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Chapter 7

Medicinal Values of Selected Mushrooms with Special Reference to Anti-Hypercholesterolemia

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Hypercholesterolemia

Hypercholesterolemia is a known major risk factor in the development of athereosclerosis [29, 32, 51]. This circumstance is caused by internal homeostasis due to foods consumed. The hypercholesterolemia may be related to high cholesterol diet or regular saturated fatty acids intake [42]. The incidence of Chronic Heart Disease (CHD) remains high despite blood pressure being controlled in hypertensive patients. Thus, in hypercholesterolemia patient's LDL (Low Density Lipoprotein) concentration increases, and the lipoprotein is more aged and more susceptible to oxidative modifications than LDL from healthy subjects [50]. These patients have been diagnosed with disability of LDL excreation and very low LDL receptor activity. The most potent inhibitors of cellular cholesterol synthesis are inhibitors of 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase and consequently elevated the cellular LDL receptors synthesis, resulting in significant reduction of plasma LDL levels. [66].

1.1.1. Relation of hypercholesterolemia with atherosclerosis

Atherosclerosis is the disease caused by accumulation of foam cells originated from the monocytes which are transformed into macrophages that engulf excessive oxidized lipoprotein cholesterol. There was an increased foam cell formation which leads to intimal thickening after migration of smooth muscle cells to the intima and lamellar calcification under the endothelium. Finally a typical plaque characterized (Voet and Voet, 1990). The lumen of the arteries was narrowed and high blood pressure induced. The formation of plaque occurrs internally and raises the risk of cardiovascular diseases (CVDs) and strokes while remaining asymptomatic.

Cholesterol is an important component and needed in development of metabolism cell, but the excess of cholesterol content in serum could be problematic. Preclinical and clinical studies have shown that high cholesterol diet is regarded as a main factor in the development of hypercholesterolemia, atherosclerosis and ischemic heart disease [14]. The cholesterol content in hypercholesterolemia cases produced extremely high risk agents such as oxygen free radicals in serum as well as erythrocytes, platelets and endothelial cells. The elevation of total serum cholesterol and LDL cholesterol along with generation of reactive oxygen species (ROS) play a key role in the development of coronary artery disease and atherosclerosis.

In term of mechanism, these vascular problems of atherosclerosis, hypercholesterolemia and hypertension are all correlated to each other. The formation of plaque is associated with a period of time and relies on homeostasis of each individual in dealing with good cholesterol (HDL: high-density lipoprotein) and bad cholesterol (LDL).

Today, strategies and remedies are available to combat CVDs. Even though changing life style with appropriate dietary intake remains the first line of defence advocated by the healthcare workers, drug treatment is still widely used because of the rapid effect especially in treating severe cases [7]. Hence, most research today focuses on screening and identifying compounds exhibiting anti-hypercholesterolemic properties.

To date, several cholesterol lowering medications were discovered and used singly or in combination to lower the LDL cholesterol and triglycerides, a type of fats in the blood that also increases the risk of atherosclerosis. Concomitantly increase of HDL cholesterol often offers protection from CVDs [21]. These types of drug include, bile acid binding resins, cholesterol absorption inhibitor, combination cholesterol absorption inhibitor and statins such as ezetimibe-simvastatin, fibrates, niacin and omega-3 fatty acids. However, almost all the anti-hypercholesteromia drugs have been reported as having various adverse effects [46]. Several side effects such as constipation, nausea, diarrhea, stomach pain, cramps, muscle soreness, pain and weakness are reported; while more severe side-effects such as facial and neck flushing, nausea, vomiting, diarrhea, gout, high blood sugar etc. are also known. The side effects of statin and niacin are similar to each other. In addition, adverse drug reactions are always encountered in multiple diseases treated with a number of drugs. If hypercholesterolemia is accompanied by other diseases, these diseases may have an impact on the response of the body to anti-hypercholesterolemia drugs and the metabolic processes of the body may be affected negatively. Later on, increased dosages may be required, which in turn would only worsen the cholesterol medication drugs. The search for cholesterol lowering medication has now turned to complementary traditional medicine. However, the traditional use of herbs in lowering cholesterol is often not verified scientifically. On the other hand, even if proven effective, such herbs should be investigated on their mechanisms of action.

1.1.2. Mushrooms as medicinal-functional food against hypercholesterolemia

Medicinal mushrooms have been scientifically proven to be safe, efficacious, and novel anti-hypercholesterolemia therapeutic agents of natural source. An abundance of scientific research

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and studies on medicinal mushrooms or edible mushrooms shed light on them as functional food due to their broad spectrum of therapeutic efficacy beside culinary demand [80].

The original term "functional food" has been defined by Martirosyan (1992) as "a natural or processed food that contains known biologically-active compounds which when in defined quantitative and qualitative amounts provides a clinically proven and documented health benefit, and thus, an important source in the prevention, management and treatment of chronic diseases of the modern age".

Medicinal Mushrooms (macrofungi), mostly members of the class Basidiomycetes fulfil the requirement of functional foods. Recently, they have become increasingly attractive as functional foods for their potential beneficial effects on human health. Hence, the food industry is especially interested in cultivating these mushrooms. The wild edible mushrooms have gained their reputation as health food due to their geographical origin in natural unpolluted environment. Nonetheless the cultivation of medicinal mushrooms using modern technology and the quality of the extracted product are crucial factors determining them as functional-medicinal food.

A plethora of potent therapeutic components in medicinal mushrooms such as fibers, phytosterols, saponins, polyphenols, flavanoids, terpenes and polysaccharides confer antihypercholesterolemic, antioxidant and anti-atherosclerotic properties [80].

The intensive study of [33] reported the hypocholesterolemic effect of the mushroom fruiting bodies and several types of these extracts exhibited different mechanisms of action, such as impairing dietary cholesterol absorption or inhibiting the endogenous cholesterol metabolism. Other reports showed that medicinal mushrooms are rich in chitin (dietary fibre) and specific β-glucans which may inhibit cholesterol absorption by increasing the faecal excretion of bile acids and reducing the amount of serum LDL-cholesterol [19, 28].

Among the most studied mushroom species are *Tricholoma giganteum* (giant mushroom), *Marasmius androsaceus* (horsehair parachute mushroom), *Grifola frondosa* (maitake mushroom), *Pleurotus* species (oyster mushroom), *Lentinula edodes* (shiitake mushroom), *Ganoderma lucidum* (reishi or lingzhi mushroom), *Sparassis crispa* (cauliflower mushroom), *Pholiota adiposa* (black tiger's paw mushroom), *Sarcodon aspratus* (yellow cap mushroom), *Hypsizygus marmoreus* (shimeji/buna shimeji mushroom), *Flammulina velutipes* (enoki mushroom), *Hericium erinaceus* (lion's mane mushroom), and *Agaricus bisporus* (button mushroom) [65].

The objectives of this article are to review the possible anti-hypercholesterolemic mechanisms of some putative bioactive compounds extracted from well known medical mushrooms particularly *G. lucidum*.

2. Selective mushrooms as anti-hypercholesterolemia agent

Ganoderma species are Basidiomycetes belonging to Polyporaceae (or Ganodermataceae) of Aphyllophorales. They differ from the ordinary mushrooms and are categorized in the order

Agaricales in that they have pores rather than gills on the surface of the fruiting bodies. *G. lucidum* has been reported to have multi-beneficial values and concerted medicinal effects in the treatment of various diseases. The recent application of modern analytical techniques has, in a number of cases, provided a scientific basic for these earlier empirical observation. Thus, many putatively effective compounds are isolated and identified from *G. lucidum*. The pharmacological activities of *G. lucidum* have been attributed mainly to its polysaccharides and triterpenes [24].

2.1. *Ganoderma* polysaccharides

As fungal wall constituents, bioactive polyglycans (polysaccharides), such as β-glucans in *G. lucidum*, are found in all parts of the mushroom, including the mycelium (Bartnicki-Garcia, 1968). Fungal polyglycans can also be secreted into the growth medium and become extracellular (Buck *et al*., 1968). Bioactive polyglycans in *G. lucidum* comprise neutral polysaccharides (β-1,3, β-1,6 homo D-glucan), acidic glucan and polyglycan [60], protein-bond heteroglucan [58], arabinoxyoglucan, a highly branched heteroglucan [61], with a heteroglycan with β-(1,4) core [62], and peptidoglycan: ganoderan A, B, C [35] in the fruiting body [84], β-D glucan [72] and lucidan, a protein-bond heteroglycan [44], as well as other polyglycans in the mycelia which have not been characterized.

In fact, *Ganoderma* glucans, β-(1,3), β-(1,6)-D-glucan is blocked by basic unit of β-(1,3)-D-glucopyronan which consists of 1-15 units of β-(1,6) monoglucosyl side chains (Mizuno, 1991). Numerous reports show that β-(1,3), β-(1,6)-D-glucans with molecular weight of 10^4-10^6 Daltons exhibit antitumor activity (Mizuno, 1991). It seems that the higher the molecular weight, the more effective anti-hypercholesterolemia activity. Anti-hypercholesterolemia activity is also linked to the frequency of polysaccharide branching which varies during different stages of mycelial growth. Different extraction and purification processes yield a variety of bioactive glycans. Identification of these large and highly complex bioactive *Ganoderma* polysaccharides, whose precise structures have not been elucidated, involved expensive process.

2.2. *Ganoderma* Triterpenes

Triterpenes are relatively simple molecules which are easy to isolate and quantify. They can be used as a measure of the quality of different *Ganoderma* samples [24] (Stavinoha, 1995). Twenty or so bioactive triterpenes have been isolated from *G. lucidum* although over one hundred with known chemical compositions and molecular configurations have been reported to occur in *G. lucidum*.

Triterpenes are produced in the fruiting body. They can also be induced in the mycelial mat on solid medium (Nishitoba *et al*., 1987) or in the still liquid culture of late stationary phase [86]. Limited amount of triterpene is formed in the mycelial pellets of liquid shaking culture [76]. It is said that strains producing basidiocarps with a light yellow underside may contain a high amount of triterpenes in their caps. Such observation has been used to grade commercial *Ganodema* fruiting bodies in Asia [37]. [75] found that the highest concentration of *Ganoderma* triterpenes was in the spore scrapings obtained from the underside of the mushroom in the

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1-2 mm tube region (the hymenial layer). Only 18-58 mg of bioactive triterpenes were obtained from 1000 g of *Ganoderma tsugae* basidiocarps, while 4.5% (w/v) of crude ethanol extract was obtained from the sample [77]; thus, Stavinoha *et al*. (1993) used spore scrapings from the mushroom underside instead of the whole mushroom for extracting bioactive triterpenes.

The bitter taste of *G. lucidum* as a traditional Chinese medicine or tonic is attributed to its highly oxygenated polar triterpenes [54]. Triterpenes as secondary metabolites are more strain specific in *G. lucidum* [64]. High temperature and prolonged oxidation should be avoided during extraction to retain intact structures of these volatile compounds [37].

3. Mechanism on different biomedical application of *Ganoderma lucidum*

There are many studies on *G. lucidum* worldwide due to its superior therapeutic value in tackling many types of diseases. However the mechanisms involved have yet to be clarified and fully understood. This review is undertaking to specify the anti-hypercholesterolemia mechanisms of different *G. lucidum* medicinal compounds.

Excretion of second metabolism products of the mushroom are used for self protection in extremely severe environment condition. The second metabolism products of *G. lucidum*, which included β-glucans and triterpenes have been known to possess a broad spectrum of health benefits from disease prevention and maintenance of health to the regulation or treatment of chronic as well as acute life threatening illness [16]. The anti-hypercholesterolemia therapeutics of *G. lucidum* extracts involved several types of mechanisms.

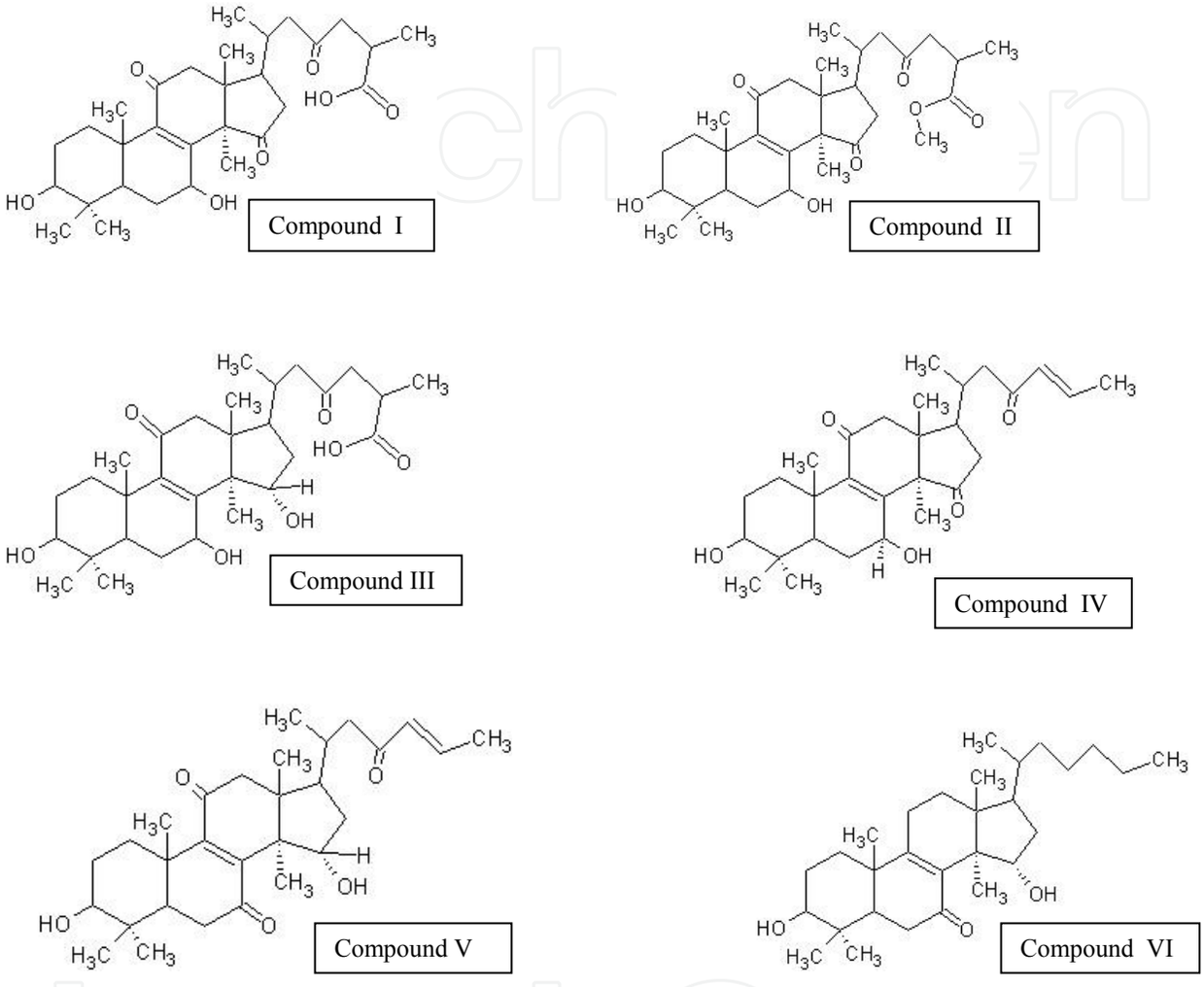
3.1. Mechanism of inhibitory effect of ganoderic acid on HMG-CoA reductase

Previous research by [85] focused on the oxygenated lanostanoid triterpenes isolated from *G. lucidum*. The pure isolated triterpenes taste bitter and some are cytotoxic. Their unique chemical structures have been studied in detail. The derivatives of these terpenes type compounds (Figure 1) were obtained through chemical conversion during inhibition of cholesterol biosynthesis.

These mushroom triterpenes inhibited histamine release from rat mast cells. Compound VI with 7-oxo and 15 α-hydroxy groups at 40 μM showed highly potential inhibition of cholesterol synthesis from [24,25-3H]-24,25-dihydrolanosterol (18 μM). This encouraging result was obtained by testing 24, 25-dihydrolanosterol on rat hepatic subcellular 10,000 xg supernatant fraction. The triterpene involved is ganoderic acid C methyl ester. Its derivative is synthesized by a complicated reaction included the yield of tri β-methoxyethoxymethyl ether (MEM) derivative under Wolff-Kishner condition to allow the 7-oxo-11-deoxo derivative further decarboxylation and deprotection of the hydroxyl group. The whole structure of compound VI has no functional group in the side chain and has both 7-oxo and 15 α-hydroxy groups on the same skeleton and showed potent inhibitory effect compared with other derivatives with carboxyl groups at the side chain.

Compound I and II showed the other derivatives with oxo group at C-23 and decarboxyl compounds at the side chain had moderate inhibitory effects. Derivatives of compond IV and

V has carboxyl group at C-25 in the side chain showed almost no inhibitory effect. These results provided an excellent clue and fundamental of specific side of triterpenes on the discovery of other *G. lucidum* bioactive triterpenes in anti-hypercholesterolemic study.



(Source: [84]

Figure 1. Ganoderic acid B methyl ester (compound II) was obtained by treating compound I with ethereal diazomethane. Decarboxylated compounds IV and V were synthesized by the reaction of compound I and III with lead tetraacetate in the presence of cupric acetate containing a drop of pyridine in refluxing benxene. In the case of V derived from III, the 7β-hydroxyl group was further oxidized to the carbonyl group.

3.1.1. Mechanism

Figure 2 showed the statins as HMG-CoA reductase inhibitor drug playing its key role in the pathway of blocking the biosynthesis mevalonate from the HMG-CoA. The mechanism involved the statin by interrupting the structure of HMG-CoA reductase binding to NADPH in HMG-CoA to produce mevalonate. Therefore the metabolic pathway that produces cholesterol and other isoprenoids has terminated.

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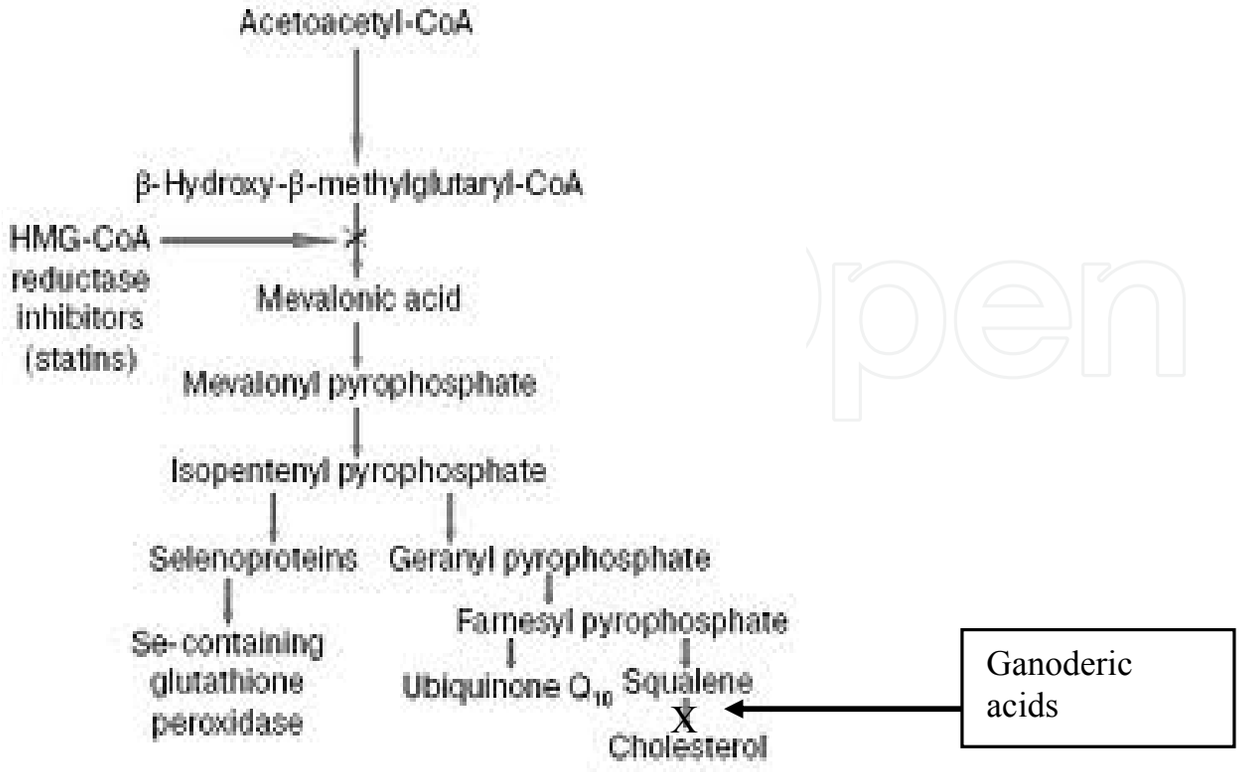

(Source: [84]; (Akira Endo, 1971)

Figure 2. Figure 2 showed the statins as HMG-CoA reductase inhibitor drug play its key role in the pathway of blocking the biosynthesis mevalonate from the HMG-CoA. Whereby ganoderic acid inhibit the cholesterol formation by competed with squalene oxido-cyclase at the last stage of cholesterol synthesis.

Akira Endo and his group discovered statins in 1976, and these HMG-CoA reductase inhibitors showed competitive effect on inhibiting HMG reductase due to their very close molecular structure to HMG-CoA. The use of statins is able to reduce the blood cholesterol levels significantly as HMG reductase is the first committed enzyme in the sequence of cholesterol synthesis cumulative process. However, since mevalonic acid (MVA) is a common precursor for many isoprenoids, blocking of MVA formation may induce undesired side effects besides inhibiting sterol synthesis. More specific inhibition of cholesterol synthesis may be attained by inhibition at some later stage of cholesterol synthesis. In this case, lanosterol was chosen in the ganoderic acid test as it originally converts from squalene by squalene oxido-cyclase at the end of cholesterol synthesis (Figure 2).

3.1.2. Comparison of structure of statins and ganoderic acid derivatives

These renowned drugs have been studied for their functional anti-hypercholesterolemic mechanism as a model for preliminary comparison of undefined natural products based on the results of the laboratory and the spectroscopy elucidation of their structure. Therefore the highest percentage of similarity of both compound structures implied similar highest effectiveness. This theory has been validated when the structure of HMG-CoA and the binding site of competitor lovastatin drug in HMG-CoA reductase inhibition was compared (figure 3).

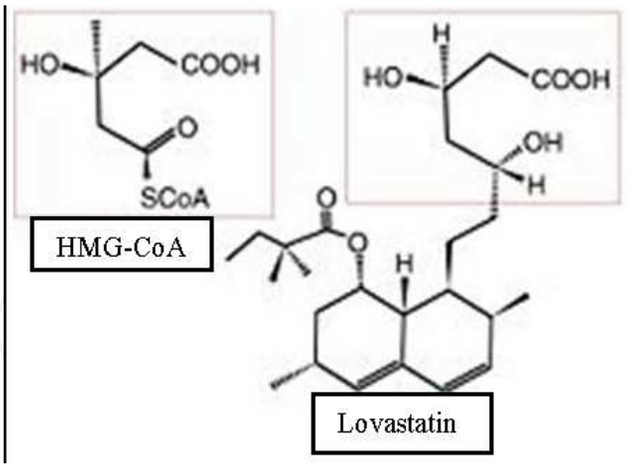

Figure 3. Comparison structure of HMG-CoA and the binding site of competitor lovstatin drug in HMG-CoA reductase inhibition.

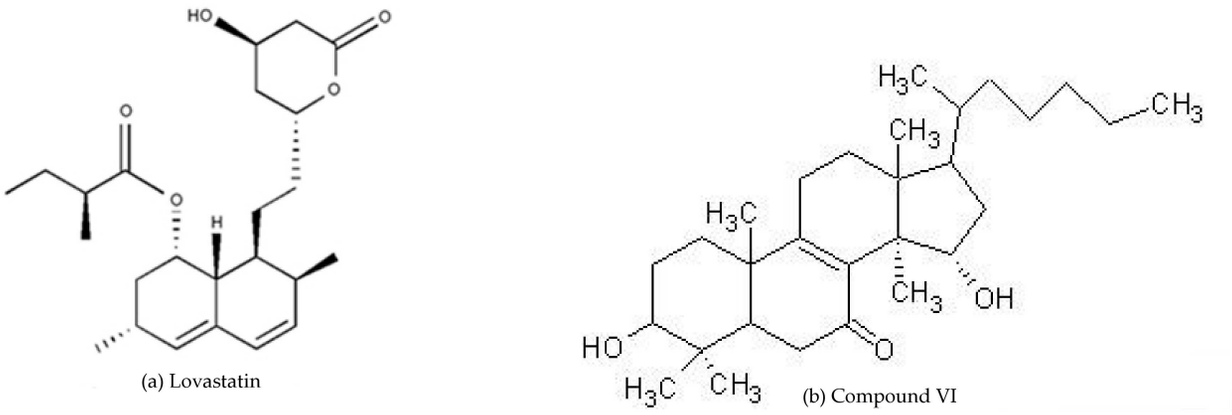

Figure 4. Comparative molecular structure of lavastatin as pharmaceutical drug and compound VI as triterpene derivative of ganoderic acid.

The active side of lovastatin is circled in box, while the structure site of the ganoderic acid derivatives automatically refers to the site chain for comparison. The important difference is statin inhibited the earlier stage of cholesterol formation whereby ganoderic acid inhibited at the late stages of cholesterol formation. Figure (4a) showed that lavostatin has 5 carbons in aromatic ring when the reaction of carboxyl group and the hydroxyl group occurred. The active sites mostly rely on the double bond of oxide group and the beta hydroxyl group. The position of both active sites is separated and impossible to react intra-molecularly. Conversely the site chain of ganoderic acid derivatives (figure 4b) is aliphatic whereby the position of carboxyl group and double bond could possibly interact. Compound IV and V (in figure 1) has close double bond at C-23 and 24 and this contribute to instability. Structure of figure (VI) shows that it is a potent inhibitor but is surprisingly without any carboxyl and double bond at the site chain. Its competitor effect could be due to the interaction of C-7 and C-15 with the binding site of 24, 25-dihydrolanosterol. The binding site of ganoderic acids with 24, 25-dihydrolanosterol is essentially similar to ganoderic acid and hence no side effect to the patient will result.

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3.2. Mechanism as angiotensin converting enzyme inhibitor in rennin angiotension-aldosterone system

The other mechanism that could apparently be involved is the inhibition of angiotensin converting enzyme (ACE) in renin-angiotensin system. This system is indirectly related to hypercholesterolemia. Atherosclerosis is the main contributory factor in this case but it may result in high blood pressure due to the narrowing of lumen of blood arteries. Therefore the regulatory mechanism of blood pressure by vasoconstriction and vasodilation may attenuate the hypercholesterolemia impact.

[62] identified five novel lanostane triterpenes, namely ganoderal A; ganoderols A and B; ganoderic acids K and S in the methanolic extract. These compounds were tested for their ACE inhibitory effect by a modification of the method described by Friedland and Silverstein (1976) and expressed in terms of IC_{50} (the amount of samples needed to inhibit 50% of ACE activity). All the newly discovered lanostane triterpenes showed IC_{50} of the order of 10^{-5} M which is considered potent. However, the earlier reported ganoderic acid F (figure 5) achieved the highest inhibitory effect with IC_{50} of 4.7×10^{-6} M.

In 2012, Abdullah *et al.* reported that the hot water extract of *G. lucidum* exhibited the best ACE inhibitory effect compared to other culinary-medicinal mushrooms. Normally the hot water extract consists of polar compounds. It has been proposed that the multitudes of phenolic substances present in *G. lucidum* contributed to this inhibitory action [62]. In addition the anti-ACE activity of the hot water extract of *G. lucidum* was enhanced when the mushroom was grown on the germinated brown rice [35].

3.2.1. Mechanism of Renin-Angiotensin System (RAS) of G. lucidum extract

The RAS is important for the aldosterone hormone system in kidneys and lung. Consequently, the blood pressure and water (fluid) balance is regulated.

Briefly, when the blood volume is low in the circulation, this scenario would be sensed by the juxtaglomerular cells at the afferent arterioles of the renal glomeruli [11] in kidneys and concurrently activate the prorenin which converts to renin directly into circulation. Plasma rennin hydrolyzes its substrate, angiotensinogen released by the liver to produce a decapeptide known as angiotensin I, which is then rapidly converted to an octapeptide, angiotensin II, by a circulating angiotensin-converting enzyme found in the lungs. Angiotensin II is a potent vasoconstrictor peptide that stimulates the cells of the zona glomerulosa of adrenal cortex to produce aldosterone [10] and [41] which causes blood vessels to constrict, resulting in increased blood pressure. When the re-intake of sodium and water in the kidneys tubules is caused by Aldosterone, the body fluid eventually increases resulting in increase of blood pressure. [4].

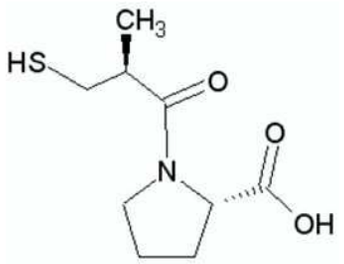
3.2.2. ACE inhibitor

Interrupting the RAS effectively control the constriction of blood vessels. When the arteries are confronted with the problem of narrowing lumen caused by the formation of plaque in hypercholesterolemia, the treatment of using ACE inhibitor or compound that blocks the

activity of ACE could reduce some risk drastically via vasodilation. Most of the synthetic pharmaceutical drug for the treatment of hypertension has no curative effect, and in fact needs prolonged administration for congestive heart failure protection. These types of drugs are usually used in combination with other medication and are usually well-tolerated by most individuals. Nevertheless, side effects such as cough, headache, drowsiness, weakness, abnormal taste (metallic or salty taste), rash are very common. By testing the active ingredient(s) in *G. lucidum*, it is possible to identify ACE inhibitor(s) which may be devoid of side effect.

3.2.3. Comparative structure of ACE inhibitor captopril and ganoderic acid F

Captopril (figure 5) is a ACE inhibitor used for the treatment of hypertension and some types of congestive heart failure. Captopril plays its role in blocking the conversion of angiotensin I to angiotensin II. Both captopril and ganoderic acid F are highly active ACE inhibitor. In term of structure, captopril contains a side chain with 3 carbons with different functional groups and an aromatic ring formed with 4 carbons and a nitrogen atom as the main attachment to the side chain. The molecule is small with a molecular weight of 217 Daltons. In contrast, ganoderic acid F is a natural product. Its structure is huge with 7 carbons at the side chain attached to the main skeleton which contains 4 aromatic rings. Its molecular weight is 2.6 times more than captopril. Both compounds have the carboxyl, ketone and methyl functional groups except captopril which, in addition has a sulphate group at the tail. There is no sulphate and nitrogen atom in ganoderic acid F.



Captopril

Figure 5. Comparative molecular structure of captopril as a commercial pharmaceutical drug and ganoderic acid F extracted from *G. lucidum*.

Comparison of the molecular structure cannot really ascertain the effectiveness of both compounds in term of anti-hypertension or indirectly on anti-hypercholesterol activity. In addition, there are many other differences in term of their configurations and binding site of both compounds. Moreover, there could be many other factors influencing the mechanism of RAS system. As the comparison is based on the isolated and characterized *G. lucidum* lanostane triterpenes which have shown ACE-inhibitory activity, this wound provide some hints on the working structure. The effect of captopril on hypertension is rapid but comes with side effect. While ganoderic acid F is a natural product, experimental studies and clinical trials are still needed. The findings from this present review contrasted the conclusion by [8] that the compounds responsible for anti-hypertensive activity have molecular weights of more than 1,000,000 Daltons, based on their in vivo data in Spontaneously Hyoertensive rats.

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3.3. Mechanism of inhibition of oxidative damage

Extracts prepared from either mycelial or fruiting body of *G. lucidum* have been accorded a prominent role as a source of natural antioxidants [57]. The antioxidant activity of these extracts was found mainly correlated with their polysaccharide content as well as with their phenolic content [21]. Several scientific reports [26, 38, 45] have proven that the mechanism involved is direct inhibition of the process of oxidation at the cell membrane of the host. The reactive compounds react directly with the free radicals and neutralize the oxidation effect in reducing oxi LDL which forms the key precursor in cardiovascular diseases included hypercholesterolemia. The physiological effects of these extracts was shown to depend on the strain and the nature of cultivation [65].

(1 → 6) or (1 → 3)-β-D-glucans from *G. lucidum* are reportedly potential drugs against oxidation. These substances seem to enhance the activity of the immune system, but there is no accepted mechanism of action nor agreement on the parameters which influence the activity (Werner *et al*., 1997). Therefore, glucans with different structures and/ or varying molar mass were characterized by spectroscopy, spectrometry coupled with size-exclusion chromatography in order to obtain the molar mass distribution and to gain an idea of the structure in solution. The activity of polysaccharides is determined by their conformation, composition and size [13]. Additionaly, polysaccharides may contribute to the oxidation properties, depending on their molecular structure, the sugar unit and conformation in whole. [78]

Many synthetic chemicals such as phenolic compounds are found to be strong radical scanvengers but they usually have side effects [33]. Most of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities of *G. lucidum* crude extract were compared with those of the well-known antioxidants such as vitamin C. At all concentrations tested, the *G. lucidum* polysaccharides exhibited a dose-dependent DPPH radical-scavenging activity. [19]

3.3.1. The mechanism of scavenging DPPH radicals

Crude hot water extract of *G. lucidum* showed its maximum scavenging activity (94.8%) at 2.5 mg/mL. However a declining tendency was also noted with higher concentrations (till 10 mg/mL). This could be caused by limitation of solubility, and increased hydrogen bonding once the concentration of polysaccharide is increased. [43] reported that the concentration of available hydroxyl groups is responsible for the scavenging ability of polysaccharides. [56] supported the direct relationship between monosaccharide composition and conformation of side chains with scavenging ability of polysaccharides. In this case, the scavenging of DPPH radicals is conducted by the arabinose linked by 1, 4 linkages of the side-chain and glucose linked to the 1,6 glycosidic linkage respectively [57].

3.3.2. Inhibition of Lipid Peroxidation (LPO)

LPO is regarded as one of the basic mechanisms of cellular damage caused by free radicals [68]. The relationship between LPO and hypercholesterolemia is well recognized. A cholesterol rich diet results in increased LPO by the induction of free radical production [3]. Hypercholesterolemia and lipid peroxidation are believed to be critically involved in development of atherosclerosis [9].

G. lucidum extract expressed the same pattern in prevention of linoleic acid peroxidation. In such experiment, the antioxidant activity of *G. ludicum* extract increased from 0.1 to 10.0 mg/mL and reached a plateau of 77.2–77.3% at 10.0–20.0 mg/mL. [56] measured the inhibition of lipid peroxidation by conjugated diene method. They concluded that strong relationship existed between monosaccharide ratio and antioxidant activity. Antioxidant activity increased with increasing concentrations of mannose and rhamnose,whereas the activity decreased with corresponding increases in concentration of arabinose and glucose.

3.3.3. Reducing power

G. lucidum polysaccharide extract exerted a high potential in hydrogen-donating ability in the ferric–reducing antioxidant power assay [57]. This extract showed promising reducing power gradually when the concentration was increased from 0.1 to 0.5 mg/mL and achieved a relative stable level of 3.2–3.4 at 5.0–20.0 mg/mL. The effectiveness of *G. lucidum* extract in reducing power test was obvious when compared to ascorbic acid tested as positive control which had a reducing power of 3.5 at 20.0 mg/mL.

[70] reported xanthan and methylcellulose showed hardly any hydrogen donating activity compared with the very huge activity of ascorbic acid. [56] also reported that weak relationship was found between monosaccharide composition and reducing ability. In fact the non-polysaccharide components of the *Ganoderma* extract play the main role in reducing power. These components could react with free radicals and eventually stabilize and block chain reactions.

3.3.4. Chelating ability on ferrous ions

The molecular masses of the polysaccharide fractions are important for the chelating ability [43]. The ferrous ions chelating ability of polysaccharides extract of *G. lucidum* is 11.0–64.6% at 0.1–20 mg/mL and achieved a maximum of 68.9 % at 10 mg/mL. A mole number of polysaccharide is required to chelate a mole number of ferrous (Fe^{2+}) ions. The absolute chelating power is inversely related with the mean molecular mass, showing that higher molecular weight of polysaccharide exhibits higher chelating ability. However, this rule excludes amylopectine and starch which have no chelating effect despite their higher molecular weight. Therefore glycoside linkage of β-D-glucan exhibited higher ranking in chelating ability compared to α-D-glucan.

3.4. Mechanism of inflammation — Hepatoprotective effect

Total triterpenes extract from *G. lucidum* have been tested on two different experimental liver injury mice models induced by carbon tetrachloride and D-galactosamine [69]. In this test model, the extract showed inhibition of liver triglyceride and serum alanine aminotransferase levels significantly. Such result is encouraging when compared to a known reference substance, malotilate for this form of protective effects. Both superoxide dismutases (SOD) activity and the glutathione content have been antagonized and decreased by *G. lucidum* extract. This

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corresponds to the reduction of malondialdehyde content in the carbon tetrachloride and D-galactosamine liver-injured mice. (Andréia *et al.,* 2013).

These data indicated that peptides and ganoderic acids which have been isolated from *G. lucidum* have a powerful protective effect against liver damage induced by carbon tetrachloride and D-galactosamine. The increased activity of free radical scavenging enzymes could be related to the hepatoprotective effects and, thus, enhancing the effectiveness of anti-oxidation.

However the other experiments revealed administration of *G. lucidum* polysaccharides dose-dependently significantly enhanced antioxidant enzymes activities in the serum of rats fed with polysaccharides compared to model group [73]. Another report reported that rats fed with ergosterol-rich and nicotinic acid-rich extract had significantly higher serum glutathione peroxidase and SOD activities (Andréia, 2013).

The liver is the main organ of detoxification and is the site of metabolic conversion of endogenous and exogenous compounds. Another major function of the liver is to synthesize bile acids from cholesterol and to secrete these compounds from the hepatocytes into the intestine, thereby generating bile flow and facilitating dietary fat emulsification and absorption [82]. The studies on hepatoprotective effect of *G. lucidum* relied on many types of substrates. Since the liver is the main organ of metabolism, many biochemical pathway are related, thus more than one substrates are involved in the respective mechanism.

3.4.1. Mechanism of hepatoprotective effect of Ganoderma extract

Whatever substrates are involved, the basic of this mechanism is promoting the release of SODs into the blood stream. Kurt and Stefan (2014) reported that plasma clearance of human extracellular-superoxide dismutase C (EC-SOD C) in rabbits was initiated in the liver which contained the most 125I-EC-SOD C, followed by kidney, spleen, heart, and lung. This scenario shows that almost all 125I-EC-SOD C in the organs was deposited on endothelial cell surfaces and was not associated with any other tissue cell surfaces, or present within the cells. [47].

Pathology studies on the hepatocytes that the SODs are a family of metalloenzymes which need mineral copper as integral component. About 50–80% copper absorption is maximal in the duodenum and may be absorbed from the stomach. Within the intestinal mucosal cells, copper can react with metallothionein, a sulfhydryl group-rich protein that binds copper through the formation of mercaptide bonds. Factors affecting copper absorption include gender, the chemical form and certain dietary constituents.

The rich and multi nutrient ingredient of ganoderma extract has provided sufficient natural supplement in enhancing the liver metabolism. Besides beta-glucan, coumarin, mannitol, and alkaloids, triterpenes isolated from *G. lucidum* included ganodenic acid which have a molecular structure similar to steroid hormones and ganoderol, ganoderenic acid, ganoderiol, ganodermanontriol, lucidadiol, and ganodermadiol which is believed to stimulate the metabolism of liver to achieve the detoxifying effect. Combination of different substrates and components also provoke the synergic in term of efficacy. It is interesting that phytochemistry profile of *G. lucidum* includes 18 types of amino acids and more than 10 minerals which could be the vital

ingredient of 50−80 % cupper and other minerals absorbed from duodenum and stomach in men.

There are three types of SODs in eukaryotic cells catalysing the same reaction. They are copper and zinc-containing SOD (CuZnSOD) exists in cytosol, an manganese-containing SOD (MnSOD) which is encoded in the nucleus, synthesized in the cytosol and imported post-translationally into the mitochondrial matrix, and an extracellular CuZnSOD.

Basically 90% of the cell's oxygen is consumed by MnSOD at the mitochondria matrix. Thus mitochondria are sensitive to oxidative damage, especially inducible by environment oxidative stress. Most probability due to mitochondria are lack of histones and an efficient DNA repair [12].

The SODs are playing their role in the initial stage of cellular anti-oxidant defense by the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen. Superoxide is the one-electron reduction product of molecular oxygen.

There are two possible equations of SOD-catalysed dismutation of superoxide where the oxidation state of the metal cation oscillates between n and n+1. The half-reactions could be written as :

$$M^{(n+1)+}\text{-SOD} + O_2^- \rightarrow Mn^+\text{-SOD} + O_2$$

$$M^{n+}\text{-SOD} + O_2^- + 2H^+ \rightarrow M^{(n+1)+}\text{-SOD} + H_2O_2$$

The H_2O_2 will be disproportionating to H_2O and O_2 by catalase which is excreted from hepatocytes to complete the oxygen radical detoxification process [12]

The mechanisms of the hepatoprotective effects of *G. lucidum* have been largely undefined. However accumulating evidences suggest most of the identified substrates of *G. lucidum* extract are able to enhance the release of SOD or other anti-oxidant enzymes from the cytosolic and extracellular which have potential hepatoprotective effect. They play their role in modulation of hepatic phase I and II enzymes, modulation of nitric oxide production, and maintenance of hepatocellular calcium homeostasis [81].

3.5. Mechanism on immunostimulation with *Ganoderma* polysaccharides

Many bioactive components in *G. lucidum* are biological response modifiers which stimulate the host's own defense system [54] by evoking favorable immune responses. The cell surface is the *Ganoderma* bioactivity target site in terms of receptors and membrane alternation. In contrast to starch and a number of other naturally occurring polysaccharides, bioactive *Ganoderma* glycans are not degraded into their component sugars in animals or humans. Thus, such fungal polysaccharides are able to produce therapeutic effects. Starch, on the other hand, is decomposed enzymatically into its component sugar, glucose, and can be used as an energy source [37].

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Of great interest is the discovery of β-D-glucan receptors on the surface of a number of white blood cells (leukocytes, monocytes, macrophages, natural killer (NK) cells, and other lymphocytes) in animals and humans [17, 22]. The broad stimulatory effects of *Ganoderma* β-glucans, via transduced cell surface receptors in the immune system, lead to the release of cytokines and lymphokines (cell mediators), such as IL-1, IL-2, IL-4 (interleukins), interferon and TNF (tumor necrosis factor). Many immune parameters are improved, e.g., increase of T-cell functions and antibody production. Potential benefits and low toxicity make *Ganoderma* polysaccharides desirable for boosting the immune system of patients undergoing chemotheraphy, radiation therapy or during recovery from major surgery [17]. Immunomodulatory effects of *Ganoderma* polysaccharides may also be useful in atherosclerosis, hypercholesterolemia and heart failure prevention.

[64] reported activation of T-cell by administering *Ganoderma* polysaccharides orally. Stimulation of cytokines [15] and activation of cytotoxic NK cells [83] by *Ganoderma* polysaccharides were subsequently reported. The major polysaccharide fraction, β-1,3 glucan has been shown to exhibit a wide-based adjuvant stimulatory activity on macrophages and T-cells, leading to IL-1 [39], IL-2 [15, 87], and TNF production [88] which play a role in antitumor immune surveillance [49]. *Ganoderma* has been reported to have some effects on immunofunctions which can stimulate the activity of acid phosphatase and β-glucuronidase of peritoneal macrophages in mice (Liu, 1993). It is also observed that there is a significant increase in plague forming cell and agglutination titer of anti-sheep erythrocytes (SRBC) antibody as well as the activity of γ interferon of mice [52]. The activity of crude *Ganoderma* extract on NK cells was of specific interest. Firstly, the effectiveness of a water soluble *Ganoderma* polysaccharide fraction derived from mycelium was shown to enhance splenic NK activity in normal mice when administered via intraperitoned, intravenous or oral route. The fraction also restored depressed NK cytotoxicity in tumor-bearing mice [83]. Secondly, the capacity of *Ganoderma* polysaccharide in activating macrophages [89] and lastly the polysaccharides markedly enhanced the cytotoxicity of T-lymphocytes [52]. The last two activities are established tumoricidal affector pathways of the host immune system.

3.6. Mechanism — *Ganoderma lanostane*-type triterpenes as potent Farnesoid-X-Receptor (FXR) agonists

[79] reported the application of *in silico* tools for the identification of natural products, namely *Ganoderma lanostane*-type triterpenes as potent FXR agonists. Intriguingly, three lanostanes secondary metabolites from *G. lucidum*, that is, ergosterol peroxide, ganodermanontriol, and ganoderiol F, dose-dependently induced FXR in the low micromolar range in a reporter gene assay.

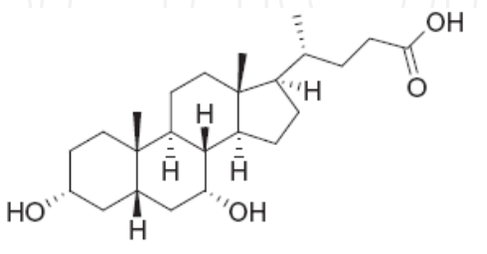
The relevance of FXR as bile acid (BA) activated receptor was illustrated with regard to the treatment of atherosclerosis and its counter-regulatory role in immunity and inflammation. FXR exhibits a regulating function in many endogenous pathways. Its active site contains specific features which are well-characterized. These important characteristics have contributed to the attractiveness of this nuclear receptor as an atypical drugable target for the development of novel therapaeutic agents which may be effective in the prevention and

treatment of, including, the metabolic syndrome, dyslipidemia and atherosclerosis. Chenodeoxycholic acid (CDCA) and other BA are natural ligands for FXR.

A dose-dependent FXR-inducing activity showed the EC_{50} of the most active lanostanes identified from *G. lucidum* activated FXR at even lower concentrations than control, the ranking from the most active is ergosterol peroxide (0.85l M), ganodermanontriol (2.5l M), ganoderiol F (5.0l M) and the control CDCA (16.8 lM). The tested *Ganoderma* compounds significantly decreased the levels of cholesterol 7α- hydroxylase (CYP7A1) mRNA. The degree of inhibition was similar to that induced by the positive control CDCA.

3.6.1. Comparative molecular structure of active lanostanes and CDCA

Four of the compounds have the basic molecular structure with 3 benzene rings and a penta ring, and the side chain is completely different (Figure 6). In term of number of carbon, ergosterol peroxide has the higher number of carbons at the side chain which is 4 carbons more than CDCA. The other two compounds contain same number of carbon. Ergosterol peroxide has 4 methyl groups at the side chain excluding hydroxyl group. The rest contain at least a hydroxyl group at the side chain with the possibility of intermolecular bond forming. Only ergosterol peroxide and ganoderiol F formed double bond at their side chain but at different position. This is important as double bond is favoured in binding and activating the compound. The position of double bond of ergosterol peroxide is ideal compared with ganoderiol F, because there is no other functional group beside the double bond. There is a carbonyl group at the side chain of CDCA compared with others. This interpretation is comparable with the possible mechanism of inhibition of HMG-CoA reductase by ganoderic acid derivatives, as the compound with carboxyl group at the side chain has lower inhibitory potential. In the component of aromatic ring, the position of carbon 7 determines the activity of the compound. In this case, ergosterol peroxide showed double bond within carbon 6 and 7. Ganodermanontriol and ganoderiol F formed double bond at carbon 7 but within carbon 8 and both compounds have another double bond of which the configuration is not as stable as ergosterol peroxide and CDCA. The interesting point is, only ergosterol peroxide showed the binding of oxygen between carbon 5 and 8 which stablises the compound and equivalent the active side of double bond between carbon 6 and 7 in the ring.



chenodeoxycholic acid (CDCA)

Figure 6. Comparative molecular structure of chenodeoxycholic acid and three types of bioactive lanostanes extracted from *G. lucidum*.

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3.6.2. Mechanism

Farnesoid X receptor (FXR; NR1H4) plays a role in the pathogenesis of cardiovascular disease [28]. It is a ligand-induced transcriptional activator and is expressed at high level in liver, intestine, kidney, adrenal glands, and also in the vasculature. It targets the enterohepatic recycling and detoxification of BA. When activated, FXR translocates to the cell nucleus, forms a heterodimer with retinoid-X-receptor and binds to hormone response elements on DNA, which produces either repression or an up regulation of gene transcription. The resulting mechanisms are affected by antagonist or agonist character of the respective ligand [79].

On the other hand, both (*CYP7A1*) and sterol response element binding protein 1c (*SREBP1c*) have shown their expression by elevation of oxysterol-induced liver X receptor-α (LXR-α) activation. This may result in increased triglyceride and BA biosynthesis, whereby the FXR-α-mediated induction of small heterodimer partner (SHP), will interrupt the activity of (LXR-α) to induce *CYP7A1* and *SREBP1c*, and hence inhibits BA and lipogenesis synthesis. Supernumerary, FXR-α-mediated induction of intestinal mouse fibroblast growth factor 15 is a surrogate SHP-independent signal from the gut to the liver to inhibit BA biosynthesis [18].

4. Conclusion

These mechanisms are possibly valid due to the promising results by comparing the treated and the control group. The activity of isolated compounds mentioned herein is very convincing and the working mechanisms mentioned above are involved. However, it is likely more than one mechanism may interplay. The method of extracting the bioactive compounds or the fraction used is closely related to the anti-hypercholesterolemia effect. There are several factors that may influence the possible mechanisms. The effective dosage of *G. lucidum* extract have yet to be standardized in animal and human clinical trial. Although many reports showed the favourable effects of *G. lucidum* extract to patients in China, such reports are anecdotal. In the absence of case-control studies, these reports could not be validated. The duration of administration of *G. lucidum* for its anti-hypercholesterolemia activity is also arguable. The mechanism responsible for the inhibition of cholesterol synthesis is also unclear, as the test subjects were provided with high cholesterol diet during the course of trial which did not reflect the real situation. Hence, to date, the effectiveness of *G. lucidum* on the hypercholesterolemia patients is still undefined. There is a lack of evidence to prove that *G. lucidum* successful dislodge the cholesterol plaque from the affected blood vessels. Whether other mechanisms are also playing a part in cholesterol lowering activity is still unclear. Another compounding factor is the different absorption rate of each test subject. In addition, the effectiveness data of *G. lucidum* acquired from testing in rats could not be applied to human, as the dosage is based on body weight. Moreover, the strain of *Ganoderma* used is also an important factor in determining the rate and type of mechanism involved, since different strain produces different contents in the extract. This becomes more prominent when the mushroom is harvested from different geographical regions.

Synthetic anti-hypercholesterolemia drug works by affecting an array of intermediate precursors which could be important for health. Therefore the side effect of such drug would be more severe than someone taking a mixture of active components present in *G. lucidum* since *G. lucidum* extract most probably exerts its inhibitory effects at the late stage of the pathway.

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