Menoufia J. Agric. Biotechnology Vol. 6 Nov. (2021): 95–105 https://mjab.journals.ekb.eg

BIOCHEMICAL STUDIES ON THE EFFECT OF ONION ON SOME BLOOD PARAMETERS OF RATS

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Received: Oct. 10, 2021 **Accepted: Nov. 18, 2021**

ABSTRACT: The objective of the present research was to study chemical composition, phenolic compounds and flavonoids of onion. Onion contained total carbohydrates 70.7%, crude protein 7.35 %, total lipids 1.43%, total ash 2.91% fibers 7.91 % and moisture 9.7% based the dry sample. Total phenols in onion bulbs was 35.23 mg /100g., while the total flavonoids was 23.18 mg/100g. HPLC results showed that onion contained 17 phenolic compounds. There were a high percentage of (benzoic acid 905 mg/kg), caticol (649 mg/kg), risvertol (425 mg/kg), perogalol (157 mg/kg) and naringinin (96 mg/kg). that help to improve some blood parameters such as liver function enzymes, kidneys function, lipids and glucose levels. Onion powder as well as ethanolic and water extracts of onion bulbs were prepared which used in the present treatments. Blood parameters of rats were tested using the methods of A.O.A.C.; (2005). Onion powder used as a treatment with concentration of 2 g% and 4 g% of diet. The ethanolic extract of onion used as a treatment with concentration of 1 g/L and 2 g/L. While water extract used as a treatment with concentration of 1 g/L and 2 g/L for hyperlipidemic rats. Blood parameters of rats were tested after the treatments. Data were subjected to statistical analysis using the SPSS (Software version no. 20). Differences between extracts were tested. The best results were obtained by comparisons of the results shown.

Treatments with onion powder and its extracts were beneficial to control the hyperlipidemia, and improved the levels of some blood parameters of rats such as, total cholesterol, HDL-c, LDL-c, TG, ALT, AST, creatinine, urea, uric acid, blood glucose.

Key words: ***Onion, HPLC, Phenolic compound, Flavonoids, blood parameters, Hyperlipidemia, rats.***

INTRODUCTION

Since the dawn of history, plants have been a source of foods, diseases treatments and prevention. Some plants are known as a medicinal plants. They are known to be the big source of many medicines in modern times. Onion (*Allium cepa*), was one of these plants, it is not only used as a food or additives for specific taste and flavor to food but also contribute for good health. Onion is rich in bioactive components that offer numerous health benefits, it also contain phenols, flavonoids, fibers, and they have antioxidant roles in life bodied. There are several reports showing that the onion has medicinal properties as, anti- bacterial, (Skerget *et al;* 2009), antivairal, anticancerous and anti-inflammatory effect, (Atkin *et al;* 2016), anticoagulant effect and reducing cardiovascular; (Tejani, 2013), antidiabetic, antihypertensive and hypolipidemic properties; (Sainani *et al;* 1979 and Ashraf *et al;* 2005). On the other hand, many epidemiological studies confirmed the connection between the consumption of onion vegetables and decrease the risk for development of many diseases, (Upadhyay, 2016). Onion has a long history of folk medicine. Modern science has recognized the beneficial medical effects of this plant, which has widespread uses in improving

some blood parameters those subjected the present study. Research has shown that the components of these plants have the ability to prevent the emergence of hyperlipidemia and some blood components and helps to reduce the side effects of chemical treatments. Blood fats produced by the liver, and it consists of triglycerides, total cholesterol, HDL-c high density lipoprotein, LDL-c low density lipoprotein and vLDL-c very low density lipoprotein …etc, (Kendrick *et al;* 1998). Sometimes it happens to be less than or more than normal levels in blood. Hyperlipidemia means increasing of fat levels in the bloo. In many cases it was the major reason of, diabetes, blood clots, obesity, hyperlipidemia, hypertension, and cardiovascular,…*etc.*), (Wouters *et al*; 2005). The present study aims to clarify the effect of onion and its extracts with different concentrations on the high levels of some blood components in experimental rats. The hypolipidemic effects of onion were investigated through food intake with program of high fat diet HFD making a hyperlipidemia in rats which caused in increasing levels of some blood components, then using onion powder in two concentrations 2 g % and 4 g %. And also using H_2O extracts and ethanolic extracts in two concentration 1 g/L and 2 g/L as treatments through experimentation. Determination the effect of onion on blood parameters in the experimental animals (mal albino rats) through comparisons between the results of blood analysis in different groups and the controls, by statistical analysis to highlight the effect of onion and its extracts on blood chemical analysis reaching to the best treatment of onion in blood parameters.

MATERIALS AND METHODS

Samples collection:

Fresh onion (*Allium cepa L.*) bulbs was purchased from local market; identified by horticultural department, Faculty of Agriculture, Menoufia University. Plant sample was washed and air-dried for 24 hours, then dried at 40 ◦C. The dried sample was grinded into fine powder to pass 100 mm filter and kept in a sterile container.

Methods:

Preparation of plant extracts:

The Frist 200 g of the dried plant powder was dissolved in one liter ethanol 95%. The second: 200 g powdered sample was extracted with one liter of H_2O. with stirring for 48 hours then, filtered through double layers of muslin, centrifuged at 9000 xg for 10 min and finally filtered again through *Whatman* filter paper number 41 to attain a clear filtrate. The filtrates were evaporated and dried at 40°C under reduced pressure using rotatory vacuum evaporator. Finally, the extract was collected and stored in refrigerator at 4°C (Gauthami *et al;* 2015).

Major chemical composition:

After bringing the samples to uniform size, they were analyzed for ash, moisture, fiber, protein and fat which determined according to A.O.A.C., (2005) and carbohydrates were by Dubois *et al;* (1956). All conducted in the Graduate Laboratory, Department of Biochemistry, Faculty of Agriculture, Menoufia University.

Determination of total phenolic compounds in different extracts:

The amounts of total phenols in the studies extracts were determined spectrometrically with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenols were expressed as mg gallic acid equivalents (GAE)/g dry weigh. (Kim *et al*; 2013).

Determination of total flavonoids amounts in different extracts:

The total flavonoids contents were determined using the method reported by Dewanto *et al;* (2002). The results were expressed as mg of catechin equivalents (CE)/g dry weight.

Quantitative fractionation analysis of phenolic compounds by HPLC:

Phenolic compounds were analyzed, fractionated, and determined at the Department of Food Science, Faculty of Agriculture, Cairo University according to the method of (Goupy *et al;* 1999), by using HPLC *Hewilet pckared* (series 1050) equipped with auto-sampler injection, solvent degasser, Ultra violet (UV) detector set at 280 nm. and quaternary HP pump (series 1050). Hewlett Packard using a column Alltima C18, 5mm (150 mm×4.6mm Allech). The column temperature was maintained at 35^{o}c. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Standards were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculate phenolic compounds concentration by the data of *Hewllet packared* software.

Experimental animal design:

Male albino rats were used in the present experiment, all rats were fed normal diet for one week after arrival `for adaptation. For induction of hyperlipidemia, all groups (except the normal control group) were fed on high fat diet (HFD). Sheep tail fat was used on the way to induce hyperlipidemia. They were then randomly assigned into 8 groups (5 rats per group) as follow, Treatment is taken by the gastric tube and the dose is one ml for rat perday. Treatment is taken from day 30 (zero time) till the end of the experiment (day 60).

Blood sampling:

Blood samples were collected after 12 - 14 hours fasting in days: 30 zero time after hyperlipidemia, 45 during of treatment and 60 at the end of experiment in dry, clean tubes without any anticoagulant from the eye plexuses of experimental rats under diethyl ether anesthesia and let samples in an incubator for 20 min., then centrifuged at 3500 xg for 10 minute to collect sera. At the end of the experiment after 60day, rats were killed by decapitation.

Blood samples were analyzed for parameters, total cholesterol, HDL, LDL, TG, ALT, AST, creatinine, urea, uric acid and blood glucose by using methods of A.O.A.C.; (2005), and results were calculated.

Statistical analysis:

The data were subjected to statistical analysis using the SPSS (Software version no. 20). Differences between extracts were tested by one-way analysis of variance (ANOVA). Probability value for the statistical test was 0.5 % .

RESULTS AND DISCUSSIONS

Major chemical composition of onion.

Composition has been analyzed three times, and the average results have been displayed.

The results displayed in Table (1) show the % of the major chemical components of onion on a dry matter base. The results show that the onion contain moisture 9.7 %, total carbohydrate 70.7%, total protein 7.35%, lipids 1.43%, fibers 7.91% and ash 2.91%.

Determination of phenolic and flavonoide compounds in onion bulbs:

Composition has been analyzed three times, and the average results have been displayed.

Table (1): Experimental groups with diet and treatment design.

Gr. No.	Diet	Treatment
1	Standard diet	No treatment.
2	HFD	No treatment.
3	HFD + treatment	Onion powder 2 % of diet.
4	HFD + treatment	Onion powder 4 % of diet.
5	HFD + treatment	One ml ingested into a rat of onion ethanolic extract 1 g/L.
6	HFD + treatment	One ml ingested into a rat of onion ethanolic extract 2 g/L.
7	HFD + treatment	One ml ingested into a rat of onion water extract 1 g/L.
8	HFD + treatment	One ml ingested into a rat of onion water extract 2 g/L.

The results displayed in Table (2) show the % of the major chemical components of onion on a dry matter base. The results show that the onion contain moisture 9.7%, total carbohydrate 70.7 %, total protein 7.35 %, lipids 1.43 %, fibers 7.91 % and ash 2.91 %.

The results in Table (3) show that the onion contain total phenols 35.23 mg/g dm. total flavonoids 23.81 mg/g dm. These results are in line with the results published by Duan *et al*; (2015).

Fractionation and identification of phenolic components in onion:

Data in Table (4) shows that the major phenolic compounds in onion.

More than 100 ppm.: Benzoic acid 905.0 ppm, Catechol 649.6, Resvertol 425.9, Pyrogallol 157.0 and Naringenin 96.6 .

Also data in Table (4) show that the minor phenolic compounds in onion (6.0 - 90 ppm.): Quercitin 18.0 ppm, Quinol 14.0 ppm, Caffiec acid 9.5 ppm, Kaempferol 5.7 ppm and Cholorogenic 5.6 ppm.

Effect of onion and its extracts on blood components of rats:

The results in Table (5) shows the effect of onion treatments on rats fed on a high fat diet. It is clear from the search results a significant increase in cholesterol, HDL-c, LDL-c, TG, levels; while all groups treated with onion showed highly significant decrease in the same parameter levels compared with positive control group.

Results also indicate that onion ethanolic extract treatment 2 g/L is the best among all treatments followed by onion ethanolic extract treatment 1g/ and both of them showed a significant decrease close to the normal control group.

These results are in line with the results published by, Kim *et al;* (2013).

Effect of onion and its extracts on kidney functions and blood glucose in rats:

The results in Table (6) show the effect of onion treatments on the kidneys functions and blood glucose rats fed on a high fat diet. It is clear from the search results a significant increase in urea, creatinine, uric acid and blood glucose levels, while all groups treated with onion showed highly significant decrease in the same parameter levels compared with positive control group.

Results also indicate that onion ethanolic extract treatment 2 g/L is the best among all treatments followed by onion ethanolic extract treatment 1g/L and both of them showed a significant decrease close to the normal control group.

These results are in line with the results published by, Jelodar *et al;* (2005), Haidari *et al*; (2008) and Kumar *et al;* (2011).

Effect of onion and its extracts on liver functions (AST & ALT) in Rats:

The results in Table (7) show the effect of onion treatments on rats fed on a high fat diet. It is clear from the search results that there are a significant increases in ALT and AST activity levels of hyperlipidemic rats while all groups treated with onion showed highly significant improvement in the same parameter levels compared with positive control group.

Table (2): The major chemical composition of onion dry matter % .

Component:	Moisture	Carbohydrate	Protein	Lipids	Ash	Fibers
	9.7	70.7	7.35	1.43	2.91	7,91
%	± 0.4	± 2.3	± 0.3	± 0.1	± 0.1	± 0.3

Table (3): Total phenolic and total flavonoide compounds in onion bulbs.

Component in the onion dry matter		
Total phenols content (TPC)	35.23 ± 1.4	mg/g dry weight
Total flavonoids content (TFC)	23.81 ± 1.3	mg/g dry weight

Table (4): Phenolic compounds in onion ppm.

No. of fractions	Phenolic compounds	Onion Conc. ppm
1	Pyrogallol	157
2	Quinol	14
3	Gallic acid	1.6
4	Catechol	650.0
5	P-Hydroxy benzoic acid	5.0
6	Catechin	4.1
7	Cholorogenic	5.6
8	Vanillic acid	4.1
9	Caffiec acid	9.5
10	Benzoic acid	905
11	Ferullic acid	3.6
12	Ellagic	Not detected
13	O-Coummaric acid	3.3
14	Resvertol	426.0
15	Cinnamic acid	2.1
16	Quercitin	18.0
17	Naringenin	96.6
18	Myricetin	Not detected
19	Kaempferol	5.7
Total amount :		2310.7 ppm

Table (5): Lipid profile of the blood in the experimental animals.

Lipids	Cholesterol			HDL-c			LDL-c			TG		
Time / Treat.	Zero time	Day 30	Day 60	Zero time	Day 30	Day 60	zero time	Day 30	Day 60	Zero time	Day 30	Day 60
-ve control	145 ± 2.8a	147 ± 3.1a	148 ± 3.3 a	52.5± 3.7e	53.5 ± 4.1e	54.4 ± 3.9e	72 ± 4.1a	80 ± 2.7a	86 ± 3.0ab	96 ± 2.6a	97 ± 2.4a	102 ± 3.9a
+ ve control	270 ± 3.3bc	298 ± 4.2c	322 ± 4.1e	38.6 ± 3.1c	35.3 ± 3.1b	30.6 ± 3.1a	194 ± 3.5cd	208 ± 3.1de	229 ± 3 f	188 ± 2.7a	272 ± 3.5a	312 ± 3.9a
Onion powder 2 %	276 ± 3.8bc	231 ± 4.1bc	216 ± 3.5b	33 ± 3.2ab	42 ± 2.1d	47 ± 3.3e	203 ± 3.2de	152 ± 3.2cd	126 ± 3 bc	194 ± 5.5ef	183 ± 3.6de	163 ± 3.5c
Onion powder 4 %	275 ± 3.6bc	229 ± 3.3b	214 ± 5.1ab	33 ± 3.1ab	43 ± 3.4d	46.7 ± 3.6e	201 ± 3.4de	150 ± 4.3cd	122 ± 4bc	192 ± 4.5e	182 ± 6.2de	162 ± 2.5c
Ethanolic extract 1 g/L	274 ± 4.1bc	229 ± 5.1b	211 ± 3.1ab	34 ± 3.3ab	44 ± 3.1d	48.3 ± 3.2e	200 ± 3.3de	149 ± 2.7cd	120 ± 2.3b	192 ± 3.7e	178 ± 3.4cd	159 ± 3.5c
Ethanolic extract 2 g/L	275 ± 3.4bc	227 ± 3.5b	208 ± 4.1a	33 ± 3.1ab	44 ± 2.1d	50 ± 3.1f	202 ± 4.3de	147 ± 3.3cd	116 ± 3.4b	192 ± 3.5e	175 ± 4.5cd	157 ± 4.5c
H_2O extract 1 g/L	277 ± 4.1 c	235 ± 3.3c	228 ± 3.5bc	34 ± 3.1bc	40 ± 3.3d	44 ± 3.7d	204 ± 3.1de	158 ± 4.1cd	140 ± 4.1c	190 ± 4.6e	184 ± 3.5de	166 ± 5cd
H_2O extract 2 g/L	277 ± 3.6 c	233 ± 3.3c	225 ± 3.1b	36 ± 3.2b	41 ± 3.1d	45 ± 3.3e	202 ± 4.2de	155 ± 3.2cd	137 ± 3.3c	192 ± 3.5ef	181 ± 3.7de	165 ± 4cd

Values represent means ± S.D. obtained from 6 treatments, means in the same column followed by the same letters don't differ significantly, and when the means followed by different letters differ significantly at ($p > 0.01$).

Table (6): The kidneys functions and blood glucose of the experimental animals

Param.	urea			creatinine			Uric acid			Glucose		
Time / Treat.	Zero time	Day 30	Day 60	Zero time	Day 30	Day 60	zero time	Day 30	Day 60	Zero time	Day 30	Day 60
+ ve control	47 ± 3.0c	44 ± 4.0 b	41 ± 3.0ab	0.98 ± 0.2bc	0.95± 0.2bc	0.88± 0.2b	6.14± 1.4d	5.63± 1.7bc	5.44± 1.2b	138 ± 4.1d	132 ± 3.1 c	123 ± 5.1b
-ve control	31 ± 4.7a	30 ± 3.2a	30 ± 3.5a	0.59 ± 0.1a	0.58± 0.2a	0.58± 0.1a	3.75± 1.2a	3.67± 1.4a	3.52± 1.3a	105 ± 4.4a	102 ± 4.3a	103 ± 5.1a
Onion powder 2 %	36 ± 3.2ab	43 ± 3.4bc	45 ± 3.4bc	0.67 ± 0.2a	0.73± 0.1ab	0.85± 0.1 b	4.31± 1.6ab	5.11± 1.3b	5.55± 1.6c	114 ± 4.2ab	119 ± 4.4ab	129 ± 4.2 b
Onion powder 4 %	35.5± 3.4ab	42 ± 3.7a	45 ± 3.2bc	0.66 ± 0.1a	0.72± 0.2ab	0.79± 0.2 b	4.27± 1.4a	5.25± 1.2b	5.50± 1.3c	113 ± 4.4ab	119 ± 4.2ab	125 ± 3.8 b
Ethanolic extract 1g/L	35 ± 4.7a	42 ± 4.1a	46 ± 3.2 c	0.64 ± 0.1a	0.71± 0.2ab	0.78± 0.1 b	4.26± 1.3a	5.31± 1.4b	5.51± 1.4c	111 ± 4.5ab	117 ± 4.1ab	122 ± 5.1 b
Ethanolic extract 2 g/L	34 ± 3.4a	41 ± 3.2ab	45 ± 4.7bc	0.63 ± 0.1a	0.71± 0.2ab	0.78± 0.2 b	4.25± 1.2a	5.21± 1.2b	5.82± 1.7c	110 ± 4.0ab	117 ± 4.4ab	122 ± 4.4 b
H_2O extract 1 g/L	38 ± 3.7ab	45 ± 3.5bc	47 ± 3.3c	0.69 ± 0.2a	0.73± 0.2ab	0.79± 0.2 b	4.35± 1.5ab	5.41± 1.3b	5.54± 1.3c	117 ± 6.2ab	122 ± 5.4 b	129 ± 4.1 b
H_2O extract 2 g/L	37 ± 3.5ab	44 ± 3.4bc	47 ± 3.1c	0.68 ± 0.2a	0.73± 0.1ab	0.77± 0.2 b	4.33± 1.5ab	5.37± 1.4b	5.51± 1.2c	115 ± 5.2ab	121 ± 5.5 b	128 ± 4.1 b

Values represent means ± S.D. obtained from 6 treatments, means in the same column followed by the same letters don't differ significantly, and when the means followed by different letters differ significantly at ($p > 0.01$).

Table (7): Liver function enzymes activity of the experimental animals:

Parameter	ALT (U/L)			AST (U/L)		
Time / Treatment	Zero time	Day 30	Day 60	Zero time	Day 30	Day 60
+ ve control	48±4.6^{e}	43±4.1^{cd}	40±4.4^{c}	51±4.6^{e}	45±4.3^{d}	42±4.1^{c}
- ve control	27±4.4^{a}	27±4.1^{a}	26±3.1^{a}	28±4.6^{a}	27±4.3^{a}	27±5.1^{a}
Onion powder 2 %	35±5.1^{ab}	38±5.1^{bc}	41±4.1^{c}	38±4.7^{ab}	39±5.3^{bc}	44±4.1^{c}
Onion powder 4 %	34±4.4^{b}	37±4.4^{bc}	41±4.3^{c}	37±4.8^{a}	38±4.7^{bc}	46±4.2^{c}
Ethanolic extract 1 g/L	32±3.8^{a}	36±4.3^{bc}	40±4.4^{c}	36±5.1^{a}	38±4.3^{bc}	45±5.1^{ce}
Ethanolic extract 2 g/L	30.5±4^{a}	35±4.4^{ab}	40±4.6^{c}	34±4.4^{a}	39±5.2^{bc}	46±4.7^{ce}
H2O extract 1 g/L	37±5.1^{ab}	37±4.1^{bc}	38±5.1^{b}	40±4.9^{bc}	41±5.1^{c}	45±4.4^{ce}
H2O extract 2 g/L	36±5.9^{ab}	37±3.6^{bc}	39±4.4^{b}	39±4.7^{ab}	40±4.4^{c}	45±4.1^{ce}

Values represent means ± S.D. obtained from 6 treatments, means in the same column followed by the same letters don't differ significantly, and when the means followed by different letters differ significantly at (p > 0.01).

Results also indicate that onion ethanolic extract treatment 2 g/L is the best among all treatments followed by onion ethanolic extract treatment 1g/L and both of them showed a significant decrease which was closed to the normal control group. These results are in line with the results published by, Stajner *et al;* (2006) and Emamat *et al;* (2016).

Conclusion

The results of the current study showed that the ethanol and water extracts of onion contain a good amounts of total phenols and total flavonoids.

In summary, treatment of onion was beneficial to control the hyperlipidemia, and improving the levels of some blood parameters such as, total cholesterol, HDL, LDL, TG, ALT, AST, creatinine, urea, uric acid, blood glucose. Those are indicators for lipid profile, liver function, kidneys function and blood sugar of the experimental albino rats.

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دراسات كيميائية حيوية على تأثير البصل على بعض قياسات الدم في الجرذان

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الملخص العربى

يهدف هذا البحث الى دراسة التركيب الكيميائي للبصل وكذلك محتوى البصل من المركبات الفينولية والفلافونويدات .

وقد وجد أن البصل يحتوي على: الكربوهيدرات 70,7 ٪ و البروتين الخام 7,35 ٪ و مجموع الدهون 1,43 ٪ و نسبة الرماد 2,91 ٪ و المحتوى من الألياف 7,91 ٪ والرطوبة 9,7 ٪ من وزن العينة الجافة.

كما اظهرت التحاليل احتواء البصل على الفينولات الكلية 35,23 مليجرام/ جم و كانت الفلافيونيدات الكلية 23,81 مليجرام/ جم من وزن النبات.

وبإجراء التحليل الكروماتوجرافى تبين أن البصل يحتوى أيضا على عدد 17 مركب فينولى ، وكانت النسبة العالية منه: حمض البنزويك 905 و الكاتيكول 649 و الريسفيرتول 425 و البيروجالول 157 و النارينجينين 96 مليجرام/كجم .وهذه المركبات بدورها تساعد فى تحسين بعض قياسات الدم مثل نشاط إنزيمات الكبد و وظائف الكلى ومستويات الدهون ومستوي الجلوكوز بالدم.

وبعد اجراء التجربة الحيوية و ذلك بالمعاملة بمسحوق البصل المجفف تجفيف هوائي و كذلك المعاملة بكل من المستخلص الايثانولى و المستخلص المائي للبصل ثم اجراء تحاليل الدم و حسابات النتائج و قد خضعت النتائج للتحليل الإحصائي باستخدام برنامج SPSS الإصدار العشرون من البرنامج و تم عمل مقارنات بين نتائج المعاملات بالمستخلصات المختلفة. وقد تم التوصل الى أفضل المعاملات من خلال مقارنات النتائج.

أظهرت النتائج أن المعاملات بمسحوق البصل وكل من المستخلص الايثانولى و المستخلص المائي للبصل مفيدة للسيطرة على زيادة مستويات الدهون بالدم مثل الكوليسترول الكلي و الدهون منخفضة الكثافة و الدهون الثلاثية و أظهرت تحسن في مستوى الدهون عالية الكثافة وقد حسنت أيضا من نشاط انزيمات الكبد ALT و AST وحسنت من مستويات بعض معاملات الدم الاخرى مثل وظائف الكلى في صورة انخفاض البولينا و الكرياتينين و حمض البوليك وخفضت ايضا نسبة جلوكوز الدم.

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