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Short communication

Antioxidant effect of plant extracts on phospholipids levels in oxidatively stressed male reproductive organs in mice

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Abstract

Background: High content of phospholipids in testes and epididymis is involved in regulation of spermatogenesis. On the other side, it makes testes susceptible to oxidative stress due to lipid peroxidation, which alters the normal mechanism of spermatogenesis.
Objective: In the present investigation, antioxidant effects of ethanolic extracts of parsley, lettuce and brahmi were tested against D-galactose induced oxidative stress in mouse testes and epididymis.
Materials and Methods: Oxidative stress was induced in six months old mice by injecting a low dose of D-galactose. Antioxidant effect of plant extracts was studied in testes and epididymis of oxidatively stressed mice in conjunction with thin layer chromatographic separation of phospholipids and quantitative estimation of phospholipid phosphorus.
Results: The results showed decrease in total phospholipids content and level of phospholipid phosphorus in the testes and epididymis of D-galactose stressed mice. The administration of plant extracts along with D-galactose showed no significant alterations in the phospholipids content in testes and epididymis.
Conclusion: Decreased phospholipids and phospholipids phosphorus in testes and epididymis of D-galactose stressed mice indicates peroxidation of lipids due to injection of D-galactose. The plant extracts helped to maintain the level of peroxidation in these organs even under stressed condition. It is postulated that ethanolic extracts of parsley, brahmi and lettuce are protective against D-galactose induced oxidative stress in testes and epididymis.

Key words: *D-galactose, Phospholipids, Oxidative stress, Male reproductive organs, Antioxidant.*

Introduction

Defective sperm function is a major cause of male infertility. It has been shown that oxidative stress is associated with this sperm dysfunction. The composition of mammalian spermatozoa is markedly different from that of mammalian somatic cells.

They are rich in phospholipids, sterols, saturated and polyunsaturated fatty acids that make them susceptible to the damage by reactive oxygen species (ROS) (1). Most common ROS that have potential implications in reproductive biology are superoxide anions (O_2-), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO-) and peroxynitrite anions (ONOO).

In normal circumstances, there is equilibrium between the generation of reactive oxygen species and natural antioxidant defense system of male reproductive tract, leaving only a critical amount of ROS required for normal sperm functions as capacitation, acrosome reaction and fusion with the

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oocytes membrane (2). But excessive production of ROS results in destruction of natural antioxidant capacity of reproductive tract (3) and peroxidation of lipids, proteins, carbohydrates and nucleic acids. These changes leads to alterations in normal spermatogenesis (4), loss of sperm motility (5), decreased sperm –oocyte fusion capacity (6) and increased chromatin damage (7). To overcome the consequences of oxidative stress, use of antioxidant therapy seems to offer benefits. In this context, the aim of present work was to study protective effects of antioxidant rich plants, parsley, lettuce and brahmi on oxidatively stressed male reproductive organs. Oxidative stress was induced in adult male mice by injecting a low dose of D-Galactose (8), a reducing sugar. Antioxidant effects of the plants were studied in the testes and epididymis of the oxidatively stressed mice in conjunction with the quantitative analysis of phospholipids.

Materials and methods

Preparation of plant extracts

Lactuca sativa (lettuce) and *Petroselinum crispum* (parsley) were obtained from the market. *Bacopa monniera* (brahmi) was obtained from the local botanical garden raised by Municipal Corporation. Fresh green leaves were separated, washed with distilled water, air-dried and crushed to make powder. The powders obtained were soaked in ethanol separately for 72 hours, filtered and evaporated in the vacuum evaporator (Buchi type). The extracts obtained in the form of thick pastes were collected in glass bottles and stored at 4°C.

Animals

Male albino mice, *Mus musculus* (Linn.) of age six months and weighing 45±2 gm were used for the present investigation. They were reared in the departmental animal house (approved by CPCSEA) in aluminum cages at 29-30°C with a light-dark cycle of 12/12 hours. They were supplied with water and food (Pranav Agro Industries, Sangli) *ad libitum*. They were divided into five groups, each consisting of six animals

Group I: Control group- Mice received subcutaneous injections of 0.5 ml sterile water for 20 days.

Group II: Oxidatively stressed group- The animal received subcutaneous injection of 5% D-Galactose 0.5 ml/day for 20 days.

Group III: Parsley extract administered group- The mice received subcutaneous injection of ethanolic extract of parsley 40mg/kg BW along with 5% D-Galactose 0.5 ml/day for 20 days.

Group IV: Lactuca treated group- The animals received subcutaneous injection of ethanolic extract of lactuca 40mg/kg BW along with 5% D-Galactose 0.5 ml/day for 20 days.

Group V: Brahmi administered group- The mice received subcutaneous injection of ethanolic extract of brahmi 40mg/kg BW along with 5% D-Galactose 0.5 ml/day for 20 days.

The animals were sacrificed by cervical dislocation. Testes and epididymis were excised out for the study of phospholipids.

Extraction of lipids (9)

The tissues were homogenized in chloroform: methanol (2:1) at room temperature. For extraction of lipids, the homogenates were kept at 4°C for 4 hrs, filtered and the filtrates were evaporated in vacuum at 40°C.

Thin layer chromatography of phospholipids

Preparation of thin layer chromatographic plates (10). The glass plates were impregnated with slurry of silica gel H without binder by using Acme applicator. The thickness of the layer was kept 0.25 mm. The plates were air dried and activated at 100°C for 1 hr prior to use. Application of samples- The sample was applied to the origin of the plates with Hamilton microsyringe (No. 8203-B). Development of chromatograms and detection of phospholipids components (11) - The solvent system used for the development of chromatogram was chloroform: methanol: acetic acid: water (75: 45: 3: 1). The dried plates were exposed to iodine vapors for detection of the components.

Determination of phospholipids phosphorus (12)

Phospholipids in the chromatographic fractions were estimated by phosphorus determination through acid digestion. Each phospholipids component separated on the silica gel plate was identified, scrapped and eluted in the solvent system used for development of chromatogram. Such elutions of all the components of phospholipids were used as samples for the determination of phosphorus. The samples were digested in 70% perchloric acid. 2.5% ammonium molybdate and amino naphthol reagent were added and the absorbance was read at 830 nm.

Statistical analysis

The results were analyzed by Student's t-test, with $p< 0.001$ considered statistically significant.

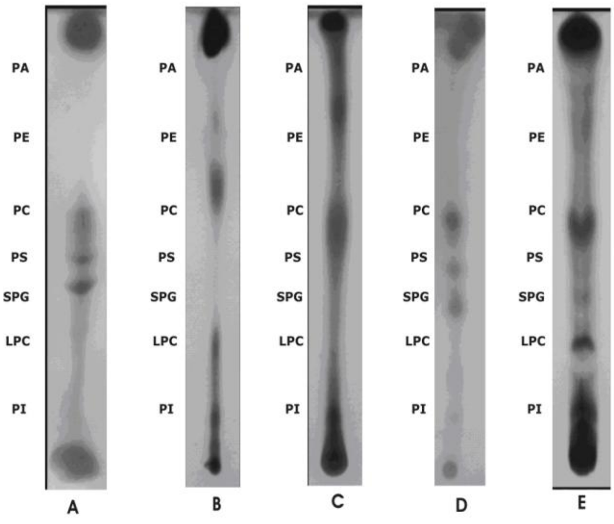
Results

Thin layer chromatographic separation of phospholipids

Plate No. I and II describe the separation of phospholipids from testis and epididymis of mice of all study groups. In control group, the phospholipids were separated into seven components, phosphatidyl inositol (PI), lysophosphatidyl choline (LPC), sphingomyeline (SG), phosphatidyl serine (PS), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidic acid (PA) from the origin to the top of the chromatographic plates in testis and epididymis. In the testis of D-galactose stressed mice only three components PI, LPC and PC were observed separated. Rest of the components was not resolved properly.

In epididymis of D-galactose stressed mice also no clear separation of the components was observed. In the testis of ethanolic extract of parsley treated mice all the seven phospholipids components were prominently separated. Same was the case of epididymis. In ethanolic extract of lactuca-administered group of mice, all components of phospholipids were resolved as compared to the D-galactose injected mice. In ethanolic extract of brahmi treated group also testis and epididymis showed clear separation of all the constituents of phospholipids.

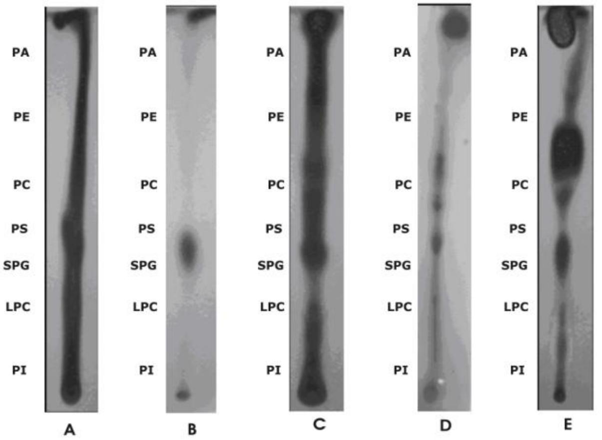
PLATE I. Effect of various plant extracts on phospholipids of testis of mice.



A: Control Testis.
B: D- galactose induced Testis.
C: Petroselinum crispum extract + D- galactose received testis.
D: Lactuca sativa extract +D- galactose received testis.
E: Bacopa monniera extract + D- galactose received testis.

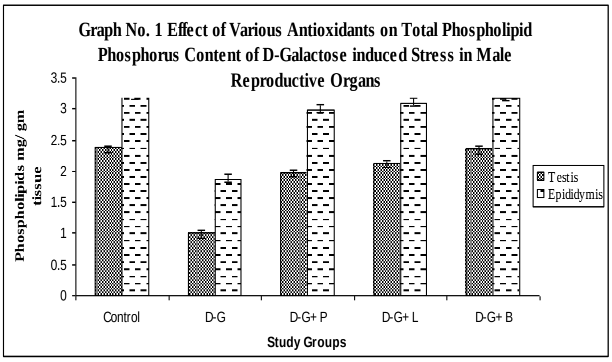
Abbreviations: Phosphatidyl Inositol (PI), Lysophosphatidyl choline (LPC), Sphingomyline (SPG), Phosphatidyl serine (PS), Phosphatidyl choline (PC), Phosphatidyl ethanolamine (PE), Phosphatidic acid (PA)

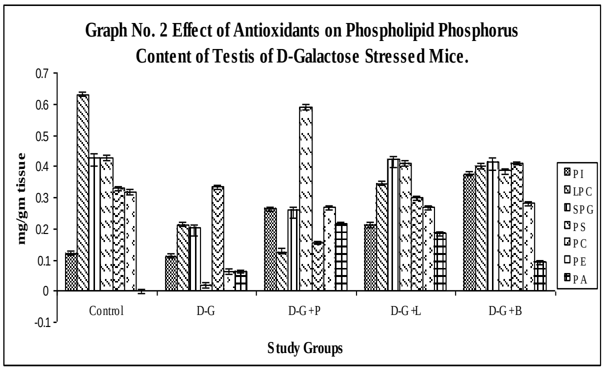
PLATE II. Effect of various plant extracts on phospholipids of Epididymis of mice.

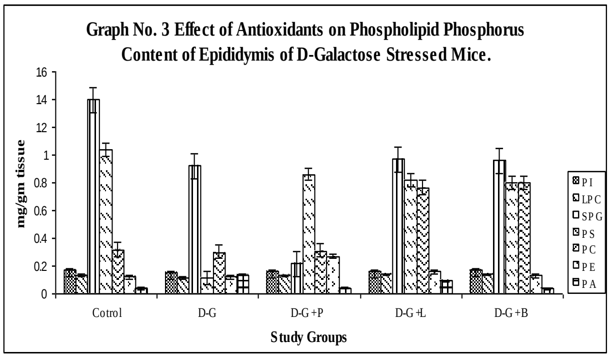


A: Control Epididymis.
B: D- galactose induced Epididymis.
C: Petroselinum crispum extract + D- galactose received Epididymis.
D: Lactuca sativa extract + D- galactose received Epididymis.
E: Bacopa monniera extract + D- galactose received Epididymis.

Abbreviations: Phosphatidyl Inositol (PI), Lysophosphatidyl choline (LPC), Sphingomyline (SPG), Phosphatidyl serine (PS), Phosphatidyl choline (PC), Phosphatidyl ethanolamine (PE), Phosphatidic acid (PA)







Quantitative determination of phospholipids phosphorus

Graph no. I shows total phosphorus content (mg/gm tissue) in both testis and epididymis. Total phosphorus content was decreased in the testis and epididymis of D-galactose injected group of mice as compared to the control group. The plant extracts treated mice showed increase in the level of total phosphorus in testis and epididymis as compared to the oxidatively stressed group.

Phosphorus content in the individual components of the testis and epididymis is shown in graph no. 2 and 3. From the graphs it was clear that phosphorus content from LPC was highest in testis while SG and PS were present in moderate amount. In epididymis phosphorus content was more from SG and PS. PA was observed in lowest concentration in both testis and epididymis. After injection of D-galactose all the components showed reduced concentration in both the organs. The extracts administered groups showed increase in the amounts of phosphorus contents of all the components from testis and epididymis as compared to the stressed group of mice.

Discussion

In the present study, it was observed that D-galactose induced alterations in the phospholipids components of testis and epididymis in mice. It has been reported that lipid peroxidation caused by increased oxidative stress induces failure in the expression of phospholipids in the gonads and spermatozoa with defective sperm count (13). It is important to note that each phospholipids component play specific role in the spermatogenesis, maturation of spermatozoa and fertilization. Peroxidation of phosphatidyl serine seems to be one of the major detrimental effects of the oxidative stress in the male reproductive tract. Phosphatidyl serine is found in the middle piece and acrosome of sperms and influences rate of capacitation (14). Lysophosphatidyl choline stimulates the fertilizing ability of spermatozoa (15). Thus it is evident that oxidative damage to the phospholipids obviously causes impairment in the spermatogenesis, morphology of spermatozoa and its functions.

The injection of D-galactose reduced the amount of phospholipids in testis and epididymis. D-galactose is a reducing sugar and is capable of reacting with macromolecules like lipids, proteins, and DNA without enzymatic intervention. The reactions are called as glycation and the end products advanced glycation end products, AGEs (8). These AGEs accumulate in the cells and provoke increase in the generation of ROS in the cells, which ultimately leads to the peroxidation of lipids.

Decrease in the phospholipids in the testis and epididymis of the D-galactose injected mice indicate peroxidation of lipids due to AGEs. When there raises a problem of oxidative stress, use of antioxidants also becomes important. The supplements of phytochemicals have been suggested to prevent the disorders caused by free radicals.

The phytochemicals like quercetin, rutin, apigenin, luteolin found in parsley, lactuca, fresh fruits and vegetables break the chain reactions of free radicals responcible for peroxidation of lipids (16). These antioxidants have the ability to maintain membrane fluidity and integrity by protecting the lipids (17, 18). Many Indian medicinal plants like *Withania somnifera* (ashwagandha), *Asparagus rosemosus* (shatavari), *Emblica officinalis* (amla), *Oscimum sanctum* (tulsi), *Bacopa monniera* (brahmi) and their formulations also possess strong antioxidant properties (19). In the present investigation, the ethanolic extracts of parsley, lactuca and brahmi were used against the oxidative stress induced by D-galactose.

It was observed that the extracts administered mice showed fairly equal amounts of phospholipids as compared to the control group. It indicates that these extracts helped to keep the balance between ROS generation and antioxidants in the testis and epididymis. Thus, it can be said that ethanolic extracts of parsley, lactuca and brahmi offer protection against D-galactose induced oxidative stress in male reproductive tract.

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