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Original Research Article

Hypolipidaemic and renoprotective effects of *Glycine max* (Soy bean) against lipid profile and renal biochemical alterations in hypercholesterolemic rat

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Abstract

Background: Hyperlipidemia can be defined as concentration of lipids in the blood of a fasted (>12hrs) patient that exceeds the upper range of normal for that species. Studies have showed that rats with hypercholesterolemia are prompt to the development of cardiovascular diseases (CVD).

Methods: Twenty-four (24) male albino Wister rats were used in this study. The rats weighed between 180-190g and were randomly divided into four (4) groups (A-D) with six (6) rats per group. For hypercholesterolemia induction, rats in group B, C and D were administered with HCD (2000mg/kg) once daily in the presence of carbimazole (1.35mg/kg) for 14days. Group B (test group) were treated with soymilk (2000mg/kg) and group C (positive control) with atorvastatin (20mg/kg) for 14 days. Group A (normal control) received water and vital feed only. At the end, fasting blood specimens were collected from all the animals in all the groups for estimation of lipid profile and renal biochemical parameters. The biochemical parameters of lipid profile: Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and Triglycerides (TG) were assayed.

Results: Results showed that the test group (soymilk) showed a non-significant decrease in TC, TG, LDL, VLDL and a non-significant increase in HDL concentrations but not as much as group C (Atorvastatin) which showed a significant decrease in TC and LDL ($p<0.01$), TG ($p<0.05$), VLDL and also a significant increase in HDL concentrations ($p<0.05$) when compared to the negative control group (HCD alone).Renal injury was assessed by measuring serum Na^+, K^+, Cl^-, HCO_3^-, creatinine and urea levels. The HCD treatment resulted in marked elevation of: K^+ (8.67±0.77mmol/l); creatinine (2.07±0.23mg/dl); and urea (89.00±14.42mg/dl) and caused deranged renal functions which were significantly improved by soymilk treatment: K^+ (6.51±0.34mmol/l); creatinine (1.03±0.24mg/dl); and urea (46.67±6.89mg/dl) with [$P<0.05$ or $p<0.01$].

Conclusion: Arguably, the phytochemicals in soy prevented dyslipidaemia and protected the kidney against the oxidative stress and the resultant renal dysfunction produced by hypercholesterolemia.

Keywords: High cholesterol diet (HCD), Cholesterol, triglyceride, creatinine, Wister rats, lipids, sodium.

1. Introduction

Connection between nutrition and health has been probably understood, at least to some extent, among all people of all places and times. For instance; around 400BC the statement "Let food be your medicine and let medicine be your food was advised by the father of medicine, Hippocrates, over two millennia ago. In 1968, one of the great minds of this century, twice Noble prize winner, Linus Pauling, coined the term *Orthomolecular Nutrition.* Orthomolecular is literally, "pertaining to the right molecule". Pauling proposes that by giving the body the right molecules (optimum nutrition) most diseases would be eradicated [1]. Of course, drugs can save the life of an ill patient, as can surgery and the other techniques at which doctors are so expert. But the paradigm is changing.

A doctor in Dublin recently said, "The evidence for nutritional therapy is becoming so strong that if the doctors of today don't become nutritionists, the nutritionist will become the doctors of tomorrow" [1]. In addition, foods like Soy bean *(Glycine max)* has been reported to have useful therapeutic effects on heart and kidney diseases [2].

The soy bean *(Glycine max)* is a legume native to the East Asia, widely grown for its edible bean which has numerous uses. The pods, stems and leaves are covered with fine brown or gray hairs. The fruit is hairy pod that grows in clusters of three to five, and contains two or four seeds. The bean is mostly consumed in the forms of *soybean milk*, a whitish liquid suspension, and *tofu*, a curd resembling cottage cheese. It is said to have antioxidant activity and chemical compound such as polyphenolics and flavonoids which protect the human tissue from free radicals released from organs, thereby reducing oxidative stress [3].

The protein gotten from legumes like soya beans helps to regulate blood sugar, body fluid, kidney, liver function, adrenaline and other aspects of metabolism [4]. In the last few decades, extensive efforts have been made towards identifying bioactive components in soy foods that are responsible for the health benefits. Among them, isoflavones and soy protein are the two major groups of components that have received the most attention [5, 6]. The dramatic increase in Soy food sales is largely credited to the Food and Drug Administration's (FDA) approval of Soy as a cholesterol-lowering food, along with other heart and health benefits.

Hyperlipidemia is a term used to describe raised serum levels of one or more of total cholesterol (TC), low-density lipoprotein cholesterol (LDL) or both. Hyperlipidemia can also be defined as concentration of lipid in the blood of a fasted (>12hrs) patient that exceeds the upper range of normal for that species. These fats are important for our bodies to function but when they are high, they can cause heart disease and stroke. Hyperlipidemia can also be hereditary. High-density lipoprotein cholesterol (HDL-C), "good cholesterol", however, confers protection. The elevation of serum TC and more importantly LDL have been implicated as primary risk factors for cardiovascular diseases like hypertension and atherosclerosis. Generally cardiovascular disease rises as the ratio of TC to HDL-C rises [7, 8].

Lipid profile contains information about several different kinds of lipid that normally circulate in the blood. Many studies have shown that serum lipid concentrations and lipid metabolisms can be greatly affected by consuming legumes; particularly soy protein [9, 10].*Glycine max* has been reported to contain important nutritive substances such as Fibre, Vitamin, carbohydrate, Fat, and Amino acids. Phytochemical content of *Glycine max* include Isoflavon, Saponin, phytic, genistein. The intake of Isoflavon, and genistein tends to decrease nephropathy risk.

Renal disease known as nephropathy or renal insufficiency is a medical condition in which kidney fails to adequately filter waste products from the blood. Most kidney disease attack the nephron, causes can include generic problems like injuries, medicines or .increase in cholesterol levels. There are few literatures or researches on the cardio-protective and/or renoprotective effect of Soy bean in rats. Furthermore, there is need for further researches to unravel more health benefits via the consumption of soybean on the heart and kidney to limit the burden of heart or kidney diseases on patients and the health workers at large.

In this research, we have hypothesized that soy milk has renoproctective effect, cardio-protective effect and also has an effect on the serum lipid concentrations in hypercholesterolemic male albino Wister rats. The aims of this study are to evaluate the anti-hyperlipidemic and the Reno protective effect of soy bean *(Glycine max)*in hypercholesterolemic rat.

2. Materials and Methods

2.1 Seed collection

The soybeans *(Glycine max*) were obtained from Bean warehouse of Ogbete main market Enugu, Nigeria.

2.2 Soy milk preparation

The soy beans (2kg) were boiled till a brownish coloration was observed. The parboiled beans were drained, weighed, and ground with a grinder; tap water was added at a ratio of 4:1 with grinded bean and then filtered to separate soy cake from soymilk. The soymilk was subsequently heated to 98℃. afterwards; the soymilk was cooled and preserved in a refrigerator at a temperature between 4-6℃.

2.3 Animal husbandry

A total of twenty-four (24) apparently healthy male albino wister rats was used in this experiment. Each rat weighed between 180g and 190g. The animals were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were made to acclimatize under standard temperature (22 ± 3°C) and 12-hour light and dark periodicities. This was performed by housing the animals at the experimental animal housing facility division of the University of Nigeria Teaching Hospital, Enugu Nigeria. The animals were fed with standard pellet diet (Guinea Growers feed) and water *ad libitum.* Experimental protocol and handling were according to Institutional guidelines describing the use of rats and in accordance with the American Physiological Society guiding principles for research involving animals and human beings [11].

2.4 Preparation of high-cholesterol diet (HCD)

A mixture of 75g of commercially available cholesterol powder and 9g of sodium deoxycholate (bile salt added to increase bioavailability) was dissolved in coconut oil and made up with the same solvent to 300ml to give 250mg/ml.

2.5 Preparation of atorvastatin solution

Ten tablets of 10mg (i.e. 100mg) atorvastatin obtained from *pfizer*® Inc., New York, USA were ground to powder, dissolved in distilled water and made up to 50ml mark in a volumetric flask to give a stock concentration of 2mg/ml.

2.6 Preparation of carbimazole solution

25 tablets of 5mg (i.e. 125mg) carbimazole obtained from *hovid*® Inc., Malaysia were ground to powder, dissolved in distilled water and made up to 500ml in a measuring cylinder to give a stock concentration of 0.25mg/ml.

2.7 Experimental design

The rats were allocated to four (4) groups of six (6) animals each in well ventilated cages. The experimental animals received the following treatment on a daily basis for a two week period together with stipulated feed and water.

Group A (Normal Control): No treatment was administered to the rats in this group. The rats continued normal feeding regimen till the end of the experiment.

Group B (Test Group): 1.35mg/kg of carbimazole, 2000mg/kg of HCD and 2000mg/kg of soy milk were administered orally once daily.

Group C (Positive Control): 1.35mg/kg of carbimazole, 2000mg/kg of HCD and 20mg/kg of atorvastatin were administered orally once daily.

Group D (Negative Control): 1.35mg/kg of carbimazole and 2000mg/kg of HCD were administered orally once daily.

2.8 Collection and preparation of blood samples

After two weeks of administration, the animals were fasted for 24hours. The animals were sacrificed under chloroform anesthesia. Approximately 5ml of blood was obtained from each animal from the heart into plain tube for biochemical analysis. Blood samples collected were carefully labeled and allowed to stand undisturbed to clot and retract. Sera were collected into plain containers.

2.9 Biochemical analysis

2.9.1 Measurement of serum lipid profile

The serum lipid parameters such as triglycerides, total cholesterol, high density lipoprotein (HDL) Cholesterol were estimated using the enzymatic end point (kit) method.

2.9.2 Triglyceride

Enzymatic method for Triglyceride as described by Fossati and Prencipe[12].

2.9.3 Total cholesterol

Enzymatic method for total cholesterol according to Fredrickson *et al*. [13]

2.9.4 HDL determination

Precipitation method as described by Albers *et al*. [14]

2.9.5 VLDL determination

VLDL was calculated using the equation

$$\text{VLDL} = \frac{\text{TG}}{2.2}$$

2.9.6 LDL determination

The low density lipoprotein (LDL) Cholesterol was calculated using the Friedewald's equation.

LDL Cholesterol = Total cholesterol, TC – (VLDL + HDL) Cholesterol

2.9.7 Measurement of renal biochemical parameters

The levels of serum electrolyte, urea and creatinine were estimated.

2.9.8 Determination of serum electrolyte:

Serum electrolytes were determined using Perlong Medical PL1000A Electrolyte Analyser. The electrolyte analyser applies the principle of advanced ion-selective electrode, which gives the instrument a stable and reliable measurement. It measures the ion concentrations of K^+, Na^+, Cl^-, Ca^{++}, HCO_3^-, and pH values in the whole blood, serum and urine sample.

2.9.9 Determination of Urea:

Serum urea concentration was determined using the diacetylmonoxime method with protein precipitation according to Natelson *et al*. [15].

2.9.10 Determination of serum creatinine concentration

Serum creatinine concentration was determined using the Jaffe Reaction according to Fabing and Ertingshausen [16].

2.10 Statistical analysis

Data was analyzed using SPSS software version 18. All data were expressed as mean ±SEM. Level Of Significance was determined by the student t-test or by the one way analysis of variance (ANOVA) followed by the Tukey's Post-HOC multiple comparison tests. $P<0.05$, $p<0.01$ or $P<0.001$ was considered significant.

3. Result

Albino wister rats that received HCD alone (negative control) for 2 weeks showed the highest serum total cholesterol levels (5.747 ± 0.245, n = 6) [Table2]. This showed that the HCD alone induced an increase in the level of serum total cholesterol in the rats. The oral administration of soy milk was shown to induce a non-significant decrease in total cholesterol levels in the rats compared with HCD alone (4.840 ± 0.471 vs. 5.747 ± 0.245, n= 6, p=0.1624). However, the positive control, Atorvastatin showed a significant decrease in total

cholesterol levels when compared with HCD alone (4.237 ± 0.118 vs. 5.747 ± 0.245, n = 6, p=0.0051).

Albino Wister rats that received HCD alone (negative control) for 2 weeks showed the highest serum LDL levels (3.570 ± 0.374, n = 6) [Table 1]. This showed that the HCD alone induced an increase in the level of serum LDL in the rats. The oral administration of soy milk was shown to induce a non-significant decrease in LDL levels in the rats compared with HCD alone (2.760 ± 0.387vs.3.570 ± 0.374, n= 6, p=0.2052). However, the positive control, Atorvastatin showed a significant decrease in serum LDL levels when compared with HCD alone (1.220 ± 0.009vs.3.570 ± 0.374, n = 6, p=0.0032).

Albino Wister rats that received HCD alone (negative control) for 2 weeks showed the lowest serum HDL levels (1.203 ± 0.199, n = 6) compared to (Soy +HCD) and (atorvastatin + HCD) [Table 1]. The oral administration of soy milk was shown to induce a non-significant increase in HDL levels in the rats when compared with HCD alone (1.483 ± 0.344 vs 1.203 ± 0.199, n= 6, p=0.2052). However, the positive control, Atorvastatin showed a significant increase in serum HDL levels when compared with HCD alone (2.117 ± 0.107 vs1.203 ± 0.199, n = 6, p=0.0156).

Albino wister rats that received HCD alone (negative control) for 2 weeks showed the highest serum Triglyceride levels (2.130 ± 0.364, n = 6) compared to (Soy +HCD) and (atorvastatin + HCD) [Table 1]. This showed that the HCD alone induced an increase in the level of serum triglyceride levels in the rats. The oral administration of soy milk was shown to induce a non-significant decrease in serum triglyceride levels in the rats compared with HCD alone (1.270 ± 0.050vs.2.130 ± 0.364, n= 6, p=0.0786). Furthermore, the positive control, Atorvastatin also showed a significant decrease in serum triglyceride levels when compared with HCD alone (1.910 ± 0.049vs.2.130 ± 0.364, n = 6, p=0.01706).

Serum Na, K, creatinine and urea levels in all groups are shown in table 2. The levels of Na, K, creatinine and urea were highly elevated significantly in the affected group (HCD alone). Administration of Soymilk (2000mg/kg) and atorvastatin (20mg/kg) separately under HCD challenge significantly lowered the elevated levels of creatinine ($p<0.05$) and urea ($p<0.05$) when compared to the affected group. Furthermore and note-worthy, the levels of K was highly elevated significantly in the affected group (HCD alone); however the administration of soymilk (2000mg/kg) under HCD challenge significantly lowered the elevated levels of K ($p<0.05$) when compared to the affected group.

Serum Ca, HCO_3^- and Cl levels; and blood pH in all groups are shown in table 3. The level of Cl^- was highly elevated non-significantly in the affected group (HCD alone). Administration of Soymilk (2000mg/kg) and atorvastatin (20mg/kg) separately under HCD challenge non-significantly lowered the elevated levels of Cl($p>0.05$) when compared to the affected group. Furthermore and note-worthy, there were no significant differences or changes in HCO_3^- level and blood pH (renal/acid-base parameters) among the groups ($p>0.05$). In addition, there was no significant difference in serum Ca level among the groups ($p>0.05$).

Table 1: Comparison of serum lipid profile parameters of treated groups with negative controls

	Serum Total Cholesterol (mmol/L)	Serum HDL (mmol/L)	Serum LDL (mmol/L)	Serum Triglyceride (mmol/L)	Serum VLDL (mmol/L)
Normal Control	2.240 ± 0.121	0.400 ± 0.058	1.760 ± 0.193	0.187 ±0.032	0.087 ± 0.015
Soy (2000mg/kg) +HCD(2000mg/kg)	4.840 ± 0.471	1.483 ± 0.344	2.760 ± 0.387	1.910 ± 0.049	0.597 ± 0.047
ATOR (20mg/kg) + HCD (2000mg/kg)	4.237 ± 0.118**	2.117 ±0.107*	1.220 ± 0.009**	1.270±0.050*	0.907 ± 0.003
HCD Alone (2000mg/kg)	5.747 ± 0.245	1.203 ± 0.199	3.570 ± 0.374	2.130 ± 0.364	0.970 ±0.165

Values given as Mean±SEM. **P< 0.01 or *P<0.05 is significant when atorvastatin + HCD (positive control) is compared with HCD alone (negative control).

Table 2: Statistical analysis of renal biochemical concentrations in different experimental animal groups

	Na (mmol/L)	K (mmol/L)	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	143.10±1.57*	6.15±0.35*	0.98±0.07*	34.67±0.88**
Soy (2000mg/kg) + HCD(2000mg/kg)	134.30±1.20	6.51±0.34*	1.03±0.24*	46.67±6.89*
ATOR (20mg/kg) + HCD(2000mg/kg)	140.33±3.74	7.17±0.12	0.97±0.12*	42.67±1.20*
HCD Alone (2000mg/kg)	130.70±2.33	8.67±0.77	2.07±0.23	89.00±14.42

Values given as Mean±SEM. **P< 0.01 or *P<0.05 is significant when Soy + HCD or atorvastatin + HCD (positive control) is compared with HCD alone (negative control).

Table 3: Statistical analysis of renal biochemical concentrations in different experimental animal groups

	Ca (mmol/L)	HCO_3^- (mmol/L)	Cl (mmol/L)	Blood pH
Normal control	1.09±0.01	25.33±2.40	103.40±2.75	7.50±0.15
Soy (2000mg/kg) +HCD (2000mg/kg)	0.91±0.04	22.00±1.16	105.33±2.40	7.27±0.02
ATOR (20mg/kg)+HCD (2000mg/kg)	0.91±0.11	24.00±2.31	104.33±0.33	7.30±005
HCD Alone (2000mg/kg)	0.88±0.03	21.33±1.33	106.67±1.20	7.17±0.09

Values are given as Mean ± SEM.

4. Discussion

Studies have showed that rats with hypercholesterolemia are prompt to the development of cardiovascular diseases (CVD) and that soy bean *(Glycine max)* altered disease progression in rats with hypercholesterolemia [17, 18]. Hypercholesterolemia and hyperlipidemia, recognized as contributing to atherosclerosis [19], are also emerging as risk factors for progression of renal disease [20]. Hypercholesterolemia increase oxidative stress and elicit vascular endothelial dysfunction, which may in turn intensify renal cellular injury, apoptosis, and interstitial fibrosis [21]. Hyperlipidemia and oxidative stress following the hypercholesterolemic diet could be prevented by endogenous and exogenous antioxidants [22]. Nutritional sources/products rich in antioxidants such as Soymilk are expected to attenuate the effects of oxidative challenges and hypercholesterolemia. The positive influence of these products in the diet is attributed to the dietary fiber and natural products content, including phenolic compounds that are associated with antioxidant activity [23].

In the present study, we examined the effect of soy bean product, soymilk against hyperlipidemia induced by a high cholesterol diet (HCD) in the presence of carbimazole (an anti-thyroid hormone drug) in albino rats. We further examined the protective effect of soy against renal oxidative stress and nephropathy induced by a high cholesterol diet (HCD) in the presence of carbimazole (an anti-thyroid hormone drug) on renal biochemical parameters in the animals.

The understanding of the impact of thyroid hormones on lipid metabolism has been considerably improved. Thyroid hormone plays an important role in the regulation of lipid metabolism. Thyroid hormone deficiency represents a well-known cause of hypercholesterolemia in hypothyroid patients [24].

Therefore, carbimazole, which is an anti-thyroid hormone drug, was used in this study to cause a thyroid hormone deficiency which enables hypercholesterolemia to develop faster in the male albino Wister rats.

The feeding on HCD plus carbimazole induced hypercholesterolemia at 14 days of treatment. Hypercholesterolemia aggravates experimental progressive glomerular injury. Evidence suggests that infiltrating glomerular macrophage is a potential effector mechanism for the harmful effects of hypercholesterolemia [25] Transforming growth factor (TGF)–beta I is secreted by activated macrophage and also stimulates fibronectin production by glomerular cells [26].

Hypothyroidism causes hypercholesterolemia which will raise the risk for coronary heart disease by many times. This can cause coronary heart disease which is one of the causes of heart failure. Hypothyroidism has also been described as the consequence, rather than the cause of renal dysfunction; thyroxin is heavily protein bound and is lost through urine in nephritic syndrome [24].

In this study, administration of Soymilk (test group) and atorvastatin (positive control group) separately under HCD challenge significantly attenuated renal injury and prevented dyslipidemia in the animals. The observed protection by soymilk could be as a result of the hypocholesterolemic effect and the antioxidant activity of soy. This may be due to individual (singular) or combined actions of one or more phytochemicals present in soy; such as Isoflavones, phytic acid, soya saponin, phytosterol etc [27-29].

A number of cardioprotective benefits have been attributed to dietary isoflavones including reduction in LDL cholesterol, potential reduction in the susceptibility of the LDL particle to oxidation, inhibition of platelet aggregation and an improvement in vascular reactivity [30]. Isoflavones, a putative health beneficial component in soy along with amino acid composition and fiber, may contribute directly to the antioxidant defense system in the body by scavenging Reactive Oxygen Species. It may also interact indirectly with other antioxidant defense system, such as enhancement of glutathione synthesis and sparing of vitamin C and E [31].

Phytic acid is a natural plant anti-oxidant constituting 1-5% of most cereals, nuts, legumes, and oil seeds. By virtue of forming a unique iron chelate it suppresses iron-catalyzed oxidative reactions and may serve a potent antioxidant function [32]. By the same mechanism dietary phytic acid has the potential ability to lower blood glucose, reduce the risk of cancer and heart disease, its addition to food inhibit lipid peroxidation and concomitant oxidative damage [32]. The antioxidant properties of soya saponins have been studied in cells *in vitro* [33].

The hypocholesterolemic effects of soyasaponins have long been recognized [34]. Two mechanisms by which saponins can affect cholesterol metabolism were suggested [35]. 1) Some saponins with particularly defined structural characteristics form insoluble complexes with cholesterol. When this complex-forming process occurs in the gut, it inhibits intestinal absorption of both endogenous and exogenous cholesterol. 2) Saponins can interfere with the entero-hepatic circulation of bile acids by forming mixed micelles. The re-absorption of bile acids from the terminal ileum is effectively blocked [36]. In animal models, soya saponins were found to significantly reduce serum total cholesterol(TC), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) concentrations, and to increase high-density lipoprotein-cholesterol (HDL-C) levels in rats [37].

Phytosterols that have long been known to reduce intestinal cholesterol absorption, lead to decrease blood LDL-cholesterol levels and lower cardiovascular disease risk. In the 1950's, phytosterols from soybeans were found to lower serum cholesterol level [38]. Since then, the cholesterol-lowering effects of phytosterols have been extensively demon strated in both humans and animals [39]. The U.S. National Cholesterol Education Program has recommended adding 2.0 g/day of phytosterols to the diet of adults to reduce LDL cholesterol and coronary heart disease risk [40]. Because phytosterols are not systemically absorbed, they are thought to act primarily in the intestinal lumen. As cholesterol analogs, phytosterols compete for cholesterol in absorptive micelles resulting in reduced solubility of cholesterol [41]. In one study specifically using soybean-derived phytosterols [42], it was found that consumption of phytosterol lowered plasma TC and LDL-cholesterol concentrations and increased HDL-cholesterol.

5. Limitation, Conclusion and Recommendation

Cardiac markers such as: troponin, LDH, myoglobin, CRP, AST etc should have been measured to truly ascertain soy's true cardio protection; however the scope of our work did not permit it. Soymilk has, in addition to its hypocholesterolemic effect; an antioxidant effect thereby abolishing the oxidative renal effect of cholesterol.

More studies should be carried out on soy bean *(Glycine max)* to further reveal its anti-hyperlipidemic properties and cardio-protective properties in the prevention of atherosclerosis. Daily dose of exercise and a healthy balanced meal become low priority in some population of individuals. This is where supplementation and nutritional awareness can play a vital role in prevention of some disorders such as hyperlipidemia and CVD. Awareness should be spread among people especially those who lead or are bound to sedentary life style. Also if possible, supplementation with soy *(Glycine max)* with at least moderate physical activity can guarantee effective prevention and improvement in the new epidemic, cardiovascular disease. It is important to enrich our diet with anti-oxidants rich food such as soymilk to protect against many chronic diseases. Further characterization and purification of the phytochemicals in soy for pharmaceutical benefits should be done.

Competing interest statement

The authors declare no competing interests.

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