British Journal of Medicine & Medical Research
4(28): 4665-4673, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antidiabetic Activity of Hydroalcoholic Extracts of *Nardostachys jatamansi* in Alloxan-induced Diabetic Rats

M. A. Aleem[1*], B. Syed Asad[1], Tasneem Mohammed[2], Riyaz Ahmed Khan[1], M. Farooq Ahmed[1], A. Anjum[3] and M. Ibrahim[1,3,4]

[1]Department of Medicinal Chemistry and Pharmacology, Nizam Institute of Pharmacy and Research Center, Deshmukhi (V), Pochampally (M), Near Ramoji Film City, Nalgonda,(AP), India-508284, India.
[2]Department of Gastroenterology and Hepatology, Ibn Sina National College of Medical Sciences, Al Mahjar, Jeddah-21418, K. S. A, Saudi Arabia.
[3]Department of chemistry, Asian Institute of Advance Scientific and Pharmaceutical Research, Hyderabad, Andhra Pradesh, India.
[4]Center for Liver Research and Diagnostics, Deccan College of Medical Sciences and Allied Hospitals Kanchanbagh, Hyderabad 500 058, Andhra Pradesh, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MI guided the entire study, author MAA designed the study, performed the statistical analysis, wrote the protocol, and author BSA wrote the first draft of the manuscript. Authors TM, RAK managed the analyses of the study. Authors MFA and AA managed the literature search. All authors read and approved the final manuscript.

Original Research Article

Received 8th May 2014
Accepted 4th June 2014
Published 20th June 2014

ABSTRACT

A review of literature indicates that diabetes mellitus was fairly well known and well conceived as an entity in India with complications like angiopathy, retinopathy, nephropathy, and causing neurological disorders. The antidiabetic study was carried out to estimate the anti hyperglycemic potential of *Nardostachys Jatamansi* rhizome's hydro alcoholic extracts in alloxan induced diabetic rats over a period of two weeks. The hydroalcoholic extract HAE1 at a dose (500mg/kg) exhibited significant antihyperglycemic activity than extract HAE2 at a dose (500mg/kg) in diabetic rats. The

**Corresponding author: Email: ibrahim_cce@rediffmail.com;*

hydroalcoholic extracts showed improvement in different parameters associated with diabetes, like body weight, lipid profile and biochemical parameters. Extracts also showed improvement in regeneration of *β*-cells of pancreas in diabetic rats. Histopathological studies strengthen the healing of pancreas by hydro alcoholic extracts (HAE1& HAE2) of *Nardostachys Jatamansi*, as a probable mechanism of their ant diabetic activity.

Keywords: Nardostachys jatamansi; diabetes mellitus; alloxan; hypoglycemic effect; hydroalcoholic extract.

1. INTRODUCTION

The flowering plant *Nardostachys Jatamansi* (*NJ)* belongs to the family Valirenaceae that grows in the Nepal, Himalayas of India and China. The plant grows to one meter in height with pink, bell-shaped flowers. It is found in the altitude of about 3000–5000 meters. In Ayurveda & uani treatment roots and rhizomes of *N. Jatamansi* are used to treat hysteria, epilepsy, and convulsions. The decoction of the *N. Jatamansi* is also used in neurological disorders, problems related to cardiovascular system and insomnia. Rhizomes are reported to contain a terpenoid ester, Nardostachysin. The sesquiterpenes (Jatamansone, Jatamansic acid), lignans and neolignans are reported to be present in the roots of this plant. To date much research has been undertaken to evaluate the drug to treat various neurological and cardiovascular disorders in different animal models and is widely used in ayurvedic & unani formulations. It is reported to possess many activities like anti depressant activit, anticonvulsant activity, antiarrhythmic activity, and possess hepatoprotective activity, usefull in Alzheimer & cereberal ischemia, have antifungal property, anxiolytic & hypolipidimic activity [1].

A common metabolic disorder known as Diabetes mellitus with micro and macro vascular complication, that results in significant morbidity and mortality. It is considered as one of the cause among five leading causes of death in the world [2,3]. A detail study of literature indicates that diabetes is quite well known and well conceived in India. The knowledge about the disease diabetes mellitus existed with the Indians since prehistoric age. The term ‘Madhumeha’ used for the disorder according to the literature in which a patient passes sweet urine and exhibits sweetness in sweat, mucus & blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20% [4]. Diabetes mellitus is a common disorder throughout the world; however, it is a big challenge to the health in the more developed countries because of their food habits. Diabetes is in the top five of the most significant diseases in the developed world and is still gaining significance [5]. Hence under these conditions exploring new cures from plants source will always be beneficial because of less side effects. Plant-based treatments have been used against a variety of diseases since prehistoric time. The ancient people used different part of plants and the extracts of the plants as therapeutic agents and medicament, which they were able to get easily. The nature has endowed thousands of plant for all living creatures, which possess medicinal potential. Though the medicinal values of several plants have been reported; but still there are plants those have to be evaluated for their biological importance. Therefore, there is a requirement to find out their pharmacological uses. By keeping the above fact in view the evaluation of new plants will give more options to treat disease like diabetes, which is a global problem nowadays.

In this study the prolonged effect (up to 14 day) of the hydroalcoholic extracts of rhizome of *N. Jatamansi* in fasting blood glucose (FBG) and biochemical parameters such as serum total cholesterol (TC), LDL, HDL, creatinine, urea, and alkaline phosphatase were studied in alloxan induced diabetic rats. On the fact no study has been carried out in detail on hydroalcoholic extracts of rhizome of *N. Jatamansi* in alloxan induced diabetic rats. Thus the present study is an attempt to test the anti diabetic activity of rhizome of *N. Jatamansi*. The other chief objective is to bring the list of anti hyperglycemic medicinal plants on a firm scientific footing, raise knowledge and contribute to the socio-economic well being at intercontinental level.

2. MATERIALS AND METHODS

2.1 Chemicals

The toxicant Alloxan monohydrate was from Sigma chemicals (St Louis, USA). The remaining chemicals used for this research work were of analytical grade.

2.2 Plant Material

The basic plant material rhizome of *N. Jatamansi used* for the investigation was obtained from authorized supplier M/s Munnalal Dawasaz and company, Hyderabad. The plant were identified and authenticated by experts in the Dept of Botany, Bhavan's New Science College, Narayanaguda, Hyderabad. AP, India.

2.3 Hydro Alcoholic Extraction

The rhizome were collected and cleaned thoroughly with distilled water to remove any type of contamination, dried and subjected to pulverization to get coarse powder. The coarsely powder of rhizome (1000 gm) of *N. Jatamansi* was used for extraction with ethanol water in a ratio 75:25 (HAE1) and 50:50 (HAE2) in soxhlate apparatus. The Hydroalcoholic extracts were dried at 45ºC in rotary evaporator to produce a semi solid mass and stored in refrigerator below 10ºC using air tight containers.

2.4 Experimental Animals

Adult Wister rats of either sex, weighing 160–200gm were used in the experiment. The animals were maintained under standard environmental conditions i.e. controlled temperature (26±1ºC) and humidity (30%–40%) in standard polypropylene cages. They were fed with a standard pellet rat diet. Water was supplied to the animals ad libitum. The rats were obtained from the animal house of the Mahaveer enterprises, Hyderabad. Experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of C.P.C.S.E.A.

2.5 Acute Toxicity Studies

Acute oral toxicity experiment was performed as per OECD-423 guidelines [1,6]. The random sampling technique was used for the selection of Wister rats (n=6) of either sex for the study. Prior to the dosing, the animals were fasted overnight for 24 hours providing only water, after that the extracts were administered orally at the dose level of 5mg, 50mg, 500mg, 1000mg, 2000mg, and 5000mg/kg/body weight and observed for 3 days. If death was observed in more than one animal, then the dose administered was assigned as lethal

dose or toxic dose. If death was observed in one animal, then the same dose was repeated again to confirm the toxic dose, If death was not observed, the procedure was repeated for further 28 days.

2.6 Oral Glucose Tolerance Test

Rats were divided into six groups each group containing six rats. All rats kept on fasting before treatment. Group I was taken as vehicle control which received 5% Tween 20 p.o., glucose only was given to group II, group III received HEA1 (500mg/kg), group IV received HEA2 (500mg/kg) and group V and VI received only extracts HEA1& HEA2 (500mg/kg) only in a vehicle, respectively. The rats of group III and IV were loaded with glucose (3g/kg, p.o.) 30 minutes after drug administration. Blood samples were collected by puncturing the retro orbital sinus just prior to drug administration, and at 30, 90, 150 minutes after loading glucose. Serum glucose level was measured immediately by using glucose estimation kit.

2.7 Experimental Design

Diabetes was induced using alloxan monohydrate (150mg/kg) [7] by a single intraperitoneal injection. Only alloxan hyperglycemic animals were used for further studies. The rats with plasma glucose levels of >140mg/dl after 48 hour of alloxan administration were included in the study. Treatment with rhizome extracts was started after 48 hour of alloxan injection. Hyperglycemic rats divided into five groups, six in each group received the following treatment schedule.

Group I: Normal control (saline).
Group II: Alloxan treated control (150mg/kg.ip).
Group III: Alloxan (150mg/kg.ip)+HAE1. (500mg/kg, p.o),
Group IV: Alloxan (150mg/kg.ip)+HAE2 (500mg/kg, p.o),
Group V: Alloxan (150mg/kg.ip)+Standard drug, Glibenclamide (5mg/kg, p.o).

2.8 Collection of Blood Sample and Determination of Blood Glucose level

Blood samples were collected from tail tip of rat at an interval of 5 days till the end of study (i.e., 2 weeks). Estimation of Fasting blood glucose and body weight measurement carried out on day 1, 5, 10 and 14 of the experiment. Estimation of Blood glucose determined by one touch select simple electronic glucometer by glucose test strips.

On 14^{th} day, blood from retro-orbital plexus was collected under ether anesthesia from overnight fasted rats and fasting blood sugar was quantified [8]. blood Serum used for the analysis serum cholesterol [9], Enzymatic DHBS colorimetric method used for the estimation of serum triglycerides [10], serum HDL [11], serum LDL [12], serum creatinine [13], serum alkaline phosphatase hydrolyzed phenol amino antipyrine method [14] and serum urea [15] was estimated. After sacrificing whole pancreas of all animal was removed and was collected in a solution of 10% formalin, and processed by the paraffin technique for histopathology studies.

2.9 Statistical Analysis

All the values body weight, fasting blood sugar, and biochemical estimations expressed as mean±standard error mean (SEM). The comparison of difference was done by using ANOVA (one-way analysis of variance) followed by Dunnet's t test. *P* values<0.01 were considered as significant.

3. RESULTS AND DISCUSSION

3.1 Glucose Tolerance

The effects of extracts HAE1 & HAE2 of *N J* (each 500mg/kg) on glucose tolerance test are shown in Fig. 1. The supplementation of *Nordostachys jatamansi enhanced* the glucose tolerance in the fasted normal rats. The serum glucose level was lowered significantly (*P*<0.05) at 90 minutes and (*P*<0.01) at 150 minutes. Extracts also showed good hypoglycemic effect after 90 minutes of treatment.

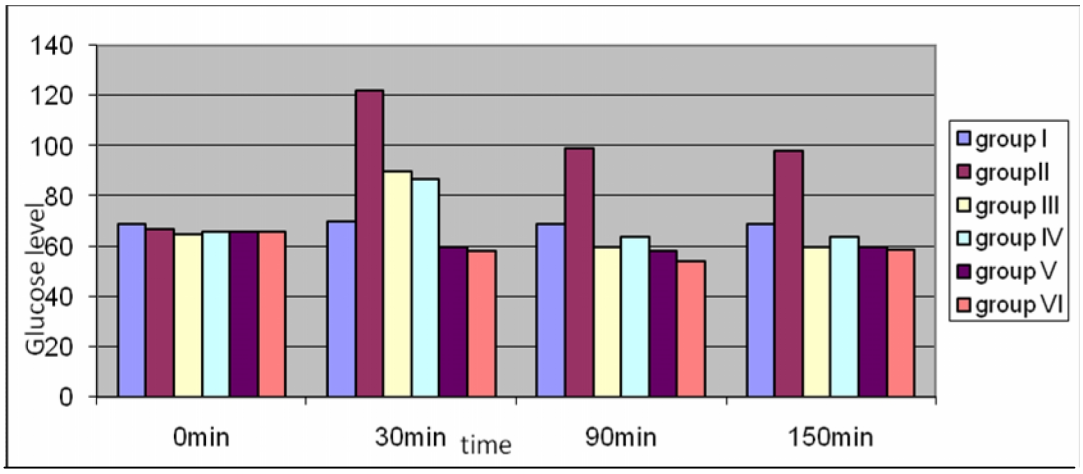

Fig. 1. Effect of hydroalcoholic extract of *N J* on glucose tolerance test

3.2 Acute Toxicity Study

This study showed no mortality up to the dose of 5000mg/kg body weight. So the extracts are safe for long term use.

3.3 Anti Diabetic Effect

The effect of plant extracts and Glibenclamide on blood glucose concentration in normal fasting and diabetic rats after treatment shown in Table 1. On last day of study (14^{th} day) blood glucose level was 78.53±1.43and 90.53±2.55mg/dl in the diabetic rats treated with 500 mg/kg body weight of HAE1 & HAE2 respectively.

Table 1. Long term effect of *Nardostachys jatamansi* extracts HAE1 & HAE2 on the blood glucose level in alloxan induced diabetic rats

Sl. No.	Groups	Blood glucose level mg/dl		
		1st day	7th day	14th day
1	Normal control	73.25±0.91	74.77±0.87	76.54±1.61
2	Diabetic control(150mg/kg)	187.56±2.94	191.86±1.27	195.16±1.76
3	HAE1(500mg/kg)	192.93±5.09	109.49±3.47*	78.53±1.43**
4	HAE2 (500mg/kg)	200.03±4.39	131.39±3.27*	90.53±2.55**
5	Glibenclamide (5mg/kg)	197.44±2.9	112.02±5.34	76.78±4.6**

*Values are given as mean±SEM for n=6, six animals in each group *P<0.05., **P<0.001*

Table 2. Effect on various groups of *Nardostachys jatamansi* on serum profile in alloxan (150mg/kg, i.p.) induced diabetic rats after 14 days of experiment

Groups	Colesterol (mg/dl)	H.D.L (mg/dl)	L.D.L (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Alkaline phosphatase (mg/dl)
Normal control	148.36±2.2	36.83±3.5	90.29±1.1	0.56±0.4	33.80±2.3	119±3.4
Diabetic control	269.14±9.4	29.01±1.6	188±11.3	2.3±0.2	61.65±2.1	269.9±4.1
Alloxan+HAE1 500mg/kg	160.46±4.9*	36.2±3.5*	92.67±4*	0.58±0.1*	31.34±1.9*	137.58±5.1*
Alloxan+HAE2 500mg/kg	179.3±2.3*	35.65±2.2*	119.27±1.6*	0.89±0.4*	41.29± 4*	142.30±5.1*
Alloxan+glibenclamide 5mg/kg	142.39±4.9*	35.69±1.3*	89.33±2.9*	0.60±0.1*	30.22±3.9*	133.75±2.8*

*Values are given as mean±SEM for groups of six animals each *P<0 .05 (Dunnet t-test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control*

Table 3. Effect of *Nardostachys jatamansi* rhizome extract on body weight in diabetic rats

Groups	Body weight of the rats in grams		
	Day 1	Day 7	Day 14
Normal control	210.4±2.34	215.2±3.44	221.5±4.96
Diabetic control	205.78±3.85	179.47±3.15	160.21±5.75
Alloxan+HAE1 500mg/kg	206.33±2.93	197.4±0.80*	183.5±2.7*
Alloxan+HAE2 500mg/kg	207.00±1.43	195.3±1.94*	180.5±1.80*
Alloxan+glibenclamide (5mg/kg)	205.5±1.26*	196.1±1.91	184.0±1.94*

*Values are mean S.E.M; n=6 *P<0.05 vs diabetic control*

3.4 Bio Chemical Estimation

Alloxan treatment increased the serum enzymes levels of LDL, cholesterol, creatinine, alkaline phosphatase and urea along with a fall in HDL level. Plant extracts HAE1& HAE2 (500mg/kg, each) and glibenclamide (5mg/kg) reversed the above alloxan induce changes Table 2 above.

3.5 Body Weight

Body weight of normal control animals were found to be slightly increased, diabetic rats showed significant loss in body weight during 14 days of treatment. Body weight reduction caused by Alloxan is well reversed by HAE1 at dose (500mg/kg) effectively than HAE2, with same dose after 14 days of treatment Table 3 above.

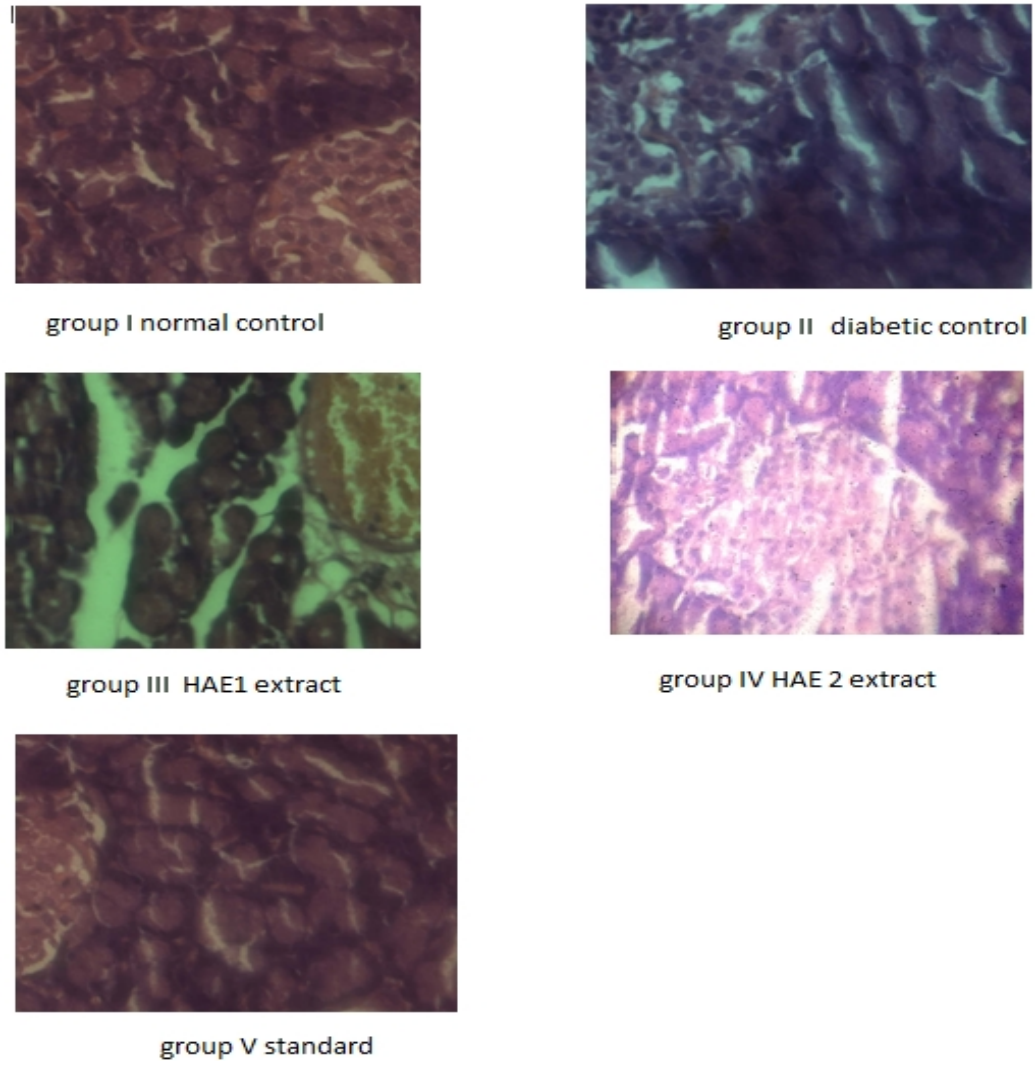

Fig. 2. Histopathological studies of pancreas

3.6 Histopathological Studies

Result of Histopathological studies of Group I showed normal acini and normal cellular population in the islets of Langerhans in pancreas of control rats. Severe damage to the islets of Langerhans observed and reduced size of islets Group II, restoration of normal cellular population dimension of islets with hyperplasia by standard glibenclamide Group V were also observed. The fractional restoration of normal cellular population and inflamed size of *β*-cells with hyperplasia were shown by HAE1 & HAE2 extracts Group III & Group IV, Fig. 2 above.

4. CONCLUSION

The analysis of results of this study reveals that *Nordostachys jatamansi* hydroalcoholic extracts posses good anti diabetic activity in alloxan- induced diabetic rats. These extracts (HAE1 & HAE2) also showed improvement in different parameters associated with diabetes, like body weight, lipid profile, histopathological studies and biochemical parameters. Further studies are required to explain in detail the mechanism of action of *Nordostachys jatamansi* at the cellular and molecular level.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mohammed Abdul Aleem, Ibrahim M, et al. Phytochemical, antioxidant and neurobehavioral study of hydroalcoholic extract of *N. jatamansi* in reversing metoclopramide-induced catalepsy in rats. International Journal of Indigenous Medicinal Plant. 2013;46(3):1338-1347,
2. Vats V, Yadav SP, Grover JK. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin induced alterations in glycogen content and carbohydrate metabolism in rats. Journal of Ethnopharmacology. 2004;90(1):155–160,.
3. Kumar GPS, Arulselvan P, Kumar DS, Subramanian SP. Anti-diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. Journal of Health Science. 2006;52(3):283–291.
4. Ivorra MD, Paya M, Villar A. A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol. 1989;27:243-75.
5. World Health Organization. Department of Non communicable Disease Surveillance. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Geneva: WHO; 1999.
6. Ecobichnon DJ. The Basis of Toxicity Testing. 2nd Ed. New York, NY: CRC Press; 1997.

7. Aruna RV, Ramesh B, Kartha VNR. Effect of betacarotene on protein glycosylation in alloxan induced diabetic rats. Indian Journal of Experimental Biology. 1999;37(4):399–401.
8. Giordano BP, Thrash W, Hollenbaugh L, et al. Performance of seven blood glucose testing systems at high altitude. The Diabetes Educator. 1989;15(5):444–448,
9. Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. Journal f ¨ ur Clinical Chemistry and Clinical Biochemistry. 1974;12(5):226.
10. Mueller PH, Schmuelling RM, Liebich HM. A fully enzymatic triglyceride determination. Journal of Clinical Chemistry and Clinical Biochemistry. 1977;15(9):457-464.
11. Allain CC, Poon LS, Chan CSG. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974;20(4):470-475.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.
13. Bowers LD. Kinetic serum creatinine assays I. The role of various factors in determining specificity. Clinical Chemistry. 1980;26(5):551-554.
14. Sasaki M. A new ultramicro method for the determination of serumalkaline phosphatase. Use of Berthelot's reaction for the estimation of phenol released by enzymatic activity. To Igaku Seibutsugaku. 1966;70(4):208-214.
15. Wilson BW. Automatic estimation of urea using urease and alkaline phenol. Clinical Chemistry. 1966;12(6):360-368.

© 2014 Aleem et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=579&id=12&aid=5024