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# Hypolipidemic effect of atorvastatin and phytotherapy on rat prostate - A histological study
# Efectul hipopolipidemic al atorvastatinei și fitoterapiei asupra prostatei de șobolan - Un studiu histologic

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**Cuvinte cheie:** prostata, descriere, sobolan, atorvastatina, catina, strugurii, HFD, citoarhitectura

**Abstract**

**The prostate is an accessory gland and a component of the male reproductive system. This is found only in some species of mammals and differs morphologically, chemically, and physiologically between species. This paper describes in chapter 1, the basic elements of the prostate (structure, functions, macroscopic and histological characteristics). In chapter 2, the description and activity of atorvastatin are presented, and in chapter 3, two recognized phytotherapeutic remedies are presented: sea buckthorn and grapes (in the form of the compound). In the second part, a study is proposed that investigates the effect of fat consumption on the histological structure of the prostate in a murine model. Through histological investigation of the prostate, this study aimed to comparatively evaluate whether atorvastatin has any healing role on the prostate mirrored in cytoarchitecture and also what is the role of phytotherapy on prostate cytoarchitecture.**

**Rezumat**

**Prostata este o glandă accesorie și o componentă a sistemului reproducător masculin. Aceasta se găsește doar la unele specii de mamifere si diferă morfologic, chimic și fiziologic între specii. Prezenta lucrare descrie in capitolul 1, elementele de baza despre prostata (structura, functii, caracteristicile macroscopice și histologice). In capitolul 2 este redata descrierea si activitatea atorvastatinei, iar in capitolul 3 sunt prezentate doua remedii fitoterapeutice recunoscute: catina si strugurele (sub forma compusului). In partea a doua, se propune un studio care investigheaza efectul consumului de grăsimi asupra structurii histologice a prostatei într-un model murin. Prin investigarea histologică a prostatei, acest studiu a urmărit să evalueze comparativ dacă atorvastatina are vreun rol de vindecare asupra prostatei oglindită în citoarhitectura și, de asemenea, care este rolul fitoterapiei asupra citoarhitecturii prostatei.**

## 1. Basic elements about prostate

### 1.1. Definition, structure, and function of the prostate

The prostate gland is an accessory gland and a component of the male reproductive system. It is only found in some mammals. It differs morphologically, chemically, and physiologically between species. It is located in the pelvic behind the urinary bladder and encircles the urethra, with the urethra crossing through it. It is covered with a surface called the prostatic capsule [Standring 2016].

It is divided into lobes in gross anatomy and zones in microanatomy. It is encased in an elastic, fibromuscular capsule and contains glandular and connective tissue.

The prostate is made up of both glandular and connective tissue. The glands' lining is made up of tall, columnar cells. These may comprise a single layer or be pseudostratified.

The epithelium is highly varied, with transitional epithelium in the outer parts of the

longer ducts and patches of low cuboidal or flat cells. The glands are made up of many follicles that drain into canals and ducts that drain into the urethra as it passes through the prostate. There are also a few flat cells that reside near gland basement membranes and act as stem cells [Standring 2016, Young 2013].

The prostate's connective tissue is consisting of fibrous tissue and smooth muscle. The gland is divided into lobules by fibrous tissue (figure 1) [Young 2013].

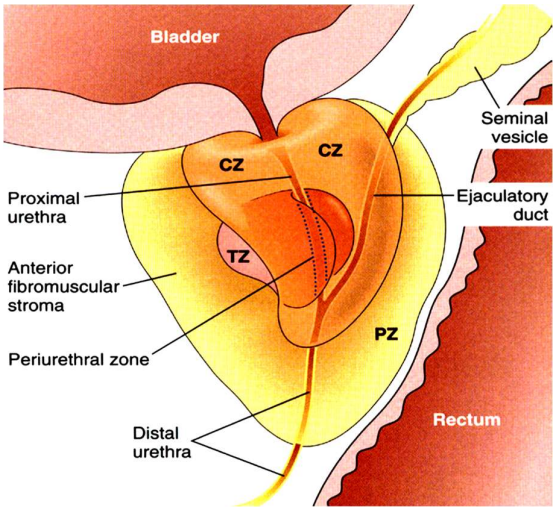


**Figure 1.** Normal prostate gland, adult [Kumar 2005]

The prostate is a vital gland that secretes a variety of substances. It is in charge of creating seminal fluid, which is essential for sperm nutrition and motility.

Although the association between obesity and an increased risk of cardiac diseases, type 2 diabetes, and various malignancies is widely documented, the link between obesity and prostatic illnesses has been overlooked. HFD-induced metabolic problems may cause prostate damage due to the formation of ROS via NADPH oxidase activation, resulting in oxidative damage that disturbs cellular homeostasis and leads to worsened metabolic syndrome characteristics. Dietary choices have an impact on prostate health, according to experimental and clinical investigations [Wu 2015].

Individuals are at risk of prostate inflammation as a result of metabolic factors such as obesity as supported by clinical and experimental evidence [Gacci 2013, Condorelli 2022].

The metabolic syndrome caused by HFD, according to Vignozzi, increases inflammation and prostate fibrosis, which is similar to benign prostatic hyperplasia. However, data on the molecular mechanisms underlying the relationship between obesity and prostate proliferative disorders is limited [Vignozzi 2012].

The very-low-calories ketogenic diet (VLCKD) simulates fasting by significantly reducing carbohydrate intake (30 g/day) while concurrently increasing fat intake increasing fat and protein consumption [Merra 2016].

This causes the synthesis of ketone molecules such as:

- *D-3-hydroxybutyrate,*
- *acetoacetate, and*
- *acetone.*

These are anorexigenic substances produced in hepatocyte mitochondria that lower cerebral neuropeptide Y and ghrelin while maintaining the cholecystokinin response to a meal. These biochemical pathways cause a decrease in both perceived hunger and food intake, which explains VLCKD's efficacy and tolerability [Paoli 2015].

### 1.1.1. Structure and function of the prostate

The prostate is the most important male reproductive gland in male fertility.

Indeed, the content of prostatic fluid generated by the prostate epithelium is inherently linked to male fertility. The prostatic fluid's important contribution to male fertility is linked to its role as a trigger for each of the molecular processes involved in ejaculation and, consequently, sperm activation and capacitation [Gilany 2015, Tena-Sempere 2013].

The prostate gland is the most important accessory gland in the male reproductive system. It is made up of two major compartments, the:

- *stroma*, and
- *epithelium*,

which impact each other through distinct signaling pathways to promote optimal prostatic development and homeostasis [Nieto 2014].

The fully developed prostate gland is made up of ducts with an inner layer of epithelium surrounded by stroma.

The prostate gland is anatomically divided into three zones as follows:

- *central zone*,
- *transition zone,* and
- *outer zone.*

The transition zone is located proximal to the ejaculatory ducts and surrounds the urethra.

The central zone encompasses the ejaculatory channels and extends beneath the bladder base [Nieto 2014].

The peripheral zone encompasses the majority of the prostate's apical, posterior, and lateral features.

The prostate is typically described clinically as having two lateral lobes divided by a central sulcus palpable on rectal examination, and a middle lobe that may protrude into the bladder in older males.

These lobes do not correlate to histologically defined structures in the normal prostate but are frequently associated with pathological processes that increase the transition zone laterally and the periurethral glands centrally [Nieto 2014].

The primary role of the prostate gland's stromal compartment is to provide an adequate milieu for the epithelial compartment. In healthy conditions or during regeneration processes, the stromal compartment delivers several supportive signals to maintain or restore gland homeostasis. Several lines of evidence suggest that activated stroma may play an important role in prostate inflammatory processes [Nieto 2014].

The main glandular function of the prostate epithelial compartment is to secrete prostatic fluid, which accounts for approximately one-fifth to one-third of the total volume of the ejaculate [Gilany 2015, Tena-Sempere 2013, Nieto 2014].

The prostatic fluid, like the other secretions of the male accessory glands, plays an important role in male fertility.

The prostatic fluid contains a variety of factors that govern proteins (from other accessory gland secretions) that trigger sperm maturation and control the ejaculation process; these factors are required for sperm liquefaction, clotting cycle, and sperm motility [Gilany 2015].

### 1.1.2. Prostate gross and histological features

The prostate is located subperitoneal, beneath the diaphragm of the pelvis.

It is located posterior to the pubic symphysis, prior to the rectum, and inferior to the urine bladder, which surrounds its neck [Lee et al. 2011].

It is pyramidal in shape, with a superior base, a neck inferiorly, and anterior, posterior, and two inferolateral surfaces. Behind the pubic arch is the anterior surface.

The urethra enters the prostate towards the base and exits on the anterior surface above and in front of the apical section. The lateral pelvic wall is related to the inferolateral surfaces. A thin layer of connective tissue known as the 'Denonvilliers fascia' separates the posterior aspect of the prostate and seminal vesicles from the rectum [Hammerich et al. 2009].

This fascia serves as a surgical excision plane for rectal malignancies. The rectum and fascia are separated anteriorly from the seminal vesicles in males and the vagina in females [Decker and du Plessis 1986].

Structure. It is surrounded by a thin layer of fibroblastic tissue, giving it an undock. The fibroblastic capsule, on the other hand, gives birth to septa that expand inward and split the prostate into five lobes: anterior, posterior, medial, and two laterals [Walsh 1992].

This lobe contains:

- *30-50 branched tubuloalveolar or secular glands,*
- *16-32 excretory ducts,*
- *thick stroma,*
- *blood arteries,*
- *lymphatics, and*
- *nerves* [Hafez 1980].

Although the normal prostate cannot be separated into lobes, when benign prostatic hyperplasia advances, there is a trend toward lobulation [Walsh 1992].

The prostate is histologically divided into two primary zones:

- *central* and
- *peripheral.*

There is also a transitional zone, an anterior section, and a periprostatic sphincteric zone [Mc Neal 1981]. Acinar cells are seen in five different types in the typical prostate gland [Sanefugi1981].

The apical surface of microvillar cells is covered with many microvilli. Secretory cells exhibit active secretion and apical cell bulging membrane. On the apical cell surface, cells have one to several tiny holes.

*Crater cells* have ruptured the apical cell membrane. Bare cells have a reasonably smooth apical surface with few microvilli at the periphery.

### 1.1.3. Main prostatic functions

The prostate gland serves several purposes:

1. Physically, it controls urine flow from the bladder and the transport of seminal fluid during ejaculation via its bulk and musculature [Williams 1976].
2. As an exocrine gland, it adds to the seminal plasma a spectrum of tiny molecules and enzymes such as fibrinolysin, coagulase, and other coagulum lysing enzymes that aid in fertility and coagulation [Walsh 1992].
3. Prostatic fluid protects sperm viability by lowering urethral acidity. It promotes and increases sperm motility by adding a factor (albumin) to seminal plasma, which stimulates the motility of epididymal and cleaned ejaculated spermatozoa [Walsh 1992].
4. Prostatic acid phosphatase is directly involved in nutrition by hydrolyzing phosphorylcholine to choline.

## 2. Atorvastatin and its activity

Atorvastatin, with the chemical formula $C_{33}H_{35}FN_2O_5$ and a molecular weight of 558.65 g/mol, is one of the most often prescribed statin medications in the world.

It is an HMG-CoA reductase inhibitor that is used to prevent cardiovascular events and lower blood total cholesterol and LDL-cholesterol in individuals with hypercholesterolemia and hypertriglyceridemia. It is a diphenyl-pyrrole, a heterocyclic aromatic molecule with a pyrrole ring connected to two phenyl groups.

In figure 2 the schematic representation of the mechanism of action of statins is presented.

Atorvastatin is a synthetic second-generation HMG-CoA reductase inhibitor that lowers plasma cholesterol levels by inhibiting the HMG-CoA reductase that catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis in the liver. It is metabolized in the body by the cytochrome P450 3A4 enzyme system. In large trials involving patients with hypercholesterolemia, atorvastatin produced greater reductions in total cholesterol, LDL-cholesterol, apolipoprotein B, and triglyceride levels than lovastatin, pravastatin, and simvastatin.

In patients with primary hypercholesterolemia, the combination of atorvastatin and colestipol tended to produce larger reductions in LDL-cholesterol levels and smaller reductions in triglyceride levels than atorvastatin monotherapy. Although atorvastatin induced smaller reductions in triglyceride levels and more modest increases in high-density lipoprotein (HDL)-cholesterol levels than either fenofibrate or nicotinic acid in patients with combined hyperlipidemia, it produced larger reductions in total cholesterol and LDL-cholesterol [Kappelle 2010].

Atorvastatin is prescribed to prevent adverse cardiovascular events and to lower blood total cholesterol and LDL cholesterol. It may be particularly suitable for patients with heterozygous or homozygous familial hypercholesterolemia because of the marked reductions in LDL cholesterol experienced with the drug. Additionally, because of its triglyceride-lowering properties, atorvastatin appears to have the potential to become an appropriate treatment for patients with combined hyperlipidemia or hypertriglyceridemia.

It is rapidly absorbed, reaching peak plasma concentration within 2.3 hours. The lipid-lowering effect of atorvastatin is not

influenced by the time of day the drug is administered, probably because of its relatively long half-life of 20 hours.

It is metabolized by cytochromes P-450 3A4 and P-450 3A5 to ortho-hydroxy atorvastatin and para-hydroxy atorvastatin.

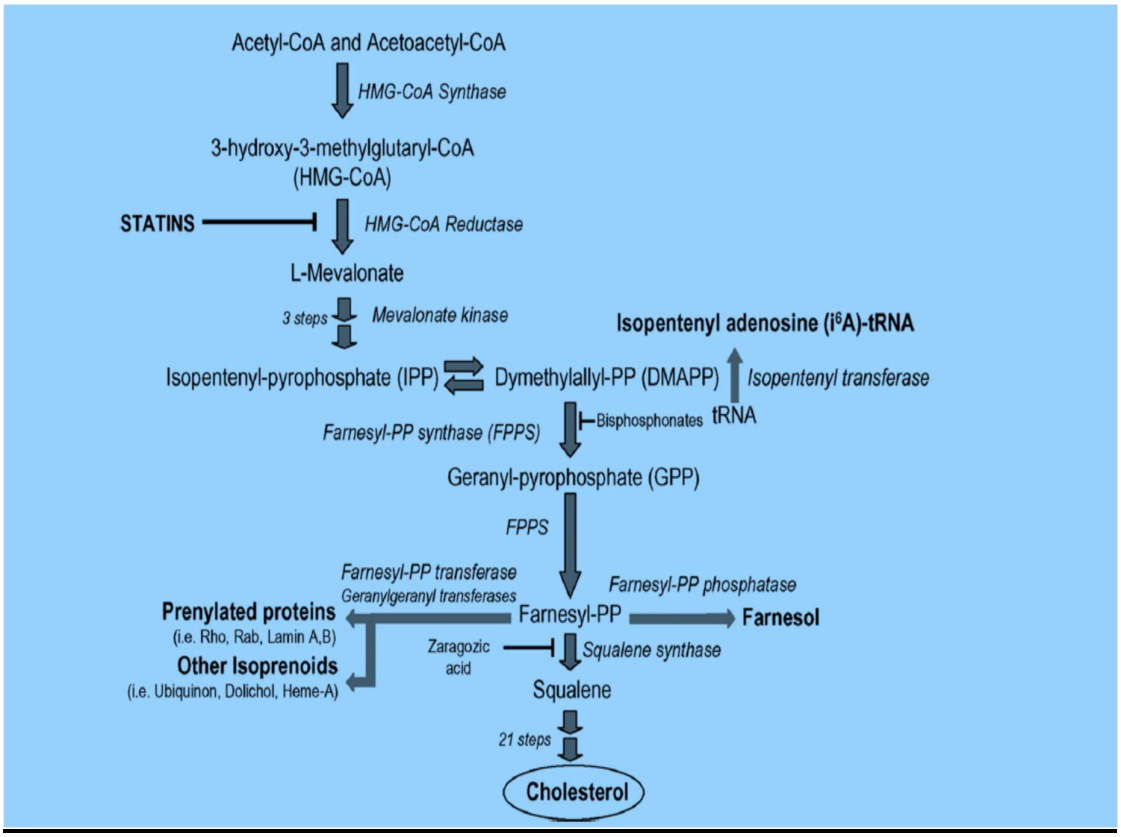


**Figure 2.** The mevalonate pathway. Statins act by inhibiting HMG-CoA reductase, the key enzyme of the mevalonate pathway. Statins could have pleiotropic effects possibly through other products of the mevalonate pathway (e.g., i6A tRNA, prenylated proteins, and other isoprenoids) that play central roles in cell signaling, protein synthesis, and cytoskeletal organization [Siobhra 2007].

These two active metabolites extend the effect of atorvastatin on HMG-CoA reductase resulting in a half-life of enzyme inhibition of 20 to 30 hours [Schachter 2005, Lins 2003].

It has been reported that atorvastatin reduces major vascular events in primary prevention populations, however, whether they reduce mortality remains controversial.

Atorvastatin is one of the most potent and extensively investigated 3-hydroxyl-3- methyl-glutaryl coenzyme A reductase inhibitors.

This is:

- lowers plasma total and LDL-cholesterol levels
- by inhibiting endogenous cholesterol synthesis and
- increasing the number of available LDL receptors in hepatocyte membranes,
- it also reduces triglyceride TG levels, through an as-yet unproven mechanism, which may offer an add-on value to treat diabetic dyslipidemia [Malhotra 2001].

Atorvastatin acts in the liver by inhibiting the rate-limiting enzyme for cholesterol synthesis, HMG-CoA reductase.

This enzyme irreversibly converts HMG-CoA to mevalonate.

This reaction is considered the third step in a sequence of reactions resulting in the production of many compounds including cholesterol and its circulating blood derivatives, LDL cholesterol, and very low-density lipoprotein VLDL - cholesterol [Gaw 2000].

Figure 3 is presented the mode of action of atorvastatin.

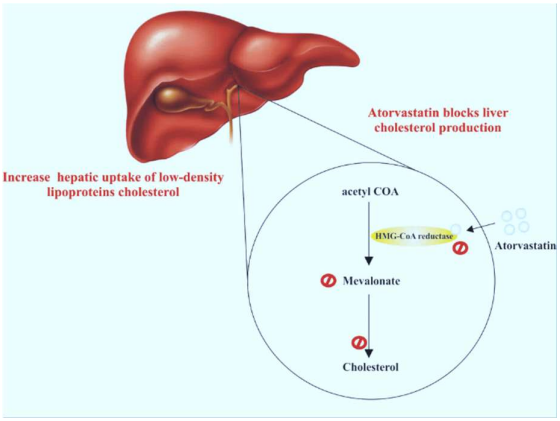


**Figure 3.** Mode of action of atorvastatin [Gaw 2000]

The prevailing hypothesis is that statins reduce mortality and morbidity in patients with occlusive vascular disease by reducing liver production of cholesterol and thus causing a reduction in blood LDL-cholesterol and a resulting decrease in atherogenesis.

However, the HMG Co-A reductase enzyme is also responsible for the production of

- *ubiquinone coenzyme Q10,*
- *heme,*
- *vitamin D,*
- *steroid hormones, and many other compounds.*

It remains possible that the beneficial effects of statins are due to actions other than the reduction of cholesterol. These other actions have been referred to as the pleiotropic effects of statins [Liao 2005].

The advantage of expressing the effect as a percent reduction as compared with an absolute reduction from baseline is that the percent reduction is a pure number, is independent of the unit of measurement, and is independent of baseline parameters.

For this review, it was established that there was no correlation between the effect expressed as percent reduction and the baseline value.

Furthermore, the percent reduction from baseline in blood LDL-cholesterol at the present time represents the best available pharmacological marker of the magnitude of the effects of statins on the enzyme, HMG Co-A reductase.

In hyperlipidemic patients, atorvastatin generated a reduction in triglyceride levels and more modest elevations in high-density lipoprotein (HDL)-cholesterol levels than fenofibrate or nicotinic acid, but it provided higher reductions in total cholesterol and LDL-cholesterol [Kappelle 2010, Malhotra 2001].

Atorvastatin is a synthetic second-generation lipid-lowering drug that works by inhibiting HMG-CoA reductase.

It is the drug of choice that is used to treat hyperlipidemia and manage cardiovascular diseases. The administered dose was 20 mg × $kg^{-1}$ BW.

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## 3. The phytotherapeutic means

### 3.1. Sea buckthorn (*Hippophae rhamnoides L.*)

*Sea buckthorn* berries are a plant that belongs to the family *Elaeagnaceae and* has many pharmacological applications due to the presence of many known and unknown bioactive substances mainly in its fruits and leaves.

*Sea buckthorn berries* contain more than 200 bioactive components:

- *many vitamins including A, B1, B2, C, E, F, K, and P,*
- *carotenoids,*
- *sterols,*
- *flavonoids,*
- *phenolics,*
- *lipids,*
- *ascorbic acid,*
- *citric acid, and*
- *more than 15 microelements (including Fe, Mn, B, Al, K, F, Ti, and so on).*

The oils are also rich in essential fatty acids, n-3, n-6, n-7, and n-9. It reduces liver

damage through antioxidant activity, making it a hepatoprotective agent [Geetha 2008].

In addition, SB oil has a cytoprotective action on liver damage induced by toxic chemicals such as carbon tetrachloride, acetaminophen, and ethyl alcohol.

In one study, the aflatoxin B1 AFB1 is a secondary metabolite of the *Aspergillus flavus* and *Aspergillus parasiticus* fungi and is found in grains and other foods and feedstuffs as a natural contaminant.

This is a potent liver toxin and an extremely potent mutagen having teratogenic effects and causing hepatocellular hyperplasia, hepatic necrosis, cirrhosis, biliary hyperplasia, and acute liver damage in animals.

Aflatoxins affect many species including humans, dogs, pigs, dairy cattle, and chickens.

Currently, there are no readily available, scientifically proven therapeutic options, and this study was designed to determine if SB oil has efficacy as a hepatoprotective action against aflatoxin-induced liver damage in chickens [Geetha 2008].

Scientific classification is presented in Table 1.

**Table 1.**
Scientific classification of Sea buckthorn

| Kingdom | Plantae |
|---|---|
| Clade | Angiosperms |
| Clade | Eudicots |
| Clade | Rosids |
| Order | Rosales |
| Family | Elaeagnaceae |
| Genus | Hippophae |
| Species | H. rhamnoides |
| Binomial name | Hippophae rhamnoides L. |

The chemical composition of the *Hippophae rhamnoides* varies according to the origin, climate, and method of extraction.

*Hippophae rhamnoides* consist of:

- *fruit acids,*
- *ascorbic acid,*
- *flavonoids,*
- *carotenoids,*
- *fatty acids, and*
- *sugar alcohols.*

Flavonoids are present in all parts of *Hippophae rhamnoides*.

Fresh fruits contain 854 mg/100 g while dried leaves contain 3888 mg/100 g of flavonoids. The main flavonoids in *Hippophae rhamnoides* are:

- *isorhamnetin,*
- *quercetin,*
- *myricetin and*
- *kaempferol.*

Polyphenols include also:

- *flavonols,*
- *catechins,*
- *proanthocyanidins,* and
- *chlorogenic acids.*

The vitamin C concentration in *Hippophae rhamnoides* ranges from 28-2500 mg/100 g of berries in various subspecies of *H. rhamnoides*.

The various factors, which affect the concentration of vitamin C, include temperature, harvesting time, origin, and method of processing. Subspecies of *H. rhamnoides* also contain vitamins:

- *A,*
- *B1,*
- *B2,*
- *K,* and
- *P.*

Seeds and berries have a sufficient quantity of tocopherols.

The concentration of tocopherols and tocotrienols ranges from 100-300 mg/1000 g in seeds and 110-150 mg/1000 g in berries Yellow-orange color of the berries is due to the presence of carotenoids.

Carotenoids in seeds present in a concentration of 1/20-1/5 to that of berries. Organic acids like malic acid and quinic acids are also present in the *H. rhamnoides* juice.

Minerals in *H. rhamnoides* juice include:

- *potassium, the most abundant,*
- *Cu,*
- *Cd,*
- *Fe,*
- *Zn,*
- *Mg, etc.*

Fatty acid distribution in the mesocarp and seed lipids is different.

The main fatty acids are palmitoleic acid, palmitic acid, linoleic acid, and oleic acid [Cakir 2004].

### 3.2. The grapes

The use of medicinal plants in medicine is increasing because of their widespread use and for their curing of various diseases.

Grapeseed is well known for its pharmaceutical properties, including:

- *anti-inflammatory,*
- *immune-modulatory activity,*
- *antipruritic effect,*
- *treatment of gastrointestinal disorders,*
- *antimicrobial activity,*
- *lipid and stress lowering effect, and*
- *anti-allergic activity.*

The grape seed oil contains 0.8 to 1.5% unsaponifiable rich in phenols (tocopherols) and steroids (campesterol, beta-sitosterol, and stigmasterol).

The grape seed oil contains small amounts of vitamin E, but safflower oil, cottonseed oil, or rice bran oil contains greater amounts.

Grape seed oil is high in polyunsaturated and low in saturated fat, it also does not contain cholesterol or trans-fatty acids.

Grape seeds contain various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers, and phytochemicals.

Polyphenols are the most important phytochemicals in grapes because they possess many biological activities and health-promoting benefits.

The phenolic compounds mainly include:

- *anthocyanins,*
- *flavonols,*
- *flavanols,*
- *stilbenes (resveratrol), and*
- *phenolic acids.*

Anthocyanins are pigments and mainly exist in grape skins.

Flavonoids are widely distributed in grapes, especially in seeds and stems.

Anthocyanins are the main polyphenolics in red grapes, while flavan-3-ols are more abundant in white varieties.

The reported evidence of beneficial health effects of phenolic compounds includes inhibiting some degenerative diseases, such as cardiovascular diseases [Majo 2008].

The scientific classification of the grape (*Vitis vinifera*) is presented in Table 2.

**Table 2.**
Scientific classification of *Vitis vinifera*

| *Kingdom* | *Plantae* |
|---|---|
| *Clade* | *Angiosperms* |
| *Clade* | *Eudicots* |
| *Clade* | *Rosids* |
| *Order* | *Vitales* |
| *Family* | *Vitaceae* |
| *Genus* | *Vitis* |
| *Species* | *V. vinifera* |
| *Binomial name* | *Vitis vinifera L.* |

Grape is a phenol-rich plant, and these phenolics are mainly distributed in the skin, stem, leaf, and seed of grapes, rather than in their juicy middle sections.

The total concentration of phenolic compounds was about 2178.8, 374.6, 23.8, and 351.6 mg/g gallic acid equivalent GAE in seed, skin, flesh, and leaf, respectively.

The total phenolic content of grape skins varied with cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections.

The compounds mainly included proanthocyanidins, anthocyanins, flavonols, flavanols, resveratrol, and phenolic acids.

Proanthocyanidins are the major phenolic compounds in the grape seed and skin of the grape.

Anthocyanins are pigments and responsible for the color of grapefruits, and flesh did not contain anthocyanins.

The chemical structures of some phenolic compounds from grapes are presented in figure 4.

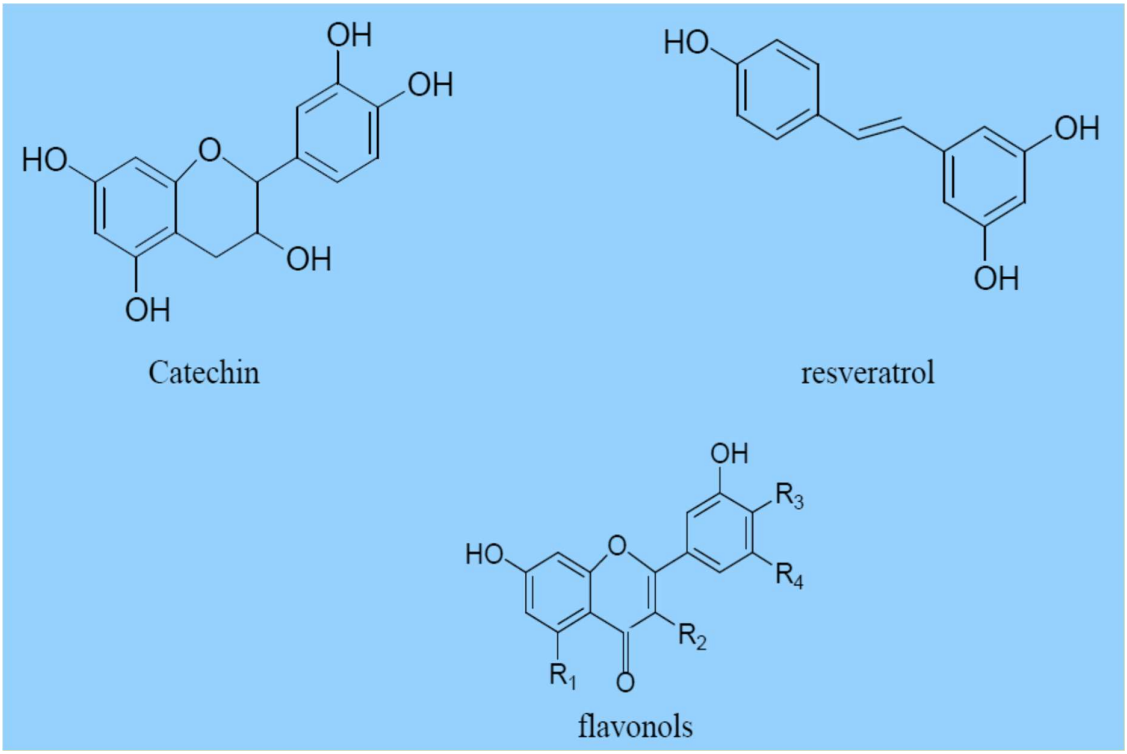



**Figure 4.** The chemical structures of some phenolic compounds from grapes [En-Qin 2010]

## 4. Aim of the study

The present study aimed to investigate the effect of fat consumption on the histological structure of the prostate in a murine model.

Through histological investigation of the prostate, this study intended to evaluate comparatively if atorvastatin has any healing role on the prostate mirrored in the cytoarchitecture and also, what is the role of phytotherapy on the cytoarchitecture of the prostate.

### 4.1. Objectives

The punctual objectives of this research are:

- *to find suitable phytotherapy in the inflammation of the prostate gland as a result of high-fat consumption;*
- *to assess the ability of phytotherapy to reduce prostatic damage induced by fat consumption;*
- *to observe if phytotherapy can restore the damaged cell tissue to its normal feature.*

## 5. Materials and methods

### 5.1. Experimental protocol

To assess the protective effects of phytotherapy against HFD and atorvastatin toxicity in a hyperlipidemic murine model, 28 white Wistar male rats were grouped into seven experimental groups, each group comprised of 16 animals.

Rats weigh between 150 and 156 g and age between 3 and 4 months. They were obtained from Cantacuzino, Bucharest, Romania.

Animals were kept in a controlled environment and housed in polycarbonate cages and provided free with a standard diet for rodents. Wood shavings were used for cage bedding.

The temperature in the laboratory was kept constant at 22 ±2°C, with a relative humidity of 55 ±10%. During the experimentation, the light cycle was a 12/12-hour light/dark period.

The rats were receiving orally the following treatment:

- Group [1] received atorvastatin as a unique oral suspension in distilled water at a dose of 20 mg × $kg^{-1}$ BW.
- Groups [2] and [3] received 20 mg×$kg^{-1}$ BW of atorvastatin in combination with 100 mg×$kg^{-1}$ BW of SBT and grape extracts, respectively.
- Groups [4] and [5] received grape and SBT extracts at 100 mg×$kg^{-1}$ BW, respectively.
- The positive control group was represented by the HFD from Group [6], and
- The negative control was Group [7], receiving a regular diet and water *ad libitum*

After six months after the administration of the therapeutic doses, rats were sacrificed and euthanized by overdosing anesthetic agents using 300 mg × kg $bw^{-1}$ of ketamine (Ketamine 10%, CP Pharma, Burgdorf, Germany) and 30 mg × kg $bw^{-1}$ of xylazine (Narcoxyl, Intervet International, Boxmeer, the Netherlands), in agreement with known methodology [Directive 2010, NRC, 2011].

### 5.1.1. Extraction of Sea buckthorn

Sea buckthorn was chosen because of the substantial amount of antioxidant, hypolipidaemic, and therapeutic compounds. As an administration dosage, the extraction technique was intended to yield 100 mg×kg-1BW of total polyphenol.

A method of extraction is illustrated by crushing 10 g of fresh fruit in a laboratory grinder, a final solution was obtained (GM 2000, Grindomix, Retsch Technology GMbH, Haan, Germany). We weighed and added 100 mL of 70% ethanol to the crushed fruit, shaking for 60 minutes, filtering, and transferring to a 70°C rotary evaporator (Labota 4000, Heidolph Instrument, Germany) until a final solution was achieved. The total polyphenol content was measured using the modified Folin-Ciocalteu technique. Gallic acid (concentration range: 2.5-250 g/mL) was used to create the calibration curve.

The results were represented in milligrams of gallic acid equivalent (GAE) per gram of sample and then converted to milligrams of GAE per 100 g of SBT berry fresh weight (mg GAE/100 g FW) [Obistioiu 2021].

**Figure 5.** Extraction steps of Sea buckthorn

Individual polyphenols were identified and quantified using LC Shimadzu (Kyoto, Japan) equipment with:

- SPD-10A UV (Shimadzu, Kyoto, Japan) detectors and
- EC 150/2 NUCLEODUR C18 Gravity SB 150 mm 2.0 mm column,
- particle size 5 m (Macherey-Nagel GmbH & Co. KG, Germany) operating at 20°C and 0.2 mL/min flow rate.
- Gradient elution of A (aqueous formic acid, pH = 3) and B (acetonitrile and formic acid, pH = 3) separated the components.
- The gradient program was as follows: 5% B (0.01).

Then, the identification of individual polyphenols were detectors and EC 150/2 NUCLEODUR C18 Gravity SB 150 mm × 20 min), 5–40% B (20.01–50 min), 40-95% B (50–55 min), 95% B (55–60 min). The injection volume was 20 µL.

Monitoring was performed at 280 and 320 nm and the detector was set at an acquisition range from 200 nm to 700 nm. Data acquisition, peak integration, and calibrations were performed with LC Solution software from Shimadzu. The calibration curves were performed in the range of 20–50 µg/mL for all individual polyphenols used (limit of detection 0.4–0.5 µg/mL, the limit of quantification 0.6–0.7 µg/mL) [Dumbrava 2020].

The SBT dose was determined as follows:

- 10 grams of SBT contained 26 mg of polyphenols, while
- 30 mL of concentrated extract had 624 mg of polyphenols.

As a result, we applied the rule of three. The provided dose was 100 m mg × $kg^{-1}$ g kg-1 BW, which corresponds to 1.5 mL of extract/rat person as an extract administration dose.

### 5.1.2. Antioxivita

Antioxivita is a nutritional supplement made using patented technology that has been recognized internationally at several invention salons. It is a novel substance that is a potent antioxidant extracted from seeds, skins, and grape brandy. It is a 100% natural Romanian product obtained by patented technology. The experimental animals were given 100 mg $kg^{-1}$ BW of it.

### 5.1.3. Histological examination

Twenty-eight male Wistar rats were slaughtered after six months of therapy.

The prostates were collected and fixed in ethanol at an 80% concentration for seven days.

Following fixation, the samples were washed, dehydrated in increasing concentrations of ethanol (80, 96, and 100 volumes, 3 baths of each type of concentration, for 1 hour each), clarified in 2 baths of xylene (or toluene or benzene, for 1 hour each), and embedded in paraffin.

The paraffin pieces with included samples were cut with the Slee-Meinz Cut 4062 microtome, yielding slices with a thickness of 5 micrometers. A portion or sections were carefully separated and put to the surface of the histology slide.

The histological slides were stained using a standard technique represented by Hematoxylin – Eosin stain method [Șincai 2000]. The Steps for the Hematoxylin –Eosin stain method:

- Deparaffination (paraffin removal) using 2 baths of xylene (or toluene or benzene), 20 minutes each.
- Rehydration using baths of ethanol in decreased concentrations (100, 96, 80, 70 volumes), 7 minutes for each concentration.
- Nuclei staining with Harris Hematoxylin, for 10 minutes, then

- Washing and differentiation in tap water, 2 baths, 5 minutes each, after that
- Washing with distilled water.
- Cytoplasm staining with Eosin 1 %, for 15 minutes.
- Washing with distilled water, 2 baths.
- Dehydration using baths of ethanol in increased concentrations: 70°, 80°, 96°, 100 volumes, 5 minutes for each bath.
- Clarification using 2 baths of xylene (or toluene or benzene), for 15 minutes each.
- Finally, all slides were mounted with Canada balsam.

#### 5.1.4. Preparation of a high-fat-diet for induction of hyperlipidemia

To prepare a high-fat diet (HFD) recipe, an earlier modified method was used to induce hyperlipidemia in all experimental animals except the normal diet group. The diet was prepared at the Laboratory of the Pharmacology discipline / FVM / Timișoara. All ingredients were thoroughly mixed and allowed to dry well [Doucet 1987, Otunola 2010].

## 6. Results and discussion

### 6.1. Total polyphenols content (TPC) and individual polyphenols

Table 3 presents the results regarding the individual polyphenols identified in the SBT and Antioxivita extracts.

Of the 11 determined polyphenols, five compounds were identified:

- *kaempferol,*
- *resveratrol,*
- *rosmarinic,*
- *rutin, acid, and,*
- *quercitin.*

Other compounds like:

- *protocatechuic acid,*
- *caffeic acid,*
- *epicatechin,*
- *p-coumaric acid*, and
- *ferulic acid*

were not detectable (concentration under the limit of detection).

SBT contains

- rosmarinic acid (43.742 µg/L),
- quercitin (40.534 µg/L),
- resveratrol (28.385 µg/L), and in lower concentration,
- kaempferol (6.208 µg/L).

Antioxivita contains a higher concentration of

- quercetin (203.798 µg/L) and kaempferol (270.556 µg/L), but lower levels of
- rosmarinic acid (26.271 µg/L), resveratrol (18.615 µg/L), and rutin (7.525 µg/L).

**Table 3.**
Individual polyphenols and LC-MS parameters of the hydroalcoholic extracts

| No. | Compound name | Rt (min) | Concentration SBT extract (µg/L) | Concentration Antioxivita extract (µg/L) | Calibration curve |
|---|---|---|---|---|---|
| 1 | Gallic acid | 4.826 | nd* | Nd | y=8.470·e-006x (r=0.9996) |
| 2 | Protocatecuic acid | 11.774 | Nd | Nd | y= 8.036·e-006x (r=0.9990) |
| 3 | Caffeic acid | 21.480 | Nd | Nd | y=7.110·e-006x (r=0.9990) |
| 4 | Epicatechin | 22.606 | Nd | Nd | y=3.881·e-005x (r=0.9996) |
| 5 | p-coumaric acid | 24.737 | Nd | Nd | y=1.1566 ·e-006x (r=0.9997) |
| 6 | Ferulic acid | 24.183 | Nd | Nd | y=1.172·e-006x (r=0.9999) |
| 7 | Rutin | 24.183 | Nd | 7.525 | y=1.813 ·e-005x (r=0.9999) |
| 8 | Rosmarinic acid | 28.203 | 43.742 | 26.271 | y=1.018 ·e-006x (r=0.9982) |
| 9 | Resveratrol | 30.274 | 28.385 | 18.615 | y=6.388·e-006x (r=0.9945) |
| 10 | Quercitin | 31.521 | 40.534 | 203.798 | y=1.001·e-005x (r=0.9992) |
| 11 | Kaempferol | 34.810 | 6.208 | 270.556 | y=3.273·e-005x (r=0.9990) |

*Note: *— non-detactable*

### 6.2. Prostate cytoarchitecture

Gross examination revealed that the control group (GVII) has a normal compound exocrine gland or tubule-alveolar, with secretory alveoli, secretory tubules, and ducts bordered by a simple epithelium with epithelial cells ranging in height from cuboidal to columnar, and basal cells on occasion. A basement membrane supports the epithelial cells.

Furthermore, the prostate has a stoma that is located surrounding the lobules and is made up of connective tissue containing smooth myocytes, as presented in figure 6.

The HFD group (GVI) had significantly expanded secretory alveoli, with the epithelium decreasing in height and stratifying in specific zones.

The basement membrane was disturbed in certain alveoli, and some desquamated cells were observed. Interstitial edema is indicated by increased gaps between secretory alveoli, as shown in figure 7.

Hematoxylin –Eosin stain showed that atorvastatin treatment minimizes the prostatic damage caused by a high-fat diet.

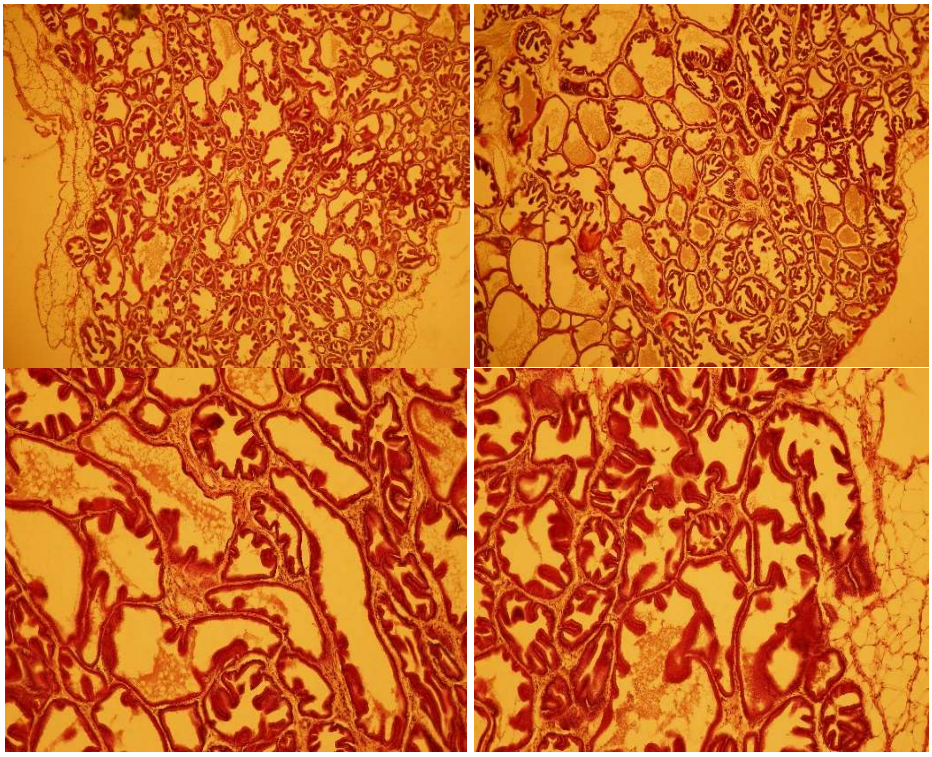
There was no evidence of interstitial edema. In general, the prostatic tissue architecture tends to be normal as presented in figure 8.

Microscopic examination of the prostate from the second and third groups revealed a normal structural aspect of this type of exocrine gland figures 9 and 10.

The presence of adipose tissue surrounding the prostate, with large monocular adipocytes, was revealed by microscopic analysis of the fourth and fifth groups.

Certain alveoli were expanded, with fewer columnar epithelial cells and stratified epithelium.

Concretions with lipid droplets of varying sizes were seen in the secretory alveoli at the prostate's perimeter. Reduced interstitial edema was observed in a small area in figures 11 and 12.



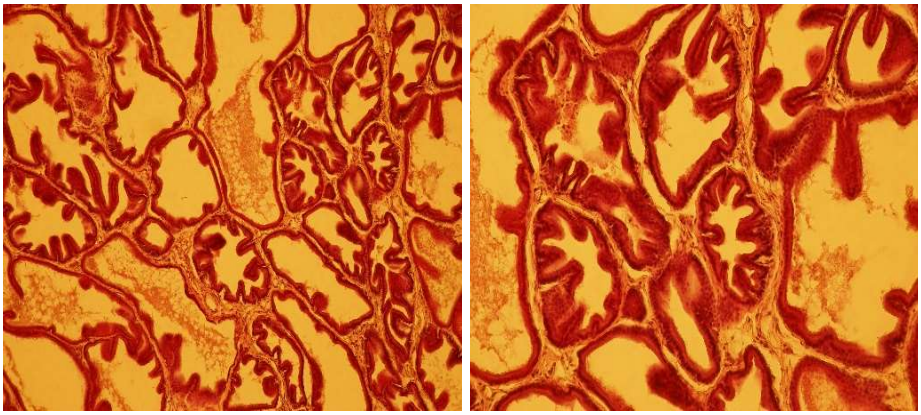


**Figure 6.** Histological section from the normal rat's prostate (GVII) after six months of treatment. (H.E. stain. ob. 4×, 10×, 20×, 40×)

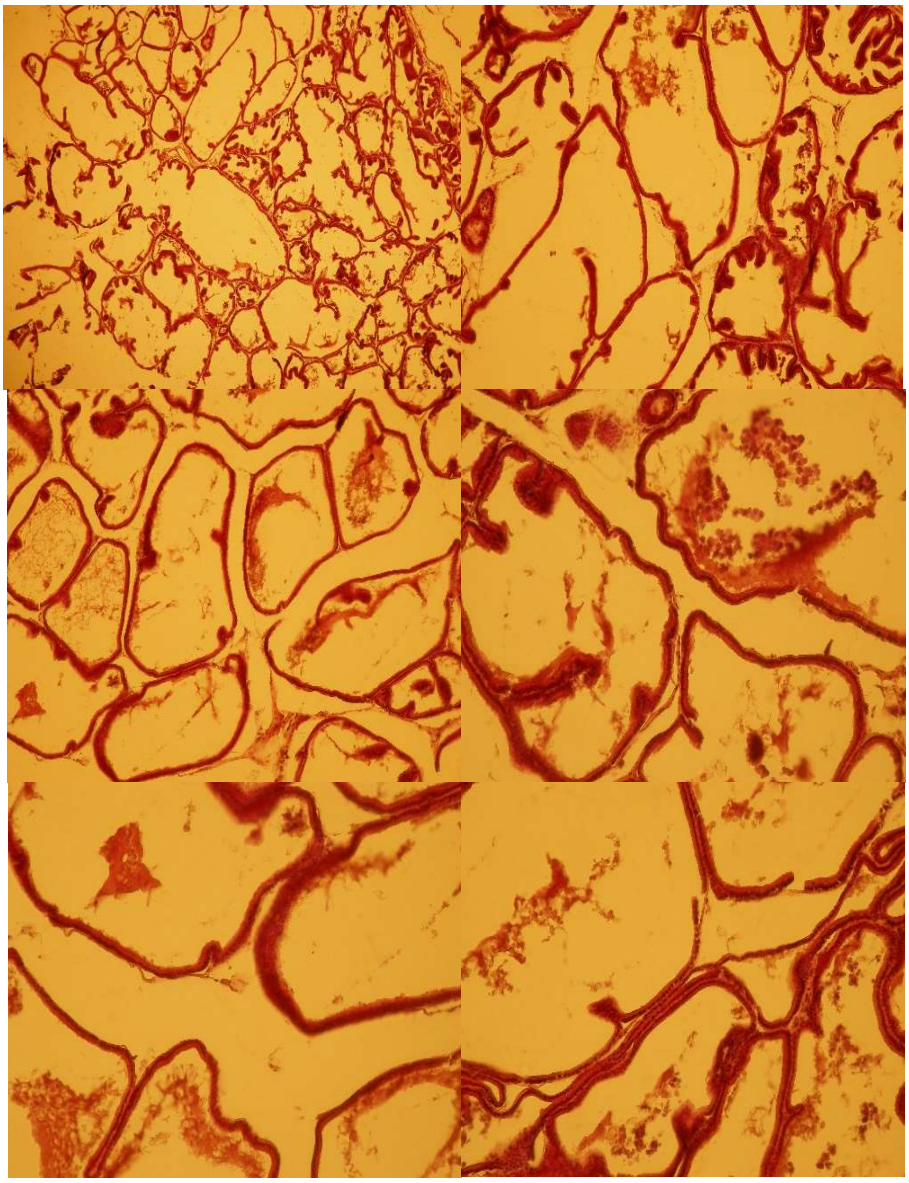


**Figure 7.** Histological section from the rat's prostate (G.VI) after six months of treatment: signs of secretory alveoli enlargement, disruption of the basement membrane, interstitial edema. (H.E. stain. ob. 4×, 10×, 20×, 40×)

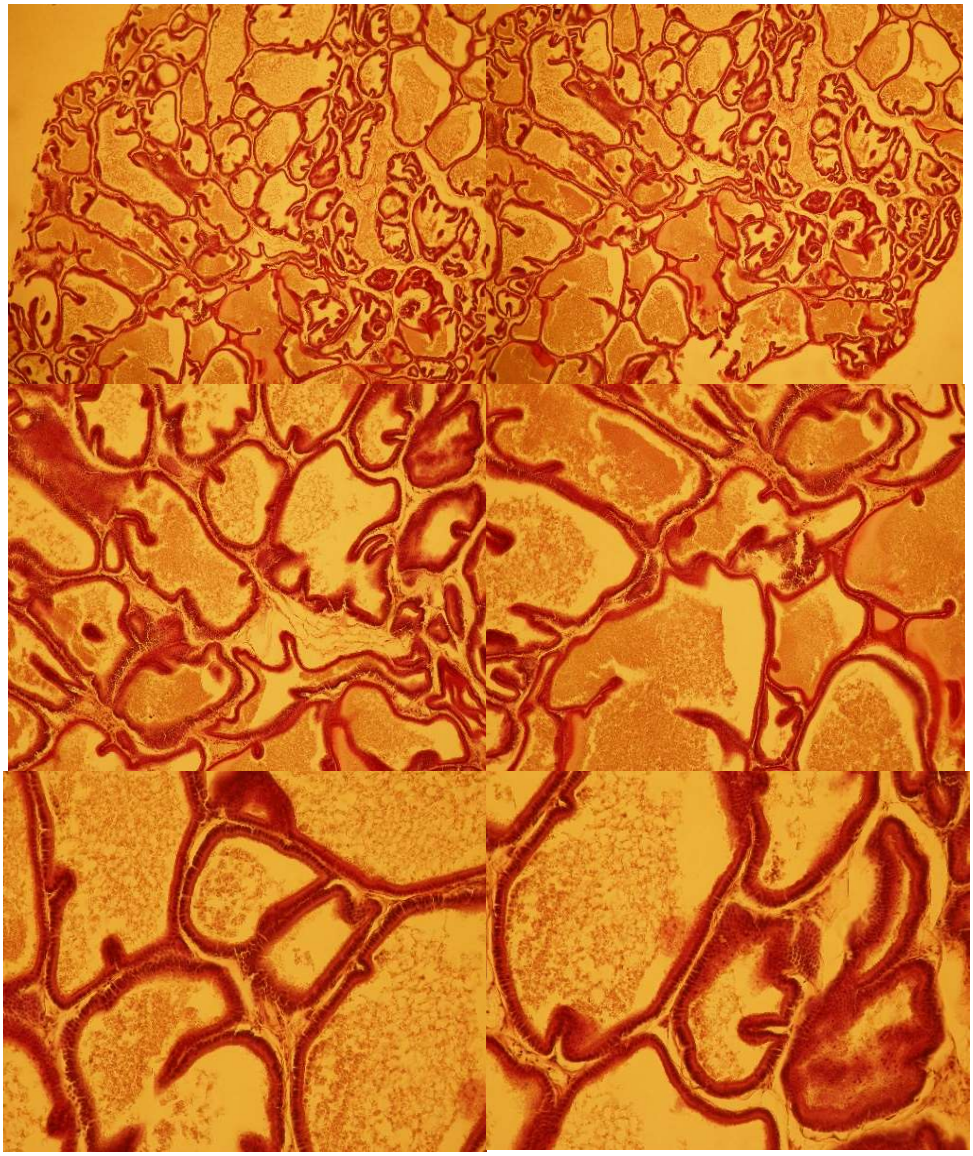
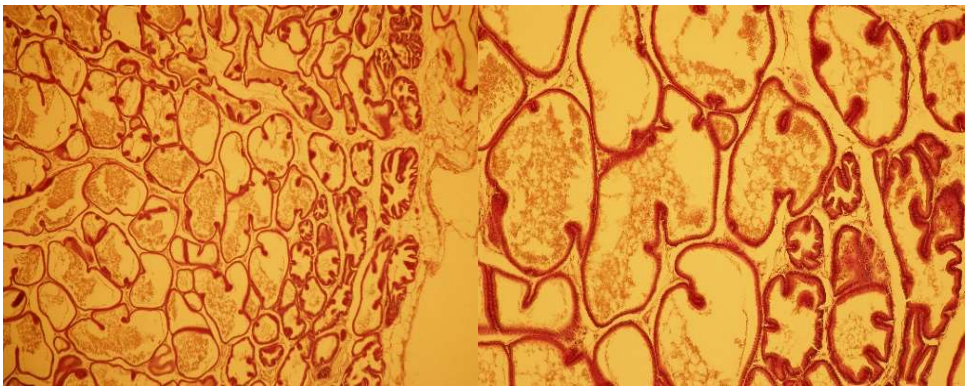


**Figure 8.** Histological section from the rat's prostate (GI) after six months of treatment: normal. (H.E. stain. ob. 10×, 20×, 40×)



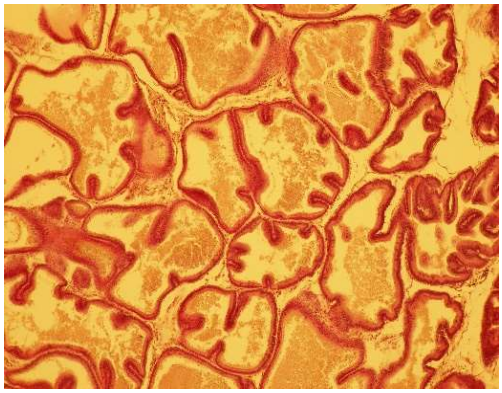
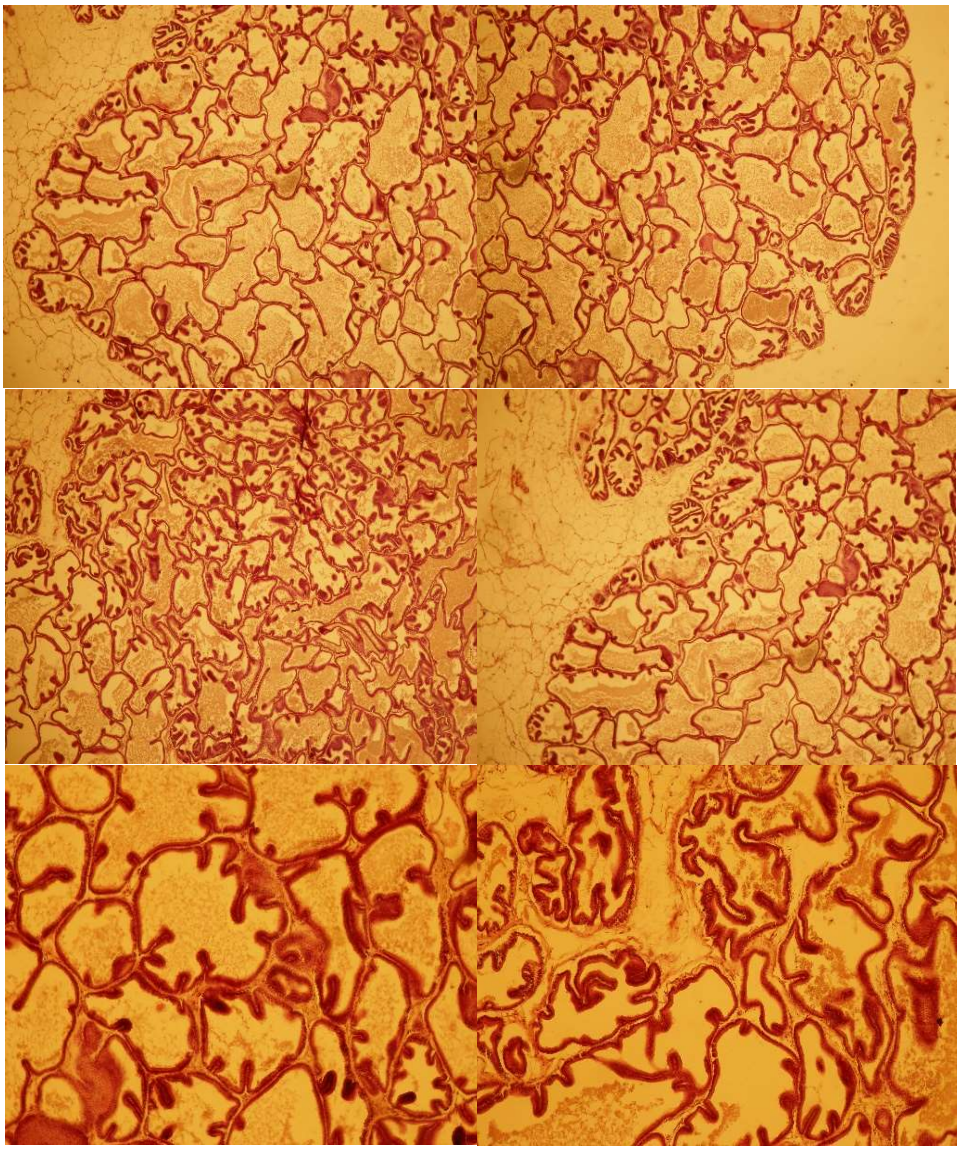


**Figure 9.** Histological section from the rat's prostate (GII) after six months of treatment: normal. (H.E. stain. ob. 10×, 20×)



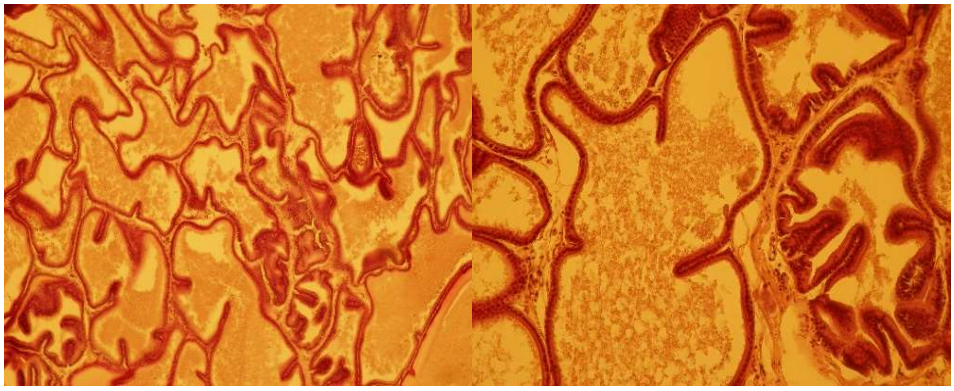


**Figure 10.** Histological section from the rat's prostate (GIII) after six months of treatment: normal. (H.E. stain. ob. 10×, 20×, 40×)

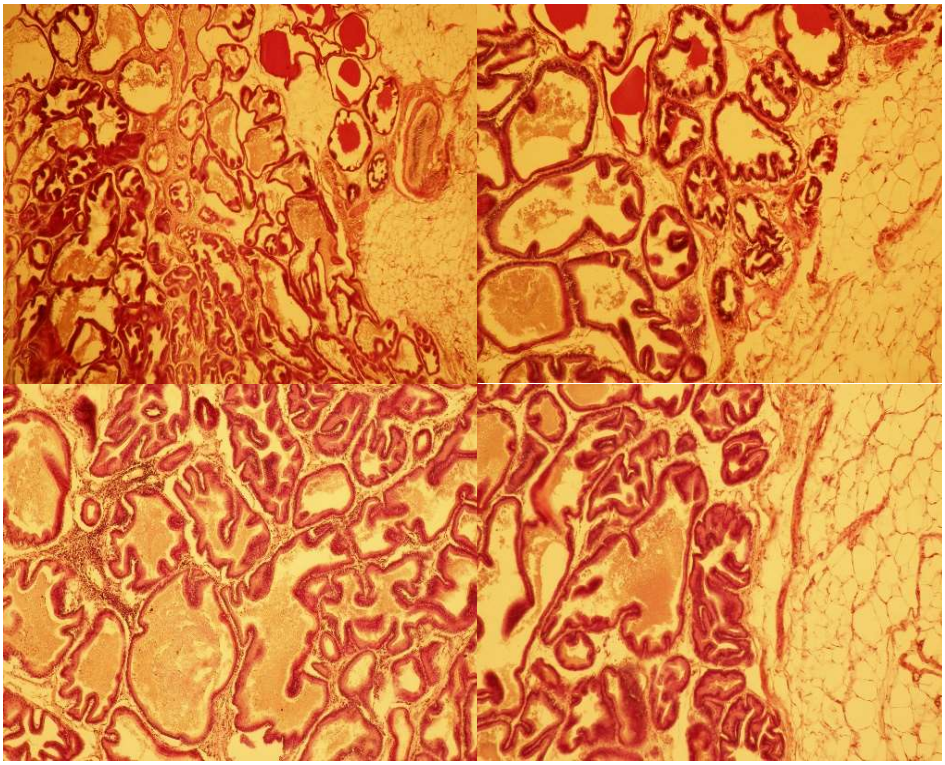
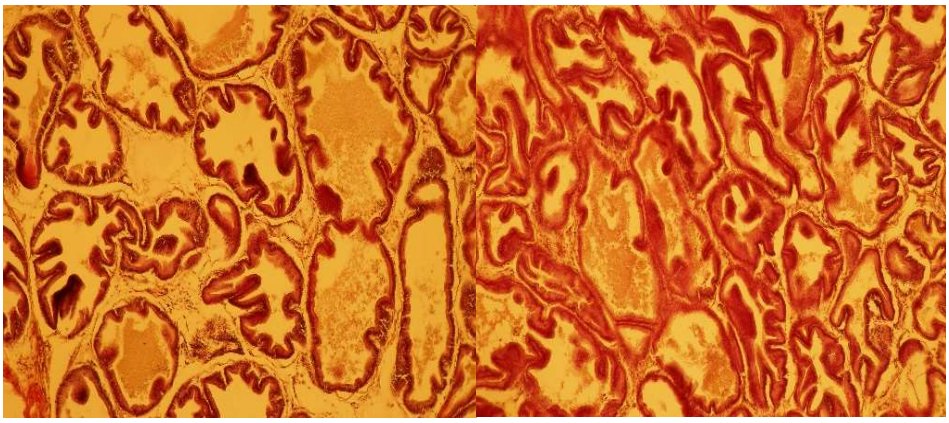


**Figure 11.** Histological section from the rat's prostate (fourth) after six months of treatment: shows signs of few secretory alveoli concretions with few lipid droplets of various size. (H.E. stain. ob. 10×, 40×)



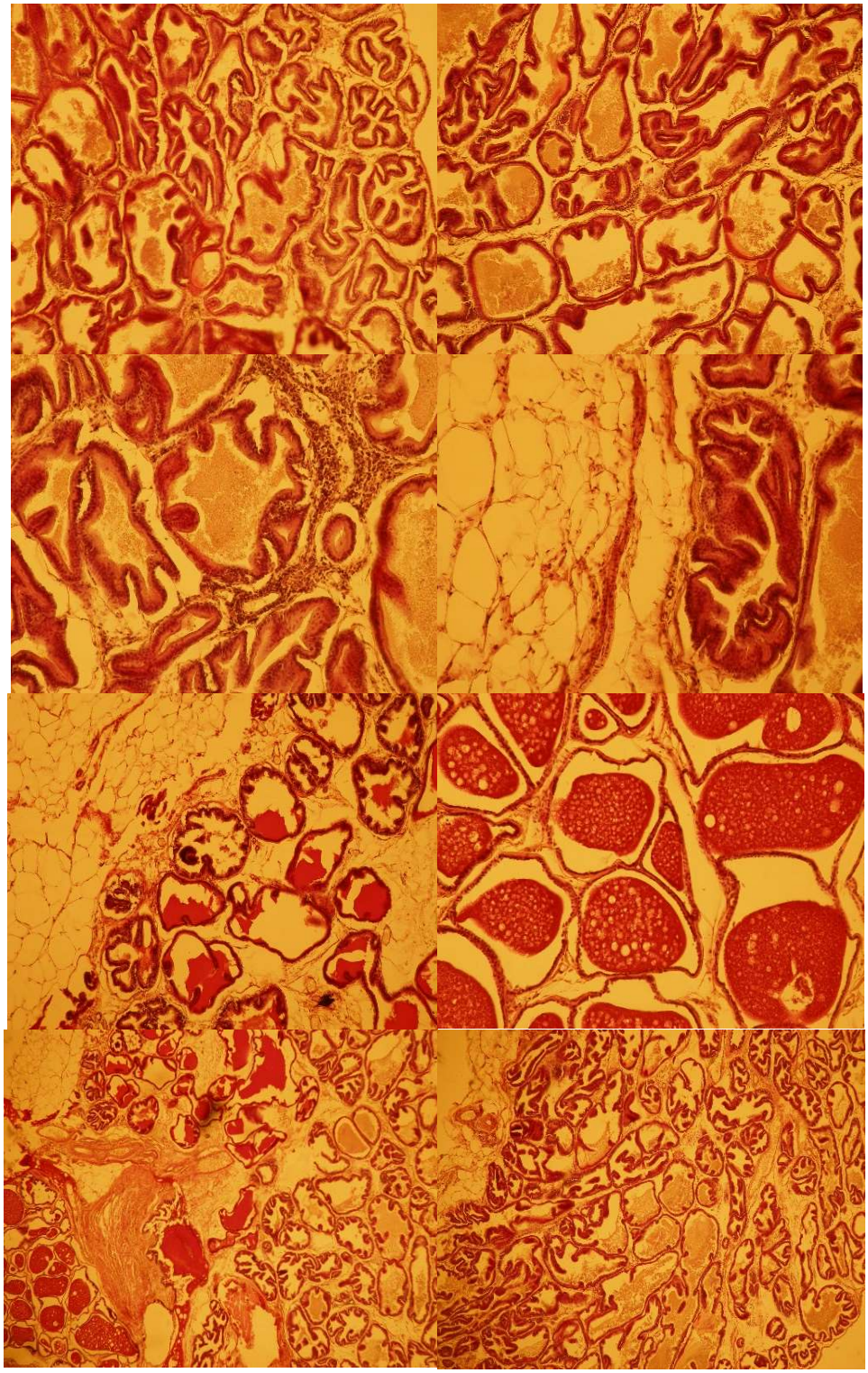


**Figure 12.** Histological section from the rat's prostate (fifth) after six months of treatment: shows signs of secretory alveoli concretions with lipid droplets in various size. (H.E. stain. ob. 4×, 10×)

## Discussion

It is generally understood that dietary alterations can have a significant impact on prostate health and enhance the benefits of traditional medical care [Tewari 2012].

We believe that polyphenolic compounds have a pharmacological role in the prevention

and treatment of HFD-induced prostatic tissue alterations.

In this study, we compared the effects of polyphenolic compounds supplementation alone or in combination with 20 mg×kg-1 BW of atorvastatin in rats submitted to an HFD.

High-fat diet consumption leads to the accumulation of lipids, which can induce prostatic tissue damage. The histological examination of prostatic sections was linked with the results in the previous chapter (biochemical analysis), which showed that HFD increased the levels of TC, TG, and LDL-*c*, which are considered important markers of lipid metabolic disorders.

Atorvastatin and polyphenolic compounds have been described as lipid-regulating agents. When rats were fed HFD and supplemented with 20 mg×kg-1 BW of atorvastatin or phenolic compounds, LDL-c and TG levels in the serum were reduced from those in the HFD group, whereas HDL-c was significantly increased as presented in some studies.

Supplementation with SBT and grape (as Antioxivita) extracts showed a therapeutic role in minimizing adverse effects on the prostatic tissue.

Some studies proved that dietary supplementation with grape powder may be an effective chemopreventive therapy for obesity-related disorders such as inflammation and prostate cancer [Joshi 2020].

## 7. Conclusions

The main conclusions who are emerging from this study are:

- We achieved that the experimental model of hyperlipidemia induced by HFD led to increased cell proliferation in the rat prostate.
- Hyperlipidemia causes marked histological changes in the prostatic tissue, and possibly changes prostatic function, thus contributing to prostatic disease pathogenesis.
- Our data showed that treatment with polyphenolic extracts from the beginning of the experiment prevented the overweight and normalized the prostate cytoarchitecture.
- Phenolic compounds most likely inhibited lipid peroxidation and reduced fat mass in prostatic tissues.
- The efficacy of the diet regimens, particularly polyphenolic-rich diets, on the main symptoms of prostatic inflammation is attributable to the benefit associated with weight loss in the prostate.

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