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Research article

Optimization of Lovastatin production under sub-merged fermentation and its effect on cholesterol blood level in rats

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Key words: lovastatin, *Aspergillus fumigates,* hypercholesterolemia, antioxidant enzymes, rats.

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Abstract

Lovastatin was endorsed for the treatment of hypercholesterolemia. In the current work, culture parameters (temperature, initial pH, incubation period, and carbon & nitrogen sources) were optimized for lovastatin production by *Aspergillus fumigatus*. Different concentrations of glucose and peptone had been also examined that enhances lovastatin production by *Aspergillus fumigatus*. The lovastatin yield of (0.869 mg/ml) was obtained at an optimum temperature of 30˚C; pH of 6; glucose concentration 35g/l; peptone concentration 7.769 g/l and at an incubation period of 10 days. Impact of produced lovastatin, had been examined on an experimental model of hypercholesterolemic rats that was established by feeding normolipidemic diet (NLD) supplemented with 4% cholesterol, 1% cholic acid and 0.5% thiouracil, w/w for three months. Current study uncovered that hypercholesterolemic rats recorded a significant elevation in total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and phospholipids (PL), whereas high density lipoprotein cholesterol (HDL-C) was decreased. In comparison the produced lovastatin instigated noteworthy decline in serum TL, PL, TC, TG, LDL-C, VLDL-C levels. On other hand, lovastatin treatment to hypercholesterolemic rats improved the lipid profile in hepatic tissues. In addition, lovastatin treatment expanded hepatic enzyme levels AST and ALT compared to Hypercholesterolemic rats. Meanwhile there was a significant decrease in lipid peroxidation alongside with significant elevation of total antioxidant (TAO) as SOD, CAT and GSH in the liver. Bringing down of these parameters, may in charge of both lipid-bringing down and antioxidative action of lovastatin, which shield liver from hyperchlorestrimia.

Introduction

Hypercholesterolemia is a regular metabolic issue connected with expanded danger for cardiovascular morbidity and mortality [1]. Statins are a class of cholesterol-bringing down medications that hinder the key enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, of the endogenous cholesterol biosynthesis [1]. Among all statins, lovastatin is the initially endorsed by USFDA for the treatment of hypercholes-terolemia in 1987 [2]. Lovastatin is a surely well-known natural statin, which is utilized for bringing down plasma cholesterol levels particularly hindering HMG-CoA reductase, a rate restricting stride of cholesterol biosynthesis [3-4]. On other hand, Kallas Ade *et al.* [5] showed that the lipophilic statin lovastatin diminishes cholesterol synthesis and is a safe and viable treatment for the avoidance of cardiovascular illnesses. Along these lines, Lovastatin is a powerful hypercholesterolemic drug utilized for bringing down blood cholesterol [6]. Lovastatin are fungal secondary metabolites, [7] and it has been accounted to be produced by submerged fermentation (SmF) and solid state fermentation (SSF). Lovastatin is acquired from various genera and types of filamentous fungi. A few fungal genera including *Aspergillus, Penicillium, Monascus, Paecilomyces, Trichoderma, Scopolariopsis, Doratomyces, Phoma, Phythium, Gymnoascus, Hypomyces* and *Pleurotus* are accounted for as lovastatin producers [8-9]. *Aspergillus terreus* is known to be the best lovastatin- producing species [10]. Moreover, even the Basidiomycetes mushrooms have been as of late reported to be utilized for lovastatin production [11]. What's more, marine actinomycete has additionally been accounted as lovastatin producer [12]. The

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clinical utilizations of lovastatin have been all archived [13-14]. For example, diminishing occurrences of fringe vascular, illnesses, avoidance of strokes, stabilization of artheromatous plaques, enhanced endothelial capacities functions and counteractive action of thrombus development [15-16]. Malignancy: The antiproliferative properties of lovastatin might be utilized as a successful anticancer medication [15]. Around 20 %– 55% diminishment in site-particular cancers (colorectal, bosom, prostate, lung and pancreatic) was seen with the utilization of statin therapy [17]. Also Kallas *et al*., [5] focused at the antitumor capability of lovastatin. On other hand lovastatin demonstrated a lessening of Alzheimer's disease level in animal cell culture; while in the human trial does not give a 100% productivity result [18]. Patients, who administrated with lovastatin, had indicated keeping from various sclerosis, furthermore there was a diminished level of inflammatory reaction [19]. Lovastatin likewise impacted the intercellular flagging pathway, which assumes a significant role in cell signal transduction and cell activation. These aides in the counteractive action of kidney harm [19]. Garrett *et al*., [20] contemplated the impact of lovastatin in the bone development; studies were done by infusing the nano lovastatin particles with the high dosage of lovastatin. On other hand, lovastatin stimulated ivigorated bone arrangement both in-vivo and in-vitro, also immunomodulation and altered endothelial capacity by lovastatin may help to decline issues connected with Rheumatoid Arthritis [14]. With its different applications, statins in future may assume a crucial role in medico-pharmaceutical field [15]. In like manner, late studies demonstrated statins in vitro antifungal action against yeasts and filamentous fungi [21]. It is worthwhile to mention that Lovastatin serves as a forerunner for other essential statins as simvastatin and wuxistatin [22]. Lovastatin has been produced commercially through fermentation just [23]. Henceforth, it is vital to quantify the content of lovastatin precisely in the fermentation broth [2]. The aim of the present research was to develop the optimization of lovastatin production by *Aspergillus fumigatus* in parallel with investigation of the effect of the produced crude lovastatin on the level of blood cholesterol in Wistar albino rats.

Experimental

Culture preparations

Lovastatin producing strain used in the present study (*Aspergillus fumigatus*) was isolated and identified in a previous study by the author Mouafi *et al.* [24]. The fungal cultures were kept at 4°C. Transfers were made at 2 weeks intervals. In 250 ml capacity Erlenmeyer flasks 50 ml of the production medium (g/l): dextrose 29.59 g/l, NH_4Cl 3.86 g/l, KH_2PO_4 1.73 g/l, $MgSO_4 \cdot 7H_2O$ 0.86 g/l, and $MnSO_4 \cdot H_2O$ 0.19 g/l [25] was autoclaved, cooled and inoculated with a spore suspension of about $2x10^5$spores/ml from routine subcultures. The flasks were incubated in a rotary shaker incubator at 150 rpm and 30°C, if not otherwise stated.

Optimization of culture conditions

Influence of different cultural parameters on lovastatin production was determined by "one factor at a time" method. Different carbon sources; glucose, dextrose, glycerol and lactose and different nitrogen sources; peptone, yeast, NH_4Cl and urea were supplemented to the basal submerged fermentative medium at equal carbon and nitrogen content, respectively. Different concentrations of glucose (20-40 g/l) and peptone (6.5 – 9.0 g/l) were tested as well. Also the production was determined at various pH (5.5, 6.0; 6.5; 7.0 and 7.5), different temperatures (from 30 to 40°C) and different incubation periods (from 0 to 14 days). For evaluation of a single factor, the other factors were kept constant for all experiments.

Extraction of lovastatin

After completion of the fermentation, the fermentation broth and fungal mycelium were filtered on Whatman No.1 filter paper for separation of fungal cell biomass. Then the filtrate was clarified, centrifuged (at 1500 rpm for 20 min), dried (at 50°C) and extracted with ethyl acetate (pH 3.0) in 250 ml Erlenmeyer's flasks. It was then, incubated at 28°C in a rotary shaker at 200 rpm for 2 h. The supernatant was stored in glass bottles at 4°C until used for further analysis [26].To 1 ml of the supernatant; 1 ml of acetic acid (1%) was added and incubated for 10 min. From the above solution, 1 ml was taken, diluted 10 times with methanol, and its absorbance was read at 238 nm, using UV-Visible spectrophotometer [27].

All the experiments carried out in triplicate and the mean values of the lovastatin yield were reported. The strategy adopted for standardization of fermentation parameters is to evaluate the effect of an individual parameter and incorporate it at the standard level before standardizing the next parameter.

Preparation of lovastatin extraction

The culture was extracted with ethyl-acetate in the same amount. After evaporation lovastatin β-open-hydroxy-acid was obtained [28].

Experimental animals

Twenty-four adult male Wistar albino rats (*Rattus rattus*) with weight of 180–200 g were obtained from the holding company for biological product and vaccines (VACSERA), Cairo, Egypt. The animals were acclimated to laboratory conditions of 20–22°C with a 12-h light/dark cycle for two weeks before experimentation. All rats were fed with a standard pellet diet and water *ad libitum*. Care and use of animals were conducted under supervision of the animal Care Committee of Mansoura University, Egypt.

Experimental design

After 2 weeks of acclimation, the animals were divided into four main groups with six rats each. In group 1, the rats were received standard diet without any treatment. In group 2, the rats supplemented diet with 4% cholesterol, 1% cholic acid and 0.5% thiouracil, w/w for 3months [29]. In group 3, the rats were fed on a chow diet with lovastatin at a dose of 0.4 mg/rat/day orally [30] for 3 months. In group 4, the rats supplemented diets with 4% cholesterol, 1% cholic acid, and 0.5% thiouracil, w/w with lovastatin at a dose of 0.4 mg/rat/day orally for 3 months.

Serum collection and liver homogenate

At the end of the experimental period (3 months), overnight fasted animals were euthanized and sacrificed by cervical dislocation. Trunk blood samples were collected from the sacrificed animals in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 5000 r.p.m. for 20min, and then quickly frozen at 20ºC for further biochemical analysis. Liver samples were rapidly excised, cleaned and weighted, then immediately homogenized in ice-cold saline [0.9% NaCl]. The homogenate was centrifuged at 5000 rpm and the supernatant was used for determination of other biochemical analysis

Biochemical analyses

Aspartate transaminase (AST), alanine transaminase (ALT), kits were purchased from Diamond Diagnostic Company (Dokki, Giza, Egypt). Total lipids, total cholesterol, phospholipids, triglycerides, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c),) were estimated using kits supplied by Bio-diagnostic Company for diagnostic reagents (Dokki, Giza, Egypt). Malondialdehyde (MDA), total antioxidant capacity (TAO), reduced glutathione (GSH, superoxide dismutase (SOD) and catalase (CAT) kits were purchased from Randox Laboratories Co. (UK).

All other chemicals were of analytical grade and were products of BDH Chemicals Ltd. (Poole, England)

Statistical analysis: All results obtained in the present study were evaluated by One-way ANOVA test and post comparison was carried out with Tukey-test. The values were expressed as means ±SE and values of $P< 0.05$ were considered statistically significant [31].

Results and Discussion

Results

Effect of incubation temperature on lovastatin production

So as to assess the impacts of the temperature, lovastatin production was researched in a range from 25°C to 40°C in SmF (Figure 1). A progressive increment in lovastatin production was observed when the incubation temperature was expanded from 25°C to 30°C. The most extreme lovastatin production, 231.50 mg/ml was seen at 30°C. While the production of lovastatin was 0.138, 0.047 and 0.054 mg/ml at temperatures 30, 35 and 40°C respectively.

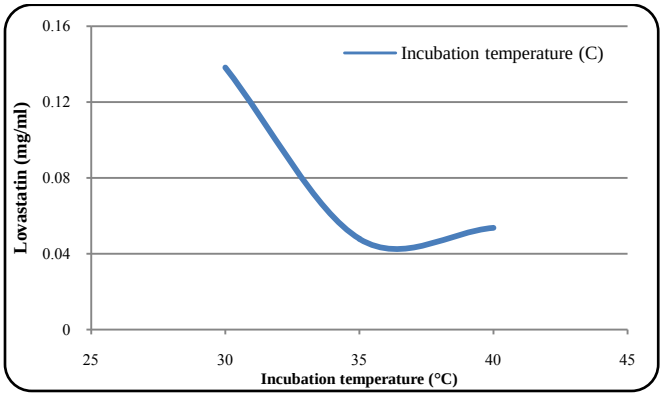


Figure 1. Effect of incubation temperature on lovastatin production.

Effect of initial pH on lovastatin production

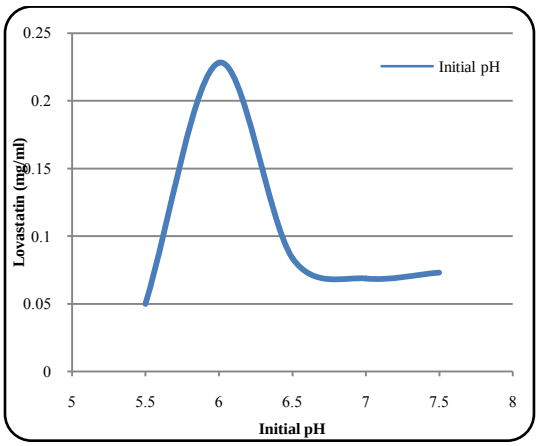


Figure 2. Effect of initial pH on lovastatin production

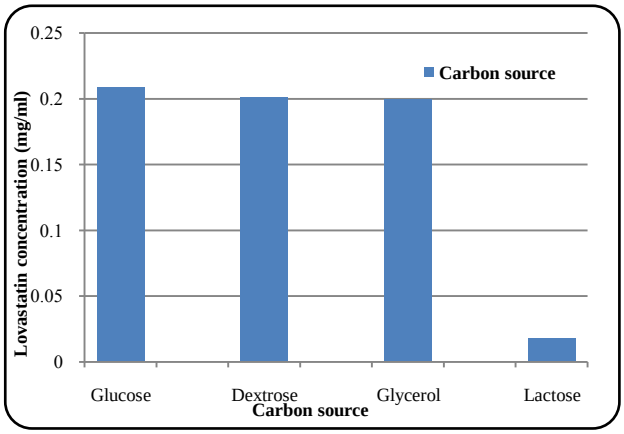


Figure 3. Effect of different carbon sources on lovastatin production

The lovastatin production by *Aspergillus fumigatus* profiles under different pHs are appeared in Figure 2. Lovastatin production was progressively increased in the range of pH

(5.5- 6.0) where maximum lovastatin production (0.228 mg/ml) was gotten at pH 6.0.

Effect of carbon sources on lovastatin production

To decide the impact of carbon sources on lovastatin yield, distinctive carbon sources as glucose, Dextrose, Glycerol and lactose, was supplemented to the basal submerged fermentative medium. Figure 3 exhibited that Glucose upgraded lovastatin yield 0.2090 mg/ml, while lovastatin yield was 0.2010, 0.1997 and 0.01802 mg/ml with dextrose, glycerol and lactose respectively. In spite of lactose addition demonstrated the most reduced yield of lovastatin

Effect of Nitrogen sources

Figure 4 exhibits the impact of nitrogen sources on lovastatin production by *A. fumigatus,* however peptone and yeast extract showed about 0.31837 and 0.30266 mg/ ml. respectively. While lovastin yield was around 0.1381 mg/ ml with urea and NH4Cl displayed around 0.09103 mg/ ml.

Effect of glucose concentration on lovastatin production

Figure 5 indicated that, as glucose concentration increased yield of lovastatin increased. It achieved maximum (0.48 mg/ml) at 3.5% glucose concentration.

Effect of Peptone concentration on lovastatin production

To optimize peptone concentration, different peptone concentrations were tested in the culture medium where maximum lovastatin production (0.39 mg/ml) was at (7.769 g/l) peptone concentration (Figure 6).

Effect of incubation period

Figure 7 showed that Lovastatin production, gradually has, increased up to 9 days and then, apparently, increased to 0.87 (mg/ml) at the tenth day. At the eleventh day, the peak decreased to 0.36 (mg/ml).

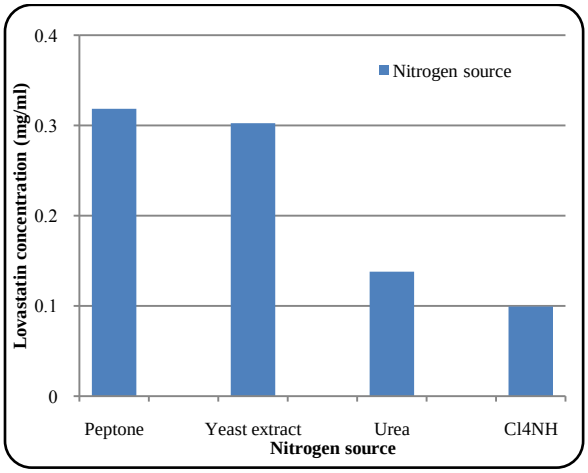


Figure 4. Effect of different nitrogen sources on lovastatin production

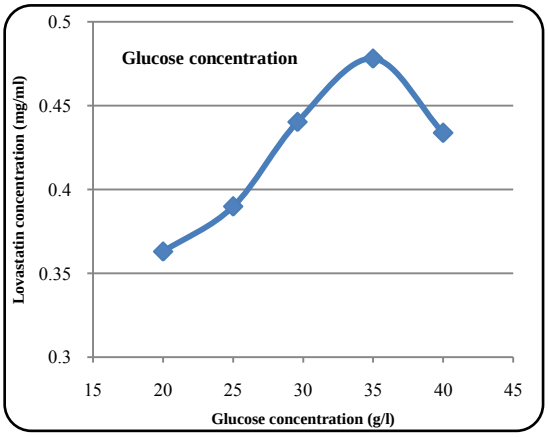


Figure 5. Effect of glucose concentration on lovastatin production

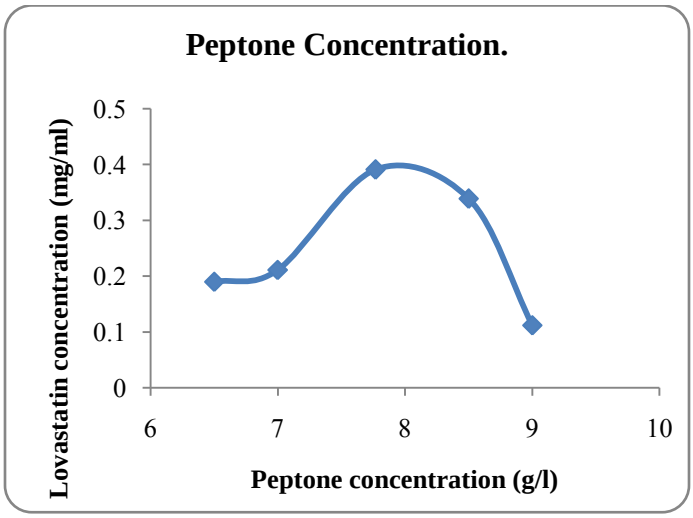


Figure 6. Effect of peptone concentration on lovastatin production

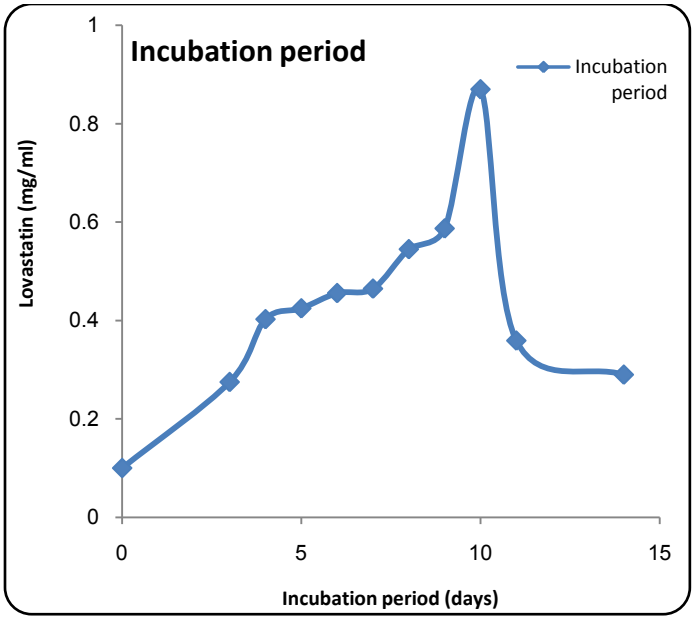


Figure 7. Effect of incubation period on lovastatin production

As shown from Figure 8, administration of lovastatin to normal rats did not produce any significant changes in all tested parameters in comparison to control rats, indicating its non toxic effect at applied dose. However feeding rats on hypercholesterolemic diet revealed a significant increase in serum total lipids, total cholesterol, triglycerides, phospholipids, LDL-C and VLDL-C levels, accompanied with a significant decrease in HDL-C level compared to the control rat group.

Figure 9 exhibited that feeding rats on hypercholesterolemic diet recorded a significant elevation in liver lipid profile (total lipids, total cholesterol, triglycerides and phospholipids).

As shown in Figure 10 Treatment with lovastatin caused an improvement in all parameter if compared to hypercholesterolemic rats group. Additionally, the hypercholesterolemic rats showed a significant increase in the activity of serum AST and ALT, together with a reduction of their activities in liver .

Figure 11 showed the activity of these enzymes was ameliorated significantly with administration of lovastatin. On the other hand, a significant increase in the level of hepatic MDA, with concomitant decreases in the GSH, SOD, CAT and TAC were obtained in hypercholesterolemic -fed rats.

Effect of fungal lovastatin on hypercholesterolemic rats

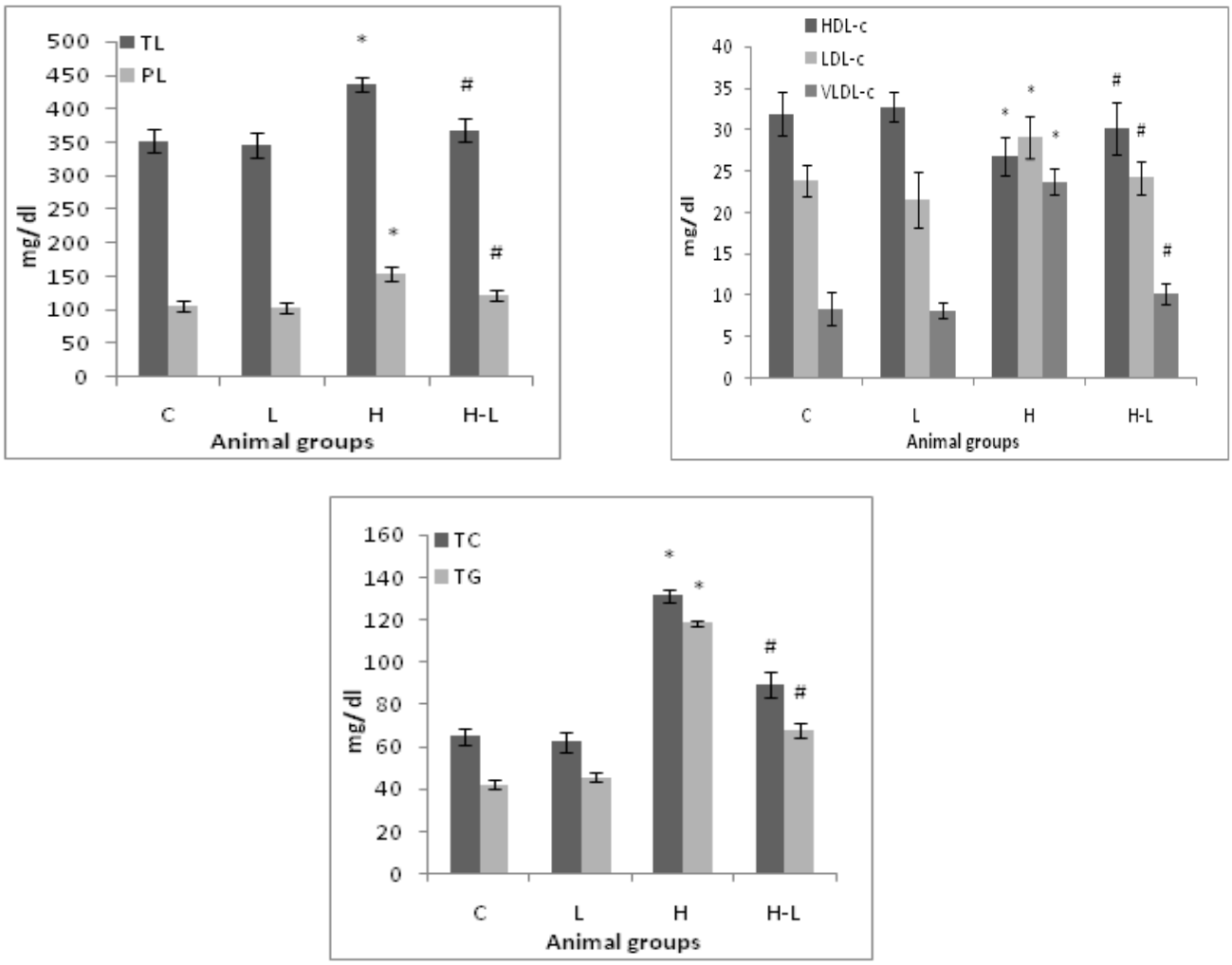


Figure 8. Effect of hypercholesterolemia and lovastatin on the lipid profile [total lipids (TL),phospholipids(PL),total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-c) high-density lipoprotein (HDL-c), and very-low-density lipoprotein (VLDL-c)] expressed as mg/dl in the serum of rats in different groups. The values are expressed as the means ± SEM.(n =6).(*) indicate comparisons with respect to the control group. (#) indicate comparisons with respect to the hypercholesterolimic group. C=control group, L=lovastatin group, H=hypercholesterolemic group and H-L= hypercholesterolemic +lovastatin group.

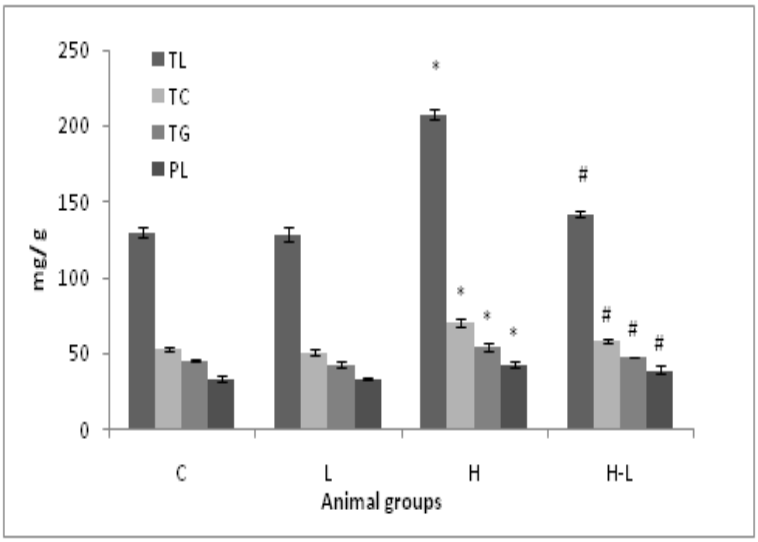


Figure 9. Effect of hypercholesterolemia and lovastatin on the lipid profile [total lipids (TL), total cholesterol (TC),phospholipids(PL) and triglycerides (TG)] expressed as mg/g in the liver of rats in different groups.The values are expressed as the means ± SEM.(n =6).(*) indicate comparisons with respect to the control group. (#) indicate comparisons with respect to the hypercholesterolemic group. C=control group, L=lovastatin group, H=hypercholesterolemic group and H-L= hypercholestereolimic+lovastatin group.

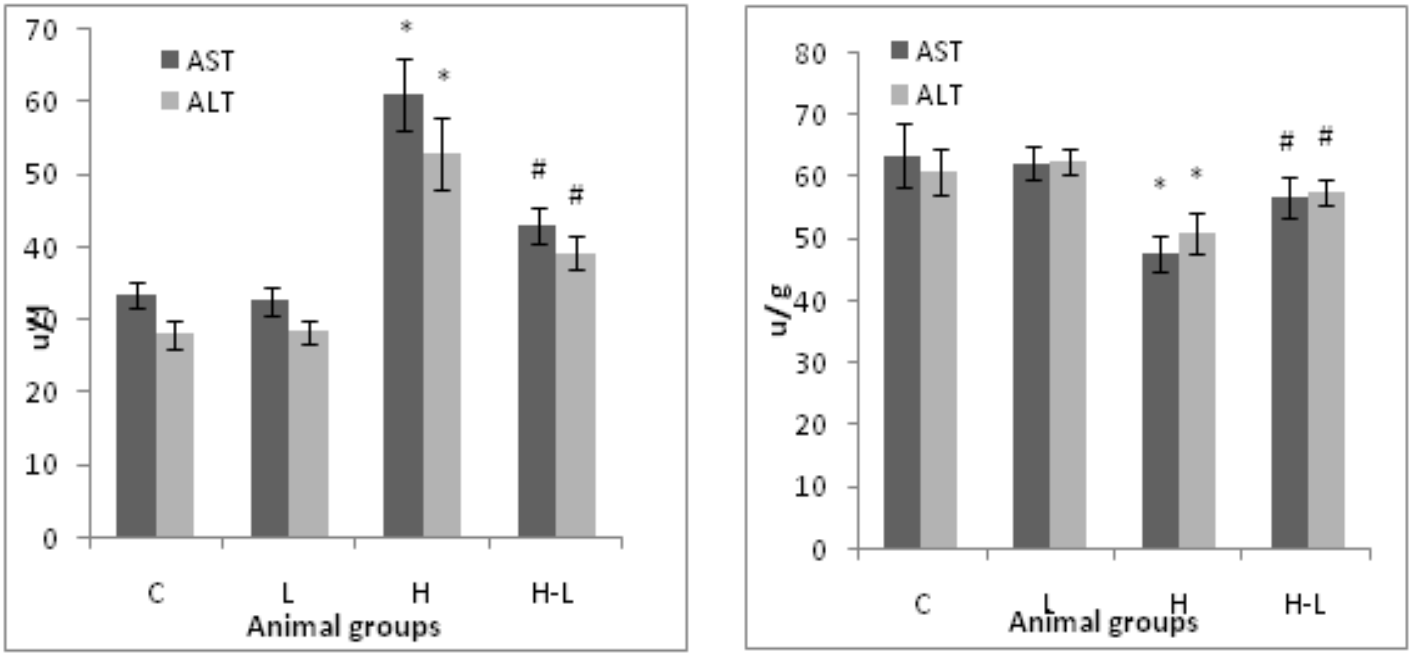


Figure 10. Effect of hypercholesterolemia and lovastatin on the activities of AST and ALT expressed as U/L and U/g in the serum and the liver of rats in different groups. The values are expressed as the means ± SEM. (n =6). (*) indicate comparisons with respect to the control group. (#) Indicate comparisons with respect to the hypercholesterolemic group. C=control group, L=lovastatin group, H=hypercholesterolemic group and H-L= hypercholesterolimic+lovastatin group.

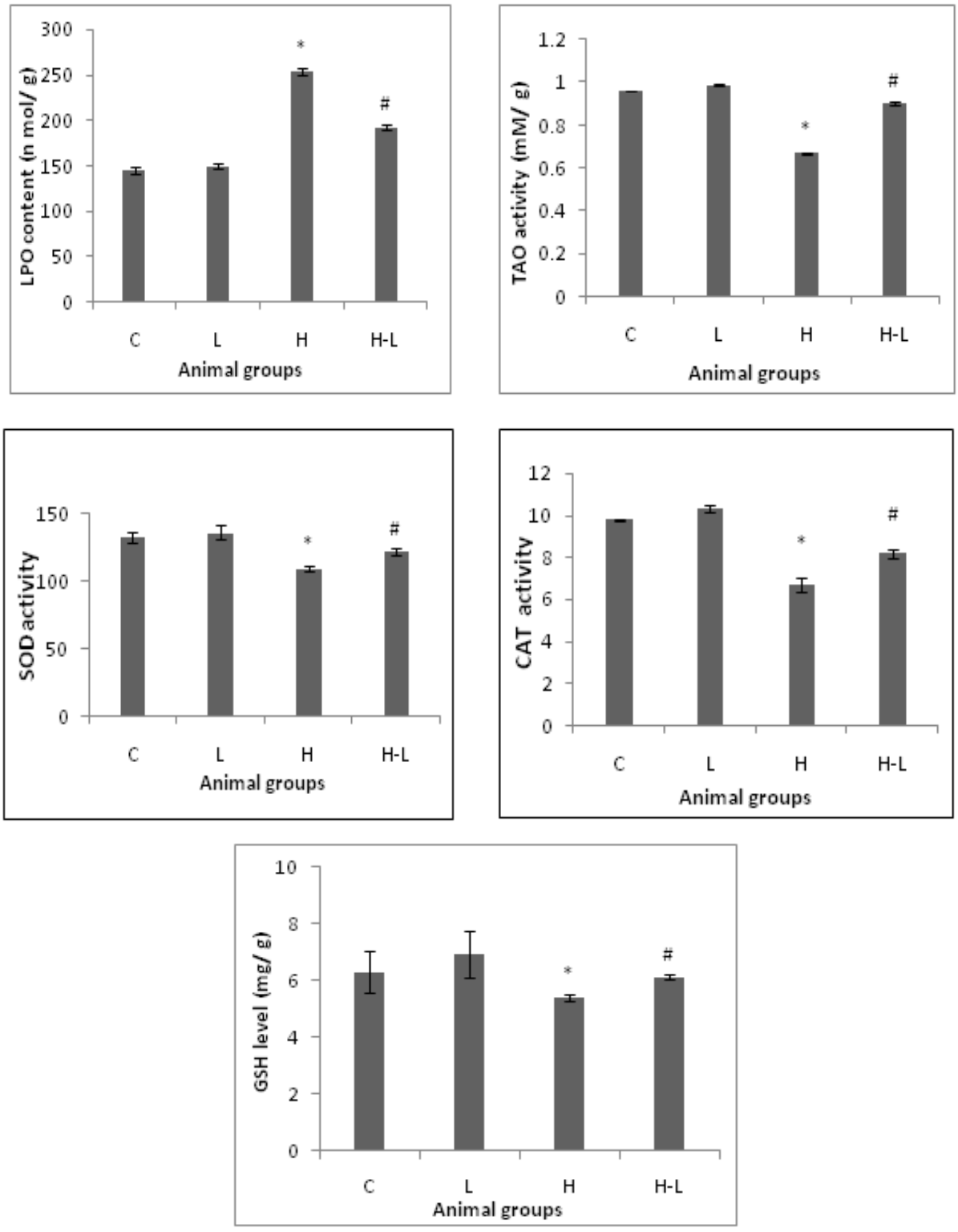


Figure 11. Effect of hypercholesterolemia and lovastatin on the on the levels of MDA (nmol/g), TAO (mM/g), SOD (U/g), CAT (U/g) and GSH(mg/g) in the liver in the different animal groups. The values are expressed as the means ± SEM.(n =6).(*) indicate comparisons with respect to the control group. (#) indicate comparisons with respect to the hypercholesterolemic group. C=control group, L=lovastatin group, H=hypercholesterolemic group and H-L= hypercholesterolemic+lovastatin group.

Discussion:

According to Figure (1) maximum lovastatin production was observed when the incubation temperature was expanded from 25°C to 30°C. it may be because of this temperature is best for the sporulation, growth and proliferation of mycelial mass for lovastatin production .This obtained result is as per [32-34] Additionally it is firmly related to the work of Praveen *et al*., [10] and Atalla *et al*., [34]. Lovastatin production was gradually diminished with an expansion of temperature from 30°C to 40°C. While further increment or diminishing in temperature lead to poor production of lovastatin. On opposite the optimum temp of lovastatin production for another species was 25°C [35].Thus, temperature was surely appeared to be an important environmental factor that enhances lovastatin titers, likely by inducing genes or activating enzymes involved in lovastatin production [32]. Besides the temperature shift might be strain and/or fermentation mode subordinate and may be entirely related to the physiological age of the organism [13].

According to Figure 2 Lovastatin production reached its maximum production at pH 6. Where most of the fungi grow become effectively at acidic pH which is fundamental for transport of various components across the cell membrane thus along these lines firmly unequivocally affecting the development of secondary metabolites. This outcome is as per [6, 10, 36]. At that point it was diminished by expanding the underlying pH above 6.0. On contrary Bizukojc *et al*., [37] reported that neutral and alkaline pH values give preferable lovastatin titers over acidic ones.

Figure 3 portrayed that Glucose upgraded lovastatin yield to respect 0.2090 mg/ml when compared to other carbon sources, where lovastatin yield was 0.2010, 0.1997 and 0.01802 mg/ml with dextrose, glycerol and lactose respectively. This result is as per [38]. In spite of lactose addition demonstrated the most reduced yield of lovastatin Figure.1 as lactose is slowly metabolized carbon source, this result is in accordance with Praveen *et al*., [10]. On contrary Chanakya *et al*. [36] found that addition of lactose has a positive effect on lovastatin production.

The results obtained with different nitrogen sources (Figure 4) indicated that however peptone and yeast extract showed the most extreme lovastatin yield these results are in accordance with [36-39]. On other hand urea displayed around 0.1381 mg/ml lovastatin yield, and NH_4Cl demonstrated the most reduced lovastatin yield. In perspective of these outcomes, biosynthesis of lovastatin has been found to rely on the carbon and nitrogen sources [40]. More over as lovastatin does not contain any nitrogen in its structure ($C_{24}H_{36}O_5$), lovastatin production is connected with the stationary phase of nitrogen restricted development when overabundance carbon can be diverted into secondary metabolism [13]. Where, the growth has been arrested and lovastatin production might be repressed by nitrogen limitation [35].

It has been accounted for that the nature and concentration of the carbon source manages lovastatin biosynthesis [40,41]. So far, another experiment was completed to advance glucose concentration of (20 – 40 g/l) (Figure 5). Where yield of lovastatin was achieved maximum (0.48 mg/ml) at 3.5% glucose concentration. This result is closely related with [35] as glucose is an accessible soluble monosaccharide gets metabolized so act as a promptly wellspring of energy.

The N-source must not be high but rather in the meantime not very low, which may likewise disable lovastatin production [8]. Therefore, optimization of peptone concentration was carried out where maximum lovastatin production (0.39 mg/ml) was at (7.769 g/l) peptone concentration (figure 6).

Lovastatin production, gradually, increased to a maximum yield 0.87 (mg/ml) at the tenth day (Figure 7) while at the eleventh day, the peak decreased to 0.36 (mg/ml).The decrease of the yield after ten days maybe due to onset of death phase of the organism due to depletion of nutrients and accumulation of toxins. This result is in accordance with Pei-Lian and co-workers 2007 [42] who reported maximum lovastatin yield at 11 days with *Aspergillus terreus*. On other hand Casas *et al*., [40] found that the maximum lovastatin yield accomplished on day 7, after which the yield was gradually diminished.

The utilization of excessive calories and eating diet containing unsaturated fats and cholesterol prompts hypercholesterolemia for human or animals [43]. Hypercholesterolemia prompts oxidative stress, which is known to have adverse effects on the integrity of cells [44]. Lovastatin is a vital compound to overcome the hypercholesterolemia disease since its action could hinder the hydroxymethylglutaril-coenzyme A (HMG-CoA) reductase, which catalyzes the rate-limiting step in cholesterol biosynthesis [32-45].

In the present study Figure 8 demonstrated that, feeding rats on hypercholesterolemic diet showed a significant increase in serum TL, PL,TC, TG, LDL-C and VLDL-C levels accompanied with a significant decrease in HDL-C level compared to control group. On other hand, there was a huge increment in hepatic TL, TC, TG and PL levels as shown in Figure 9. These findings were in the same line as with those outcomes reported by [46, 47]. Also oxidation of LDL is a lipid peroxidation process bringing about arrangement of an extensive variety of naturally active products, including peroxides and malondialdehyde. oxidation of cholesterol fractions (specifically, LDL) has been acknowledged as an imperative part in the atherosclerotic process [48]. In addition the oxidatively lipids and their debasement are believed to have pro inflammatory genius incendiary, immunogenic, and cytotoxic properties which add to both the initiation and progression of atherosclerotic sores [49]. Fungal lovastatin from *A. fumigatus* could essentially diminish serum cholesterol, triglycerides and LDL when contrasted with hypercholesterolemic rats, while HDL level

was expanded in treated group. This outcome is in concurrence with [30]. Whereas, Lovastatin blocked cholesterol biosynthesis. An attractive characteristic of these inhibitors is that they selectively reduce levels of low-density-lipoprotein LDL (bad lipoproteins). These results are in agreement with Kusmana *et al*., [30] who also found that the lovastatin extract produced by *Aspergillus flavus* diminished total cholesterol, triglycerides and LDL contents. Additionally it was suggested that LDL contents decreased, while, levels of high-density lipoprotein HDL (good lipoproteins) stay unaffected where, HDL cholesterol can anticipate atherosclerotic disease [50], since it serves as a transporter of overabundance carrier of excess cholesterol from cells to the liver [14-30]. The present work uncovers that capability of lovastatin to standardize the lipid and antioxidant profiles, elevated cholesterol fed rats, while cholesterol- fed rats delivered liver harm as demonstrated by checked rise in serum hepatic enzyme levels AST and ALT (Figure 10). These results are in agreement with Arhan *et al.,* [51]. On other hand the obtained results indicated that rats fed cholesterol –enriched diet exhibited significant decrease in the activity of hepatic GSH, SOD and CAT accompanied by significant increment in hepatic lipid oxidation (as measured by malondialdehyde (MDA) content) in liver of rats. Additionally Lovastatin treatment for these rats diminished tissue lipid peroxidation alongside fundamentally lifted of antioxidant enzyme activities SOD and CAT and non-enzymatic antioxidant GSH (Figure 11). These outcomes are in concurrence with Sanjay *et al.,* [52]. In like manner Cooper and Kristal [53] reported that, GSH assumes a pivotal guarded role against oxidative put-down as an endogenous scavenger of free radicals. Likewise, its level in the blood is a touchy pointer of antioxidant status in circulation [54]. These results are accompanied by significant increment in hepatic lipid oxidation as the level of malondialdehyde (MDA) in liver of rats (Figure 11) that considered as a biomarker of lipid peroxidation [55]. On other hand, Arhan *et al*., [51] observed that cholesterol-induced steatosis leads to a weakened antioxidant defense system and caused peroxidation in the hepatic tissue. What's more, oxidative anxiety prompts a debilitated cancer prevention agent so enhances lipid peroxidation and free radical generation era [44]. The present examination demonstrates that lovastatin enhances the lipid and antioxidant profiles of hypercholesterolemic rats showing its antihyperlipidemic, antiperoxidative and antioxidant properties (Figure 11). As superoxide (O^{-2}), one of the parental types of intracellular ROS, is an extremely responsive molecule that can be changed over to H_2O_2 by superoxide dismutase (SOD) then to oxygen and water by a several enzymes include catalase (CAT) [56]. In spite of administration of lovastatin to those animals succeeded to induce a significant improvement in all these parameters through overcoming the oxidative stress. (Figure 8-11), it is noteworthy that administration of lovastatin to normal rats did not produce any toxic effect at applied dose (0.4mg/rat/day orally) for 3 months.

Conclusion

In the present study, the point was to explore the impact of various environmental and physiological parameters (the temperature, initial pH, incubation period, carbon and nitrogen sources) so as to establish optimum growth conditions for the maximum production of lovastatin by the *A. fumigatus* under submerged fermentation. In like way, to assess the viability to investigate hypolipidemic, free radical and antioxidative activity of the produced lovastatin on hypercholesterolemic exploratory rats. As such, to evaluate the adequacy of advanced lovastatin, through examining its effect on TL, PL, TC, TG, HDL, LDL, VLDL, AST, ALT, LPO, GSH and TAO levels on rats. In this manner, Lovastatins may be a specialist key to control or deal with numerous real diseases later on.

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References

1. Csaba C. *et al.*: Modulation of Hypercholesterolemia-Induced Oxidative/Nitrative Stress in the Heart. Oxidative Medicine and Cellular Longevity 2016; Article ID 3863726, 23 pages.
2. Seenivasana A, Sathyanarayana N. Gummadib, Tapobrata P. and Thomas T: Quantification of lovastatin produced by Monascus purpureus. The Open Biotechnology Journal 2015; 9:6-13.
3. Mishal S, Rani Faryal and Ian M: Exploitation of Aspergillus terreus for the production of Natural Statins J. Fungi 2016; 2:1- 13.
4. Rashmi D., Padmavathi T: Statistical optimization of lovastatin and confirmation of nonexistence of citrinin under solid-state fermentation by Monascus sanguineus. Journal of food and drug analysis 2016; 24:430 - 440.
5. Kallas K., Annika T. and Toivo M: Lovastatin Decreases the Expression of CD133 and Influences the Differentiation Potential of Human Embryonic Stem Cells 2016; Article ID 1580701, 16 pages.
6. Prasanna Latha D and Hemalatha K: Production of lovastatin by Aspergillus fischeri NCIM 509 using barley bran, wheat husk, rice bran and rice husk under solid state fermentation. European Journal of Experimental Biology 2015; 5(8):8-17.
7. Praveen VK, Savitha J: Solid state fermentation: an effective method for lovastatin production by fungi over submerged fermentation. E3 Journal of Biotech Pharma Res 2012; 3(2):15-21.
8. Bizukojc M, Ledakowicz S: A macrokinetic modelling of the biosynthesis of lovastatin by Aspergillus terreus. J Biotechnol 2007; 130(4):422–35.
9. Upendra RS, Khandelwal Pratima, Amiri ZR, Shwetha L, Ausim M: Screening and molecular characterization of natural fungal isolates producing lovastatin. Microbial Biochem Tech 2013; 5(2):025-30.
10. Praveen V, *et al.*: Optimization of Culture Conditions for Maximal Lovastatin Production by Aspergillus terreus (KM017963) under Solid State Fermentation. Journal of Bioscience 2016; 22:174-180.
11. Burcu A, Yamaç Mustafa, Zeki Y, Isikhuemhen Omoanghe S: Statistical optimization of lovastatin production by Omphalotus olearius (DC.) singer in submerged fermentation. Preparative Biochemistry & Biotechnology 2016; 46(3):254-260.
12. Srinu M., *et al.*: Screening of HMG CoA reduc-tase inhibitor producing marine actinomycetes. J. Pharm. Sci. Health 2010; 2(1):66-74.

13. Kelly CL M.,*et al.*: Lovastatin production: From molecular basis to industrial process optimization. Biotechnology Advances 2015; 33:648–665.
14. Sreenivasan A, Shubahgar S, Arvindan R, Viruthagiri T: Microbial production and biomedical applications of lovastatin. Indian J Pharm Sci. 2008; 70:701-709.
15. Tandon V, Bano, G, Khajuria V, Parihar A, Gupta S: Pleiotropic effects of statins. Ind J Pharmacol 2005; 37:77-85.
16. Barrios GJ, Miranda RU: Biotechnological production and applications of statins. Appl. Microbiol. Biotech. 2010; 85:869-883.
17. Glynn SA, O'Sullivan D, Eustace AJ, Clynes M, O'Donovan N: The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and in- vasion of melanoma cells. BMC Cancer 2008; 16:8-9.
18. Carrie, *et al.*: A randomized placebo-controlled lovastatin trial for neurobehavioral function in neurofibromatosis. Annals of Clinical and Translational Neurology 2016; 3(4):266–279.
19. Radha KV, Lakshmanan D: A review: lovastatin production and applications. Asian J Pharma Clincal Res 2013; 6(3):21-26.
20. Garrett IR., *et al.*: Locally delivered lovastatin nanoparticles enhance fracture healing in rats. J. Ortho. Res. 2007; 25(10):1351-1357.
21. László Galgóczy, Ildikó Nyilasi, Tamás Papp, Csaba V: Statins as antifungal agents. World J Clin Infect Dis 2011; 1(1):4-10.
22. Zhuge B, Fang HY, Yu H, *et al.*: Bioconversion of lovastatin to a novel statin by Amycolatopsis sp. Appl Microbiol Biotechnol 2008; 79: 209-216.
23. Nigovi B, Pavkovi I: Preconcentration of the lipid-lowering drug lovastatin at a hanging mercury drop electrode surface. J Anal Chem 2009; 64:304-309.
24. Mouafi, F. E., Ghada S.I. and Mostafa M. A: Screening and optimization of Lovastatin Production by isolated Aspergillus fumigatus under solid state fermentation. Journal of Genetic Engineering and Biotechnology (accepted 17 October) 2016; Number: JGEB-D-15-00058
25. Sayyad S. A., Bibhu P. P., Saleem J. and Mohd A: Optimization of nutrient parameters for lovastatin production by Monascus purpureus MTCC 369 under submerged fermentation using response surface methodology. Appl Microbiol Biotechnol 2007; 73:1054–1058.
26. Raghunath R., Radhakrishna A., Angayarkanni J., Palaniswamy M.: Production and cytotoxicity studies of Lovastatin from Aspergillus niger PN2 an endophytic fungi isolated from Taxus baccata. IJABPT 2012; 3:562-570.
27. Mielcarek J., Naskreni M., Grobelny P: Photochemical properties of simvastatin and lovastatin by radiation. J Thermal Analysis Calorimetry 2009; 96:301-305.
28. Ouda SM, *et al.*: Optimization of Different Parameters on Lovastatin Production by Aspergillus niger I4 and Aspergillus terreus I8 and its Hypocholesterolemic Effect. R. J. Ph. Biol. and Ch. Sci. 2015; 6(5):1439-1448.
29. EL-Wakf, M.A., Ibrahim, H.A., Hassan, H.A., Serag, H.M., Gumaih, H.S: Favorable Alteration Of Cardiovascular Disorders With Virgin Olive Oil Compared To Refined Olive Oil In Atherosclerotic Male Rats. J. Egypt. Ger. Soc. Zool. 2010; 61(A):25-47.
30. Kusmana D, Raflianti Y, Mangunwardoyo, W: The Effect of Lovastatin Produced from Aspergillus flavus UICC 360 on Decreasing Cholesterol Level in Blood of Rattusnovergicus L. Sprague Dawley strain. Int. J. Sci. Rese. Publications 2012; 2:1-5.
31. Snedecor, G. W. and Cochran, W. G: Statistical Methods. 1982; 7th ed. The State University Press American, Iowa.
32. Lai LS, Tsai TH, *et al.*: The influence of culturing environments on lovastatin production by Aspergillus terreus in submerged cultures. Enzyme Microb. Technol. 2005; 36:737–748.
33. Osman ME, Khattab OH, Zaghlol GM, El-Hameed RM: Screening for the production of cholesterol lowering drugs (lovastatin) by some fungi. Aus J Basic Appl Sci 2011; 5:698-703.
34. Atalla MM, Hamed ER and El-Shami AR: Optimization of a culture medium for increased mevinolin production by Aspergillus terreus strain. Malaysian J. Microbiol 2008; 4: 6-10.
35. Jahromi MF, Liang JB, Ho YW, Mohamad R, Goh YM, Shokryazdan P: Lovastatin production by Aspergillus terreus using agro-biomass as substrate in solid state fermentation. J Biomed Biotechnol. 2012; 1–11.
36. Chanakya P, Latha PM, Manipati Srikanth M: Solid state fermentation for the production of lovastatin by Aspergillus fischerii. Res J Pharm Sci Biotech 2011; 1: 9-13.
37. Bizukojc M, Pawlak M, Boruta T, Gonciarz J: Effect of pH on biosynthesis of lovastatin and other secondary metabolites by Aspergillus terreus ATCC 20542. J Biotechnol 2012; 162(2–3):253–261.
38. Hajjaj H, Niederberger P, Duboc P: Lovastatin biosynthesis by Aspergillus terreus in a chemically defined medium. Appl Environ Microbiol 2001; 67(2):596–602.
39. Xu B, Wang Q, Jia X, Sung C: Enhanced lovastatin production by solid state fermentation of Monascus ruber. Biotechnol Biochem Engg 2005; 10:78-84.
40. Casas JL, *et al.*: Production of lovastatin by Aspergillus terreus: effects of the C:N ratio and the principal nutrients on growth and metabolite production. Enzyme and Microbial Technology 2003; 33:270–277.
41. Jia Z, Zhang X, Zhao Y, Cao X: Enhancement of lovastatin production by supplementing polyketide antibiotics to the submerged culture of Aspergillus terreus. Appl Biochem Biotech 2010; 160:2014-2025.
42. Pie-Lian WEI, Zhi-nan XU, and Pei-Lin CEN: Lovastatin production by Aspergillus terreus in Solid-State fermentation. J Zheijan Univ 2007; 9: 1521-1526.
43. Kabiri N, Asgary S, Setork M: Lipid lowering by hydroalcoholic extracts of Amaranthus Caudatus L. induces regression of rabbits atherosclerotic lesions. Lipids in Health and Disease 2011; 10:89.
44. Stapleton PA, Good Will AG, James, ME, Brock RW, Frisbee JC: Hypercholesterolemia and microvascular dysfunction: interventional strategies. Journal of inflammation 2010; 47:54.
45. Raghunath, P M: Study on anti-cholesterolemic activity of lovastatin based on a mice model. International Journal of Comprehensive Research in Biology Sciences 2015; 2(1):41-47.
46. Frantz E, Menezes HS, Lange KC, Abegg MP, Correa CA, Zangalli L, Vieita JL, Zetter CG: The effect of maternal hypercholesterolemia on the placenta and fetal arteries in rabbits. Acta Cirurgica Brasileira 2012; 27(1):7-12.
47. Fyiad AA, El-Sayed ST: Effect of Allium Sativum Extract on Serum Lipid and Antioxidant Status in hypercholesterolemic Rabbits. Life Science Journal 2012; 9(3):187-196.
48. Durak I, *et al.*: Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. J. Nutr. Biochem. 2004; 15:373– 377.
49. Sezer ED, Sozmen EY, Nart D, Onat T: Effect of atorvastatin therapy on oxidant- antioxidant status and atherosclerotic plaque formation. Vascular Health and Risk Mangment 2011; 7:333-343.
50. Daniels TF, Killinger KM, Michal JJ, *et al.*: Lipoproteins, cholesterol homeostasis and cardiac health. Int. J. Biol. Sci. 2009; 5(5):474-488.
51. Arhan M, Öztürk HS, Turhan N, Aytac B, Güven MC, Olcay E, Durak I: Hepatic oxidant/antioxidant status in cholesterol- fed rabbits: effects of garlic extract. Hepatology Research 2009; 39:70-77.
52. Sanjay S., Sanjay B., Rupal A., Narasimhacharya A: Lovastatin improves fluoride-induced hypercholesterolemia in Albino Rats. Research report Fluoride 2014; 47(1)69–77.
53. Cooper AJL, Kristal BS: Multiple roles of glutathione in the central nervous system. Biol Chem 1997; 378:793-802.
54. Piemonte, F. *et al.*: Glutathione in blood of patients with Friedreich´s ataxia. Eur J Clin Invest 2001; 31:1007-1011.
55. Lykkesfeldt J: Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin. Chim. Acta. 2007; 380:50–58.
56. Kumar P, *et al.*: Simultaneous estimation of statins like pravastain, atorvastatin and simvastatin in bulk and pharmaceutical dosage form by means of High-Performance Liquid Chromatography. Journal of Chemical and Pharmaceutical Research 2013; 5(5):359-364.