3 animals. The first group consider as control and treated on normal laboratory diet and water ad labium. The second group till to 5th animals were treated with 0.75% ethylene glycol (EG) in water to induce renal damage for 30th day (12). Group III to V group were served as curative regimen and received flavonoids, glycosides and alkaloids extract of the Annona at a dose of 100 mg/kg B.W. from 15th day to 30th day. The extracts was administered twice daily by oral ingestion.

- Kidney function tests

The serum was collected from the blood sample for the determination levels of total protein & albumin by using (17). The estimation of globulin levels and Albumin/globulin ratios, urea(18) and creatinine (19).

- Estimation Lipid peroxidation and antioxidant enzyme in serum.

Estimation of malondialdehyde (MDA) levels via Thiobarbituric acid reactions technique. Thiobarbituric acid (TBARS) in the serum was measured by the technique of(20)& estimating the GSH by the method of (21).Antioxidant enzyme activities (CAT, SOD and GPx), the activities were estimated by the methods of (22,23) .

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- Histopathology

Tissue samples , were collected from the kidney and fixed in Neutral Buffered formalin 10 % solution for 3-5 days then trimmed to suitable sizes , washed with tap water, dehydrated in ascending concentration of ethyl alcohol (70 ,90, 100%) , cleared with xylene , Embedded by paraffine wax, Sectioned by microtome at 5-6 μ M , stained with routine hematoxylin & Eosin and finally examined under a light microscope (24).

Finding

Table 1 showed that rabbits exposed to the EG alone developing significant kidney injury as demonstrated by a significant elevation (P= 0.05) in serum activities urea, creatinine, total protein and calculated globulin levels. Meanwhile there was significant drop (P=0.05) in albumin, and A/G ratio compared to the control and treated group. Table 2 revealed levels of lipid peroxidation were significantly elevated in Serum of EG group comparing with control animals . Also the result showed a significantly elevation in GSH level in the serum of rabbits. While the level of the SOD, CAT and GPx were significantly lower than the normal animlas , were EG animals which treated with Annona significantly elevated the antioxidant protection mechanisms compared with animals treated with EG.

Table1: Changes in kidney function tests ethylene glycol group and treated ethylene glycol group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
urea(mg/dl)	18.95±0.805	38.17±1.02*	21.61±0.19	31.55±2.325 *	30.01±1.14*
creatinine(mg/dl)	2.283±0.08	4.133±0.16*	2.13±0.13	4.33±0.20 *	2.113±0.29
Total Protein (g/dL)	10.72±0.03	12.46±0.11*	11.89±0.11	12.11±0.10	10.79±0.11
Albumin (g/dL)	6.35±0.03	5.79±0.55*	7.17±0.61	7.77±0.42	6.47±0.42
Globulin (g/dL)	3.57±0.05	4.44±0.23*	4.54±0.12*	4.44±0.12*	4.21±0.11*
A/G Ratio	1.78±0.04	1.31 ±0.23*	1.57±0.20	1.75±0.12	1.53±0.12*

Blood samples were taken after 30 days of oral administration, number of rabbit each group = 5 Values are given as mean± SD

. * means P value <0.05 = significant level

Table 2: Changes in kidney lipid peroxidation and enzymatic antioxidants of ethylene glycol group and treated ethylene glycol group as compared to control.

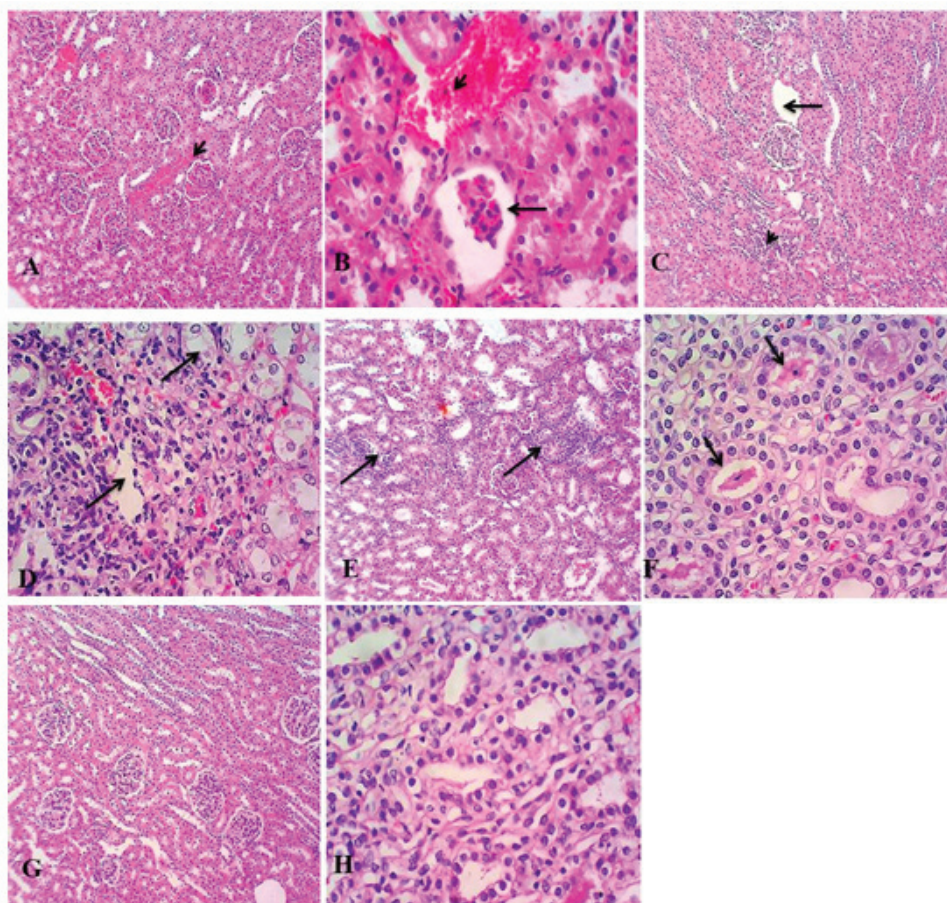
Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
MDA (mM/100 g tissue)	0.52±0.02	0.92±0.02	0.95±0.03	0.87±0.03	0.58±0.02
GSH (mg/100mg tissue)	85±3.21	47±2.8	78±3.3	79±3.3	81±2.9
CAT (mmol/l)	0.170±0.01	0.09±0.01	0.132±0.02	0.169±0.02	0.136±0.02
GPx (U/mg protein)	6.80±0.51	3.43±0.32	6.55±0.42	5.43±0.49	6.98±0.49
SOD	2.30±0.2	3.23±0.2	2.07±0.12	2.01±0.13	2.06±0.12

MDA = Malondialdehyde; GSH = glutathione; CAT= Catalase; GPx = glutathione peroxidase; SOD = superoxide dismutase, tissue samples were taken after 30 days of oral administration, number of rabbit each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level.

Histopathological finding:

The histopathological examination of renal sections taken from kidney specimens of animals treated with ethylene glycol indicated that the administration of ethylene glycol for thirty days lesions involved the cortical renal tubules which characterized by deposition of oxalate crystals in the lumen of renal tubules , degeneration and necrosis of the epithelial cells lining the renal tubules as well as tubular dilatation with cystic formation in renal paranchyma , Other sections revealed infiltration of inflammatory cells in the interstitial parenchyma , vascular changes also observed which characterized by generalized blood vessels congestion

with vasculitis. The histopathological lesions in renal glomeruli characterized by expansion of the bowman’s space which appear as a result of atrophy and shrinkage of the glomerular tuft. While the group of animals which treated with ethylene glycol and flavonoid showed improvement in the histopathology of the kidney , these histopathological changes were limited dilatation of renal tubules with vascular changes which were blood vessels congestion and interstitial hemorrhage , with reduced renal damage revealed by less degenerative and necrotic lesion in tubular epithelium and no inflammatory reaction and the glomeruli appeared normal compared with ethylene glycol sections (Figure 1).



Discussion

This study, showed that Annona extract have an ameliorative effect and reduction on kidney damage caused by ethylene glycol. Ethylene glycol cause damage in the renal functional capacity , kidney fail to carry out their normal metabolic and endocrine functions

, this will cause alteration in tubular function or structure affect glomerular function and vice versa (Tsujihata, 2008). Also this occur due to deposition of oxalates in the lumen of the renal tubules which induced inflammatory reaction then leads to increase in the concentration of proteins (globulins and albumins) in the blood

(Tugcu et al., 2008). Annona is a species of the Annonaceae family that has been commonly studied in the last periods due to its therapeutic actions. Table No. (1) illustrates kidney function in rabbit treated with ethylene glycol and Annona extract as demonstrated that decreasing in serum levels, urea, creatinine, total protein and calculated globulin levels, compare with ethylene glycol and control rabbits. While table No. (2). Natural antioxidants from plant species have gained interest due to their protective effect against oxygen-derived from free radicals involved in the development of many diseases. This result agreement with that of Abdul Wahab (2018) and Correa-Gordillo et al. (2012) were demonstrated that the Annona extract have an antioxidant protective and improve organs properties. A histopathological study of kidney showed severe damage and crystal formation occur by ethylene glycol treated animals as compare with animals treated with flavonoid of Annona extract that reverted and ameliorative the renal damage, and these results were agreement with (Abdul Wahab, 2018) That demonstrated the organ protection, antioxidant and anti-inflammatory properties of Annona extract. Histological alteration of renal tissue reported in this study occur because the kidney is a critical organ for the toxicity of ethylene glycol in both human and experimental animals. Consistently cause metabolic acidosis, degeneration and necrosis and deposition of calcium oxalates in the lumen of the renal tubules, these changes have been observed in our study. (Song et al., 2017; Khatib et al., 2010; Thapa and Walia, 2007). The treatment with ethylene glycol cause deposition Ca⁺ oxalates because the end product of ethylene glycol metabolism is oxalates which cause histological changes in the renal tubules and glomeruli causing degeneration and necrosis (Thamilselvan and Khan, 1998; Hadjzadhe et al., 2007). The protection against free radicals are done by preventing their formation, by blocking the chain reaction or by repairing the oxidative damage of these biomolecules (Gyesi et al., 2018).

Conclusion

The conclusion of this study showed that Annona extracts have ameliorative effect of renal histopathological changes and biochemical changes induced by ethylene glycol.

Conflict of Interest: non

Source of Findings: was done by the researcher himself and without any government funding

Ethical Clearance: non

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