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Research Article

Effects of Aqueous Extract of Cymbopogon Citratus Leaves on Exercise-Induced Oxidative Stress and Lipid Profile in Wistar Albino Rats

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A B S T R A C T

Physical activity induces oxidative stress which causes fatigue which can be acute or chronic in athletes, responsible for a reduction in physical performance. The objective of the study was to evaluate the antioxidant activity of the aqueous extract of Cymbopogon citratus and its effects on the lipid profile in albino rats of the Wistar strain subjected to forced swimming. Twenty (25) male rats were fed a standard laboratory ration and divided into 5 batches of 5 rats, then subjected to a 14-day treatment with distilled water, 100 mg/kg, 200 mg/kg or 400 mg/kg of extract and swam every 2 days. On the 14th day, the rats except the control groups were subjected to the forced swimming test with a load of 10% of body weight attached to the tail. The results showed that Cymbopogon citratus reduces the DPPH radical and prevents tissue lipid peroxidation, dose dependent by decreasing Malondialdehyde level ($p < 0.001$) in liver, lung and gastrocnemius muscle, increases dose dependent cellular concentrations of reduced Glutathione in liver ($p < 0.05$) and studentthe specific activity of Superoxide dismutase in the lungs ($p < 0.001$). C. citratus reduced serum levels of total cholesterol ($p < 0.05$) and LDL-cholesterol ($p < 0.01$). C.citratus fights against oxidative stress induced by physical activity in rats. It would be interesting to test its action on other rat strains and endurance models.

Keywords: Cymbopogon citratus, Oxidative stress, Forced swimming test, Wistar albino rats.

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INTRODUCTION

Physical exercise induces fatigue which can be either acute or chronic, thus plunging the body into a state of oxidative stress. Numerous studies have made the relationship between exercise, oxygen consumption and the production of oxidative stress. The higher the oxygen consumption, the more oxidative stress increases [1,2]. Oxidative stress causes damage to the body. It has been associated with the denaturation of the body's cells, notably the alteration of membrane lipids (lipid peroxidation), the denaturation of DNA and proteins, the increase in fatigue (asthenia), the appearance cramps and even premature aging [3]. It is also the result of overtraining, insufficient recovery and other states of exhaustion likely to compromise an individual's state of health [4]. Oxidative stress occurs when an imbalance is created between the body's antioxidant system and excess free radicals in the body. It has been reported that an exogenous intake of antioxidants such as vitamins and polyphenols reduces damage linked to oxidative stress [5,6]. Therefore, the consumption of food plants seems to be a solution approach. They are numerous on the market, at A lower cost, have medicinal virtues and the desired ergogenic effects [7].

In Cameroon there are many food plants that are well adapted to environmental conditions, rich in phytonutrients. Some are abundantly cultivated and used to prepare all kinds of meals, thus contributing significantly to human nutrition, however their nutritional values and biological virtues remain empirical.

Thus, we identified Cymbopogon citratus, also called lemongrass, is a herbaceous plant from the grass family. It is used in Cameroon as a herbal tea, made from a decoction or infusion of leaves, to soothe digestive disorders. Its essential oil helps relieve joint and muscle pain, soothe stress or insomnia, treat fungal infections, and as a mosquito repellent. Several studies have evaluated its biological properties, including its antioxidant effects [8,9].

With regard to the literature, studies aimed at evaluating, in vivo, the antioxidant effects and on the lipid profile, associated with physical activity, in a mouse model, have not yet been reported. We postulate that the aqueous extract of the leaves of C. citratus attenuates the effects of oxidative stress and improves the lipid profile in rats subjected to chronic fatigue. Thus the general objective of this study is to evaluate the antioxidant effects of Cymbopogon citratus on oxidative stress induced by physical effort and on the lipid profile in albino wistar rats.

More specifically, this will involve: evaluating the phytochemical composition; to evaluate the antioxidant activity in vitro and to evaluate the effect of the aqueous extract of C.citratus leaves on serum biochemical parameters (oxidative stress, lipid profile) in rats.

MATERIALS AND METHODS

Materials

Plant material and extraction of the aqueous extract

The plant material consisted of fresh leaves and stems of Cybopogom citratus harvested in Yaoundé, southern region of Cameroon, more precisely in Ba'aba in February 2022. The plant was identified at the National Herbarium in comparison with the material of APM Van der Zon from the specimen in the herbarium collection No. 36148/HNC. Fresh leaves collected at dawn were sorted, washed and dried in the shade at room temperature for 14 days. They were then crushed and reduced to powder. The extraction was done by decoction according to the method used by Nwosu [10]. Two hundred grams (200 g) of powder was mixed in 1000 ml of distilled water and boiled for 20 minutes. The mixture was then filtered with Whatman No. 3 paper. The solution obtained was freeze-dried. After freeze-drying, the aqueous extract obtained was 24 g, or a yield of 12%. The doses to be administered to the rats were 100 mg, 200 mg and 400 mg/kg body weight. They were prepared from the lyophilisate by dilution of 1 g, 2 g and 4 g respectively in 10 ml of distilled water.

Animal material

It consisted of healthy, male Wistar strain albino rats. Rat weights at randomization ranged from 130 to 180 g. The animals were aged 8 to 10 weeks and came from a local pet store. They were housed in Plexiglas cages under ambient temperature conditions (25°C). Ventilation was sufficient with a natural light cycle. The animals had ad libitum access to water and food. The rat food formulation was based on the recommendations of the American Institute of Nutrition (1997) [11] with some local adaptations from the National Veterinary Laboratory (LANAVET) of Cameroon. Dried fish replaced casein while cottonseed meal replaced methionine and choline. The metabolizable energy content of the rat pellets was 3000 kcal/kg, i.e. 22% crude protein, 6.5% fat, 4.5% maximum crude fiber, 6% maximum mineral matter and 11% maximum moisture.

Methods

To measure the antioxidant effects of the extract, a protocol was set up by subjecting rats to chronic physical activity (training).

Experimental protocol: fatigue induced by chronic physical swimming training

Distribution and treatment of rats

The male rats were subjected to a one-week acclimation period during which they were accommodated to a 15-minute swim three (3) times a week. Twenty-five (25) Wistar strain rats whose weights were between 130g and 180g with swimming times similar to the third session were retained and divided into five (5) batches of 5 rats each. Treatments received over 14 days were as follows: a no-training control group received distilled water; a control group subjected to training also received distilled water; three trained groups received respectively the dose of 100 mg, 200 mg and 400 mg ofthe extract.

Rat training protocol

The rats underwent training according to the protocol described by Qi [12]. Swimming training was carried out every two (2) days between 11 a.m. and 5 p.m. for 14 days in a glass swimming pool measuring 90 cm × 45 cm × 45 cm filled with water to a depth 35 cm. The water was at room temperature and was changed each time. Rats (groups 2, 3, 4, and 5) were weighed before each swim training session and then swam with a load of 10% of body weight attached to their tail. Rats were considered tired when they could no longer keep their snouts out of the water for more than 10 seconds. The average swimming time of each group during the training sessions was recorded in seconds.

Organ harvesting and preparation of homogenates

One hour after the last swimming training session, the rats (groups 1 to 5) were sacrificed. Arteriovenous blood was collected in dry tubes then centrifuged at 3000 rpm for 15 minutes to obtain serum which was used for measuring serum biochemical parameters. The liver, lungs and gastrocnemius muscle were removed and washed in a 09% NaCl solution, then drained onto toilet paper, then weighed, wrapped in aluminum foil and stored on ice for the preparation of the homogenates. The homogenates were prepared by grinding the collected organs, then centrifuged at 3000 rpm for 25 minutes and were used to assay oxidative stress parameters (MDA, SOD, CAT and GSH).

Determination of the in vitro antioxidant activity of the aqueous extract

The DPPH radical scavenging assay by the aqueous leaf extract was measured as described by Zhang [13] with some modifications. In the analysis protocol, 2 mL of DPPH (0.1 mM prepared in methanol) was introduced into a test tube containing 0.5 mL of extract (0.1 to 1 mg/mL). Then the mixture was shaken well for 5 min and incubated in the dark

for 60 min at room temperature (20°C). For the control tube, methanol was used instead of the extract. The reference used was gallic acid at concentrations of 0.1 mg/mL to 1 mg/mL. A calibration curve was drawn from this reference. The absorbance was read at 517 nm. The antioxidant activity of the extract was expressed as percentage inhibition.

% I = [(Control Abs – Test Abs) / Control Abs] x 100.

Measurement of oxidative stress parameters during chronic exercise

Serum markers of oxidative stress assessed were specific superoxide dismutase (SOD) activity, catalase activity, serum and hepatic levels of reduced glutathione (GSH) and malondiadehyde (MDA).

Malondialdehyde is a marker of oxidative stress and its determination was made according to the Wilbur method [14]. During the final stages, the peroxides break down into volatile compound responsible for the rancid smell. Malonic aldehyde is formed which reacts with thiobarbituric acid to form a pink complex which absorbs at 530 nm.

Reduced glutathione was measured using the protocol described by Ellman [15]. 2,2-dithio-5,5-dibenzoic acid (DTNB) reacts with the SH groups of glutathione forming a yellow colored complex which absorbs at 412 nm.

Superoxide dismutase (SOD) activity was measured according to the method of Misra and Fridovish [16]. The presence of superoxide dismutase in the sample inhibits the oxidation of adrenaline to adrenochrome. The increase in absorbance is proportional to the SOD activity noted between 20 and 80 seconds at 480 nm wavelength.

Catalase was measured using the Sinha method [17]. Hydrogen peroxide is broken down in the presence of catalase. The residue binds to potassium dichromate to form a blue-green precipitate of unstable perchloric acid which will be decomposed by heat and form a green complex which absorbs at 570 nm.

Lipid profile

Total cholesterol was measured using the KIT (LABKIT, Spain). Cholesterol is present in the serum in the form of cholesterol ester and free cholesterol. The cholesterol ester is hydrolyzed by cholesterol oxidase to form hydrogen peroxide which reacts with phenol and 4-aminoantipyrine thus forming quinoneimine. The intensity of the coloring is directly proportional to the cholesterol level present in the sample. The absorbances of the samples and the standard were read against the blank on a spectrophotometer at 500 nm.

The determination of triglycerides was carried out as follows: under the action of lipase, triglycerides (TG) are hydrolyzed into glycerol and fatty acids. The glycerol will then be transformed into hydrogen peroxide under the successive action of glycerolkinase and glycerol-3-phosphate oxidase. Quinoneimine, which serves as an indicator, provides information on the formation of hydrogen peroxide, aminoantipyrine and 4-chlorophenol under the catalytic action of peroxidase. The absorbances of the samples and the standard were read against the blank on the spectrophotometer at 546 nm.

The determination of HDL-cholesterol was carried out by the following method: in the presence of Mg2+ ions, LDL, VLDL and chylomicrons precipitate quantitatively by addition of phosphotungstic acid and HDL-cholesterol is measured in the supernatant according to the same method principle that cholesteroltotal.

Statistical analysis of results

The results were expressed as mean ± SEM (Standard Error of the Mean). ANOVA was used for comparison of means followed by Dunett's post test. Graphpad prism software version 8.01., was used. The difference is considered significant at $p < 0.05$.

RESULTS

Phytochemical screening of the aqueous extract of C.citratus leaves

The result of the phytochemical screening of the aqueous extract of C. citratus leaves revealed the presence of numerous compounds such as: alkaloids, flavonoids, saponins, glycosides, phenols, steroids, triterpenes, anthocyanins and quinones (Table 1). The result, however, did not reveal the presence of tannins.

Table 1: Classes of Phytochemicals Present In The Leaves Of C. Citratus

Compounds	Identification
AlKaloids	+
Flavonoids	+
Saponins	+
Tannins	-
Glycosides	+
Phenols	+
Stéroids	+
Triterpenes	+
Anthocyanins	+
Quinones	+

(+) : Present ; (-) : Absent

In vitro antioxidant potential of the aqueous extract of C.citratus leaves

The in vitro antioxidant potential of the extract was based on the DPPH test. The test analysis results show that the sample reduces DPPH with an IC50 of 390 ± 0.36; but its action is relatively weaker than that of the reference antioxidant gallic acid (IC50 = 130 ± 20.38). This reduction power is determined by a reduction in absorbance induced by anti-radical substances [18]. The reducing activity of food plant extracts is linked to the fact that these extracts donate a hydrogen atom or an electron to DPPH [19]. Cymbopogon citratus is therefore endowed with anti-radical activities, it could thus prevent the production and propagation of free radicals.

Oxidative stress parameters

The lipid peroxidation test in the liver, lung and gastrocnemius muscle homogenates was carried out by measuring the optical density at 530 nm in the thiobarbituric acid (TBA) and trichloroacetic acid (Benzie) and Strain test (1996). Citratus at doses of 100, 200 and 400 mg/kg caused a significant decrease ($p < 0.001$; $p < 0.001$; $p < 0.01$ respectively) in the level of MDA in the liver compared to the AP group. The aqueous extract of C. citratus (100, 200 and 400 mg/kg) also led to a significant decrease ($p < 0.001$; $p < 0.01$; $p < 0.01$ respectively) in the MDA level in the gastrocnemius muscle compared to the AP group. At a dose of 400 mg/kg, the extract caused a significant reduction ($p < 0.001$) in the level of MDA in the lungs compared to the AP control.

The extract at a dose of 200 mg/kg in the lungs caused a significant increase ($p < 0.001$) in SOD activity compared to the AP group; which suggests a protective effect of the extract on the lungs at a dose of 200 mg/kg.

The aqueous extract of C. citratus at doses of 100 and 400 mg/kg caused a significant increase in GSH levels in the liver ($p < 0.005$) and lungs ($p < 0.001$) respectively compared to the AP group.

Catalase activity was significantly ($p < 0.005$) increased in the liver of rats receiving the extract (400 mg/kg) compared to the AP control.

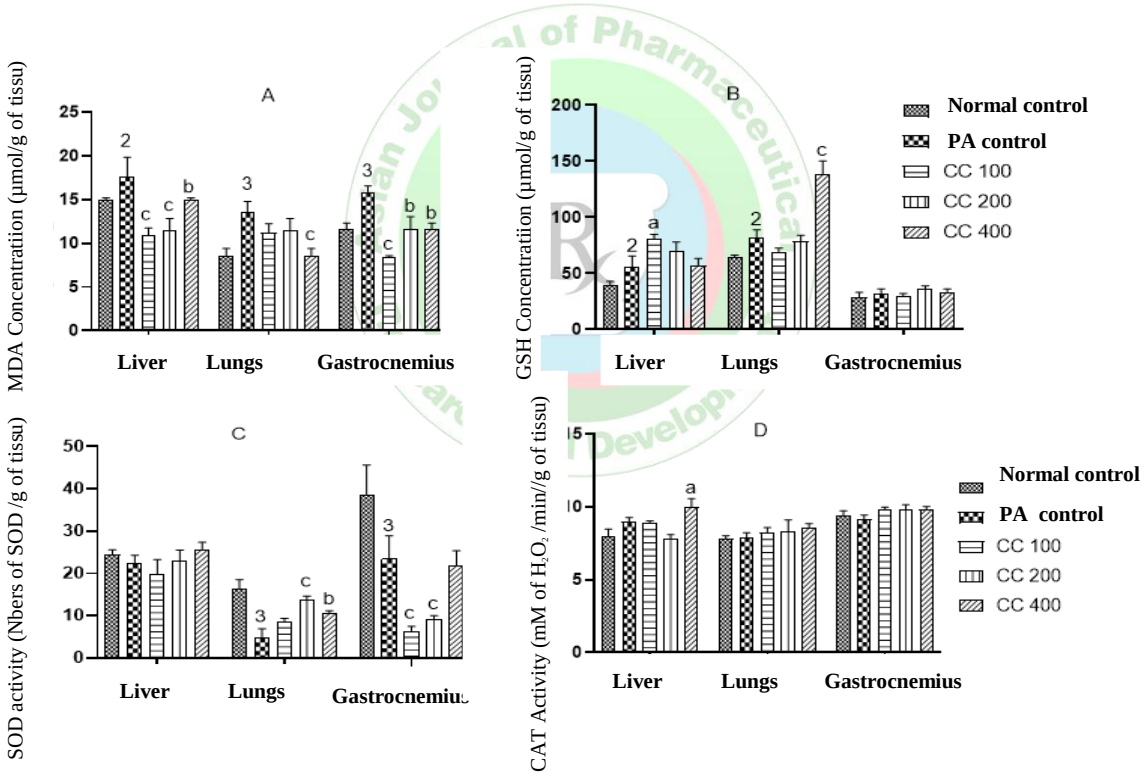

Figure 1: Effects of C. citratus aqueous extract on MDA (A) and reduced glutathione (B) levels and on SOD (C) and catalase (D) activity.

Table 2: Effects of C. citratus aqueous extract on lipid profile

Settings	Normal control	PA control	CC 100	CC 200	CC 400
CT (mg/dL)	128,10 ±2,42	134,60 ±3,28	124,81 ±3,98	114,80 ±2,88[a]	119,40 ±2,71
TG (mg/dL)	75,30 ±1,20	76,69 ±4,66	72,41 ±1,63	74,86 ± 2,16	76,04 ±1,02
HDL-Chol (mg/dL)	52,34 ±3,02	55,62 ±1,95	49,91 ±2,77	51,69 ±1,97	49,10 ±1,67
LDL-Chol (mg/dL)	60,60 ±1,23	63,64 ±1,98	53,85 ±1,25[a]	48,14 ±2,20[b]	55,09 ±3,58
Atherogenic Index	2,44 ± 0,26	2,42±0,13	2,5± 0,30	2,22± 0,36	2,43± 0,22

Each value represents the mean ± SEM (n = 5). CT: Total Cholesterol, LDL-Chol: LDL-Cholesterol, HDL-Chol: HDL-Cholesterol, TG: Triglycerides. Normal control: rats having not undergone physical activity and receiving distilled water (10 mL/kg), PA control: rats having undergone physical activity and receiving distilled water (10 mL/kg) , CC 100, CC 200 and CC 400: rats having undergone physical activity and receiving the C.citratus extract at doses of 100, 200 and 400 mg/kg. 1 $p < 0.05$: significant difference compared to the normal control. a $p < 0.05$; b $p < 0.01$: significant difference compared to the PA control.

DISCUSSION

The objective of this study was to evaluate the effects of the aqueous extract of Cymbopogon citratus on oxidative stress parameters and on the lipid profile in albino rats of the Wistar strain subjected to a forced swimming endurance test. Phytochemical screening of the aqueous extract of C. citratus leaves revealed the presence of numerous compounds such as alkaloids, flavonoids, saponins, glycosides, phenols, steroids, triterpenes, anthocyanins and quinones. Tannins were absent from the aqueous extract. These results are similar to those obtained by Omorogiuwa [20]; which revealed the presence of saponins, flavonoids, glycosides, carbohydrates, steroids, terpenoids and alkaloids in the raw powder sample, but not that of tannins, phenols and anthraquinones. Similar results were also reported by Tcheutchoua [21]. Plant secondary metabolites are well known to exert beneficial health effects in humans. Phenolic compounds, in particular, are a wide range of plant-derived substances with diverse biological activities, ranging from antioxidant and cancer properties, to the ability to inhibit and kill pathogenic bacteria [22]. Flavonoids act either as chelators, free radical scavengers or as pro-oxidants on proteins. They are associated with numerous biological activities such as anti-inflammatory, antiviral, anti-hepatotoxic, anti-tumor, anti-hypertensive, anti-bacterial and anti-allergic activity [23]. The biological activity of the aqueous extract of C.citratus could be attributed to the numerous classes of phytochemical compounds highlighted.

The in vitro antioxidant potential results show that C.citratus aqueous extract reduces DPPH with an IC50 of 390 ± 0.36; but its action is relatively weaker than that of the reference antioxidant gallic acid (IC50 = 130 ± 20.38). This reducing power is determined by a reduction in absorbance induced by anti-radical substances [18]. The reducing activity of food plant extracts is linked to the fact that these extracts donate a hydrogen atom or an electron to DPPH [19]. Cymbopogon citratus is therefore endowed with anti-radical activities, it could thus prevent the production and propagation of free radicals.

The results of oxidative stress parameters in chronic swimming at exhaustion showed that the aqueous extract of C. citratus a at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg induced a significantly ($p < 0.001$; $p < 0.001$; $p < 0.01$ respectively) lower level of MDA in the liver compared to the AP group. The aqueous extract of C. citratus (100 mg/kg, 200 mg/kg and 400 mg/kg) also resulted in a significantly ($p < 0.001$; $p < 0.01$; $p < 0.01$ respectively) lower level of MDA in the muscle (gastrocnemius) compared to the AP group. The extract resulted, at a dose of 400 mg/kg, in a significantly ($p < 0.001$) lower score for the level of MDA in the lungs compared to the AP control. These results suggest that the aqueous extract of C.citratus at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg would have a protective effect on the liver and muscles by preventing lipid peroxidation of cell membranes by oxygenated free radicals [24]. Some studies have demonstrated that the blood concentration of oxidative stress markers (MDA) is significantly lower in trained subjects compared to sedentary people. On the other hand, the level of antioxidants (SOD, GPx, CAT, GSH) is significantly higher in the trained than in the untrained [25]. These results show that in trained subjects, antioxidant activity at rest is higher, both endogenous and exogenous, and therefore more efficient than in untrained subjects. The same results are obtained with athletes in long-duration events with a very large training volume [18]. The extract at a dose of 200 mg/kg in the lungs induced a significantly ($p < 0.001$) higher score of SOD activity compared to the AP group; which suggests a protective effect of the extract on the lungs at a dose of 200 mg/kg.

The aqueous extract of C. citratus at doses of 100 mg/kg and 400 mg/kg resulted in a significantly higher level of GSH respectively in the liver ($p < 0.005$) and lungs ($p < 0.001$) compared to the group AP. Catalase activities were significantly ($p < 0.005$) higher in the liver of rats receiving the extract (400 mg/kg) compared to the AP control. These results suggest that the aqueous extract of the leaves of C. citratus could improve the antioxidant system of rats subjected to chronic physical activity. This improvement could be due not only to endogenous antioxidants, but also to those exogenous found in large quantities in this extract. Flavonoids act either as chelators, free radical scavengers or as prooxidants on proteins. They are associated with numerous biological activities such as anti-inflammatory, antiviral, anti-hepatotoxic, antitumor, antihypertensive, antibacterial and antiallergic activity [23]. The phytonutrients contained in plants (carotenoids, flavonoids, phenols, phytosterols and glucosinolates) because of their protective role for athletes are called adaptogenic plants [26]. These extracts play a regulatory role in the antioxidant system by trapping or inhibiting lipids free radicals, superoxide anions, singlet oxygen [27].

Examination of the lipid profile showed that the extract resulted in a significantly ($p<0.005$) lower level of CT at a dose of 200 mg/kg compared to rats in the AP group. This lower CT score could be due to the consumption of C.citratus extract which would contain physiologically active substances which would stimulate lipolysis. The extract also caused at doses of 100 mg/kg and 200 mg/kg a significantly ($p<0.005$; $p<0.01$ respectively) lower level of LDL-cholesterol compared to the AP control. these results are similar to those found by Abbas [28] who showed that aqueous extracts of C.citratus roots decreased lipidemia in rats. The increase in serum cholesterol levels could cause cardiovascular problems through the development of atherosclerotic plaques [29]. The levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides did not vary significantly in all animals gavaged with the extract compared to their respective controls. These results suggest that the aqueous extract of C. citratus leaves would not have

harmful effects on the cardiovascular system and lipid metabolism.

CONCLUSION

The aqueous extract of C. citratus leaves fights oxidative stress and improves the lipid profile. Phytochemical screening of the aqueous leaf extract of C. citratus revealed the presence of numerous compounds such as: alkaloids, flavonoids, saponins, glycosides, phenols, steroids, triterpenes, anthocyanins and quinones. The extract has good antioxidant capacity in vitro by reducing the DPPH radical. c. citratus showed a protective effect on tissues in rats by preventing lipid peroxidation by decreasing MDA levels, increasing cellular GSH concentrations and elevating SOD specific activity. c. citratus has shown a protective effect on the body by lowering the level of total cholesterol and LDL-cholesterol, thus preventing hypercholesterolemia.

However, it would be important to study the effects of C. citratus extract on weight status associated with physical activity.

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