



## Beneficial effect of *Bacopa monnieri* against alcohol induced cardiac toxicity in albino rats

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### Abstract

The current investigation has been conducted to investigate the influence of *Bacopa monnieri* on cardiac tissue antioxidant enzymes system in Alcohol treated rats. Alcohol significantly decreased the Superoxide dismutase, catalase, Glutathione peroxidase, Glutathione reductase activities and Glutathione content were estimated in the cardiac tissue. This effect was reversed by a treatment with *Bacopa monnieri* for 4 weeks in rats by improved antioxidant status which suggest that treatment of *Bacopa monnieri* may have protective role against the Alcohol induced myocardial toxicity.

**Keywords:** Alcohol, *Bacopa monnieri*, antioxidant enzymes, cardiac tissue, rats

### Introduction

Chronic alcohol consumption has been reported to have detrimental effect on behavior and cognitive processes such as learning and memory. Myocardial infarction (MI) is an acute condition of necrosis of the myocardium that occurs as a result of sudden or persistent interruption of blood supply to the demand of myocardium [1]. Every year, worldwide more than 7 million people have been affected with MI [2]. However, the occurrence of myocardial damage is mainly due to hyperlipidemia, loss of plasma membrane

integrity and membrane peroxidation [3]. Alcohol is one of the major risk factors for incidence of myocardial damage, and chronic alcohol consumption has been reported to have J or U shaped relationship with myocardial damage [4]. Wannamethee and Shaper [5] reported a strong correlation between alcohol intake and sudden cardiac death. An oxidative stress may represent a fundamental mechanism in the production of myocardial injury [6]. The increased conversion of XD into XO that has been detected in the heart after administration of a single ethanol dose, may contribute to this lipid peroxidation [7].

*Bacopa monnieri*, (a member of the *Scrophulariaceae* family) also referred to as *Bacopa monnieri*, *Herpestis monniera*, water hyssop, and “Brahmi,” has been used in the Ayurvedic system of medicine for centuries. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration [8] and to provide relief to patients with anxiety or epileptic disorders [9]. The compounds responsible for the pharmacological effects of *Bacopa* include alkaloids (Brahmine and herpestine), saponins (d-mannitol and hersaponin, acid A, and monnieri) and other active constituents such as betulic acid, stigmastanol, beta-sitosterol, as well as numerous bacosides and bacosaponins. The constituents responsible for *Bacopa*'s cognitive effects are bacosides A and B [10]. In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory [11] and neuropharmacological [12] disorders like insomnia, insanity, depression, psychosis, epilepsy and stress. It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative [13] free radical scavenging and anti-lipid peroxidative [14]. The present study was undertaken to assess the cardio protective role of *Bacopa monnieri* against oxidative stress in the cardiac tissue of rats exposed to alcohol by measuring the enzymatic antioxidants.

## Materials and Methods

**Animals:** The study involved male young (3 months; 200±220g) albino rats of wistar strain purchased from Sri Venkateswara Traders Pvt. Limited, Bangalore, maintained in the animal house of the department in polypropylene cages. Standard conditions of humidity (50± 9% relative humidity), room temperature (25-28°C) and 12 h light/ dark cycle (6:00 AM to 6:00 PM) were maintained. A standard rodent diet (M/s Hindustan Lever Ltd., Mumbai) and water were provided *ad libitum*. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee (Regd No.438 /01 / a / CPCSEA/dt.17.07.2001) in its resolution number

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## Preparation of *Bacopa monnieri* Plant Extract and Dose

Fresh *Bacopa monnieri* plant was obtained from the Tirumala hills, Andhra Pradesh, India, and the whole plant was dried under shade dust-free conditions, and was ground into fine powder. 120g of powder has taken and macerate in 1000 ml of 95% ethanol for 12 h at room temperature, then filtered and squeezed with muslin cloth to obtain ethanol extract. This process was repeated three times and finally collection of this extract were dried in rotary evaporator (Model: HS-2005V) and obtained jelly like material and then this jelly was converted to powder. We has done dose dependent studies by using, 50 mg/kg, 100 mg/kg, 150 mg/kg, 200 mg/kg, 250 mg/kg and 300 mg/kg, of this 200 mg/kg dose showed good antioxidant activity. So this study we selected dose of 200 mg/kg of ethanol extract of *Bacopa monnieri*.

## Experimental Design

The rats were divided in to four groups, 8 animals in each and were treated as described below.

Group I: Normal Control (NC): rats were rats received normal 0.9% saline orally via orogastric tube for equivalent handling.

Group II: Alcohol treatment (At): rats received alcohol orally at the dose of 2g/kg body weight via orogastric tube for 4 weeks.

Group III: *Bacopa monnieri* treatment (Bmt): All rats in this group were treated with BM at the dose of 200 mg/kg b.w. *via* an orogastric tube for 4 weeks

Group IV: Alcohol + *Bacopa monnieri* treatment (At+Bmt): alcohol intoxicated rats received *Bacopa monnieri*, as described group III for a period of 4 weeks.

## Isolation of tissues

After completion of 4 weeks of treatment, the animals were sacrificed by cervical dislocation and the cardiac tissues were excised at 4°C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80°C for further biochemical analysis.

## Analytical procedure

Cardiac tissue superoxide dismutase (SOD) activities were assayed in the heart homogenates by the method of Misra and Fridovich [15] at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Catalase (CAT) activity was determined at room temperature by using the method of Aebi [16]. Activity of glutathione peroxidase (GPx) was determined by the method of Flohe and Gunzler [17]. Glutathione reductase (GR) activity was determined according to the method of Carlberg and Mannervik [18]. Glutathione content was measured at 340 nm as per the method of Theodorus [19]. All the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry et al., [20] using bovine serum albumin (BSA) as a standard.

## Chemicals

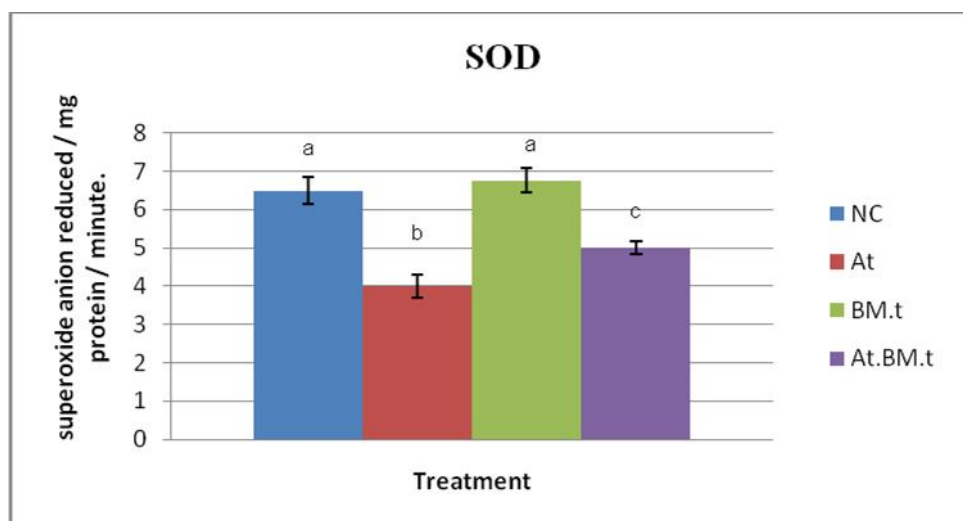
All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburgh, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), and Qualigens (Mumbai, India).

## Statistical analysis

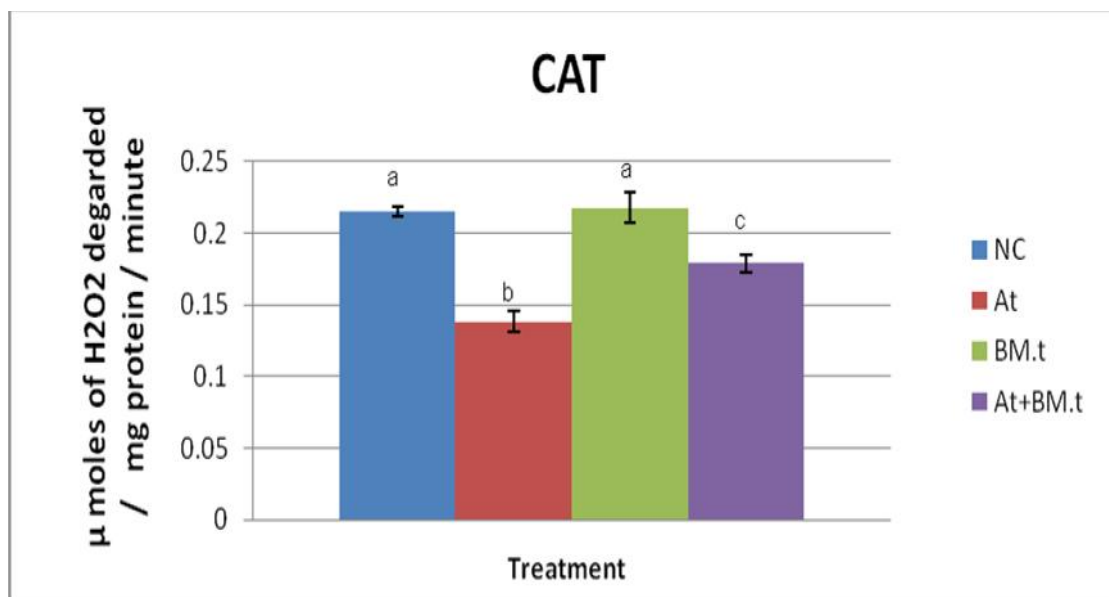
The data has been analyzed by using SPSS (Version 16.0; SPSS Inc., Chicago, IL, USA) and M.S. Office, Excel Software for the significance of the main effects (factors), and treatments along with their interactions. The data has been compared using one way ANOVA with Dunnett's multiple comparison test and differences were considered significant at  $p < 0.001$ .

## Results and Discussion

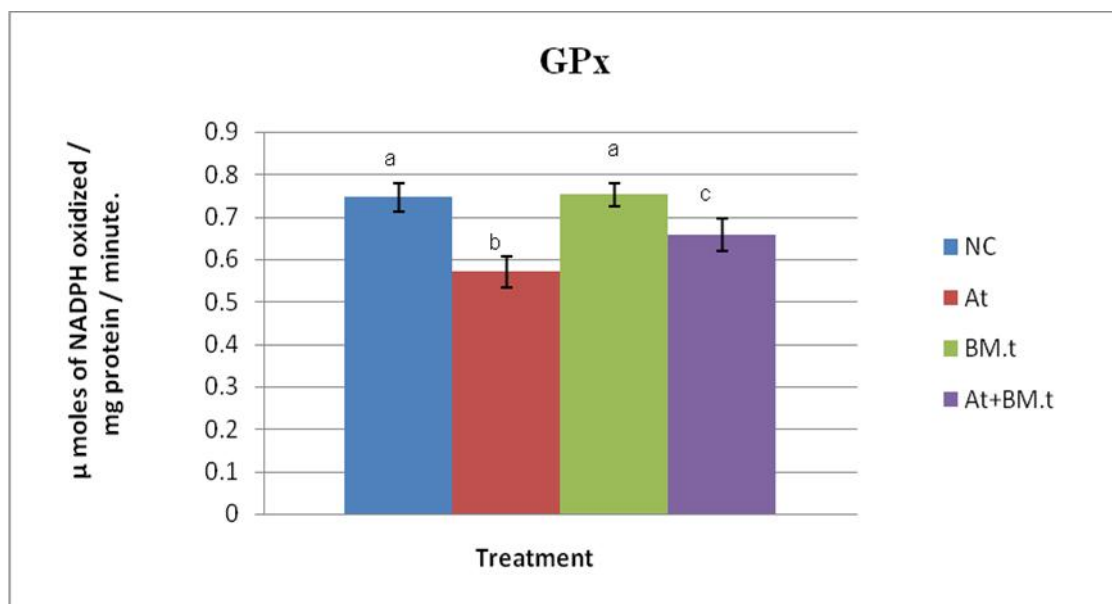
In the current study, we observed significant ( $p < 0.001$ ) decrease in SOD, CAT, GPx, GR activities and GSH level in the alcohol treated rats compared with normal control rats. However *Bacopa monnieri* treatment increased SOD, CAT, GPx, GR activities and GSH level in alcohol treated rats. (Figs. 1–5).



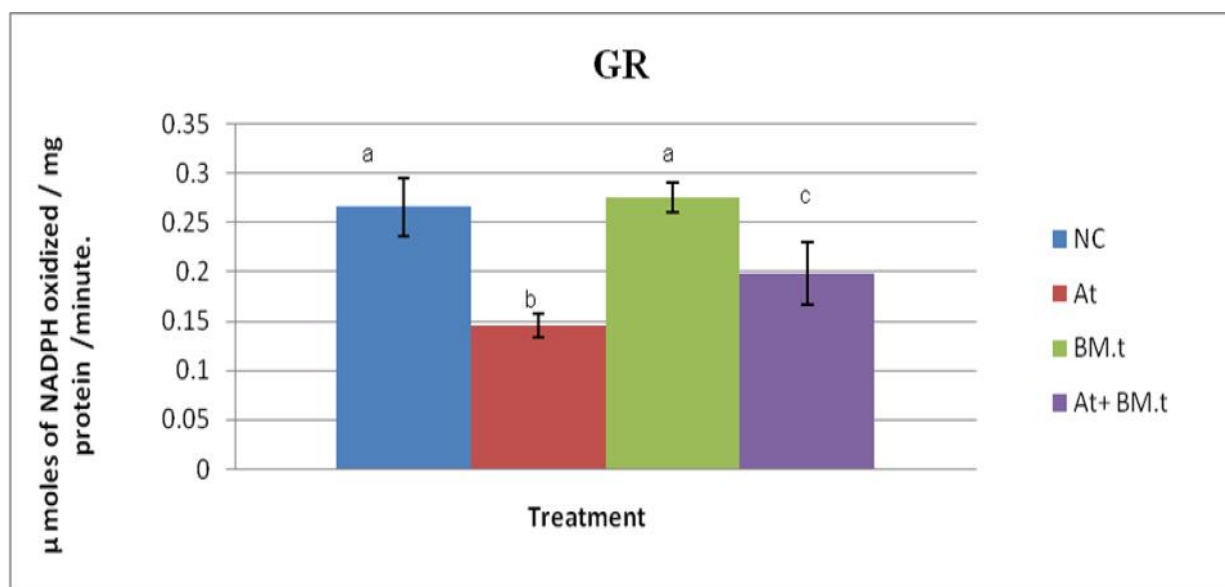
**Fig 1:** Changes in SOD activity in the heart of Normal Control (NC), Alcohol treated (At), *Bacopa Monnieri* treatment (BM.t), Alcohol rats treated with *Bacopa Monnieri* ( At + BM.t). Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



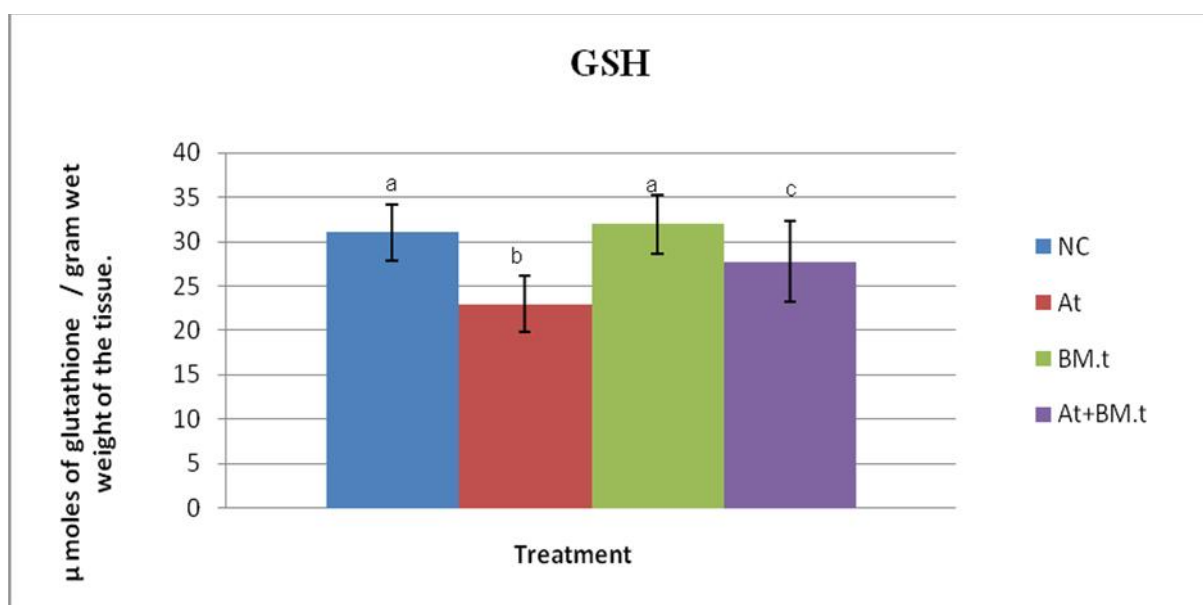
**Fig. 2:** Changes in CAT activity in the heart of Normal Control (NC), Alcohol treated (At), *Bacopa Monnieri* treatment (BM.t), Alcohol rats treated with *Bacopa Monnieri* ( At + BM.t). Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



**Fig. 3:** Changes in GPx activity in the heart of Normal Control (NC), Alcohol treated (At), *Bacopa Monnieri* treatment (BM.t), Alcohol rats treated with *Bacopa Monnieri* ( At + BM.t). Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



**Fig. 4:** Changes in GR activity in the heart of Normal Control (NC), Alcohol treated (At), *Bacopa Monnieri* treatment (BM.t), Alcohol rats treated with *Bacopa Monnieri* ( At + BM.t). Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .



**Fig. 5:** Content in GSH in the heart of Normal Control (NC), Alcohol treated (At), *Bacopa Monnieri* treatment (BM.t), Alcohol rats treated with *Bacopa Monnieri* ( At + BM.t). Each vertical bar represents the mean  $\pm$  SD (n=6). Top of the vertical bars having the same letter do not differ significantly at  $p < 0.01$ .

The direct effect of Alcohol consumption in body is achieved by the formation of free radicals, which react with various cellular components and cause damage to the tissues. Among other antioxidant enzymes, SOD is the first enzyme in antioxidant defense that scavenges superoxide radicals to form  $H_2O_2$  and hence diminishes the

toxic effects of the radical. In the present study, rats which received 2.0 g of alcohol for a period of 4 weeks showed a significant decrease in the heart SOD activity. Similar decrease in SOD activity was also reported by previous studies in the heart of rats with chronic ethanol feeding [21].

The significant decrease in SOD activity due to alcohol indicates inefficient scavenging of reactive oxygen species (ROS) which might be implicated to oxidative inactivation of enzymes [22]. The SOD activity was elevated in group III and group IV rats which received *Bacopa monnieri* in alcohol intoxicated rats. Recently Abhishekh mathur et al., 2010 [23] indicated that phenolic compounds Bacoside-A of *Bacopa monnieri*. These compounds may be responsible to scavenge the superoxide anion radicals and thereby maintain the high activity of SOD even in alcoholics.

CAT can detoxify the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) to less toxic H<sub>2</sub>O and O<sub>2</sub>. We also found that the administration of alcohol has considerably decreased cardiac tissue CAT activity. The lower activity of CAT may be due to lower levels of SOD or may be due to inactivation of catalase owing to excess production of free radicals, especially hydrogen peroxide. Bindu et al., [24] reported a significant decrease in CAT activity with 4 g/kg EtOH treatment for a period of 50 days in rats. The decreased CAT activity with alcohol treatment indicates inefficient scavenging of hydrogen peroxide due to oxidative inactivation of enzyme [25]. The groups of rats which received *Bacopa monnieri* for a period of 4 weeks showed significant elevation in CAT activity in the brain tissues which indicates the antioxidant activity of *Bacopa monnieri*. These results are in agreement with that of previous investigations by Tripathi et al., 1996 [26], which report the antioxidant property of *Bacopa monnieri*.

In the current study, we observed decreased activity of GSH-Px in alcohol treated rats. Decrease in GSH-Px activity may be implicated to either free radical dependant inactivation of enzyme [27] or depletion of its co-substrate i.e., GSH and NADPH [28]. The reduced GSH-Px activity may also be due to reduced availability of GSH as observed in the current investigation. The activity of GSH-Px was significantly increased with *Bacopa monnieri* and also with alcohol and *Bacopa monnieri* combination treatment groups which indicates that *Bacopa monnieri* could

inhibit and/or scavenge the free radicals in rat brain tissue. Shanmugham and colleagues reported that ethanol extracts of Ginger were able to increase SOD, CAT and GPx activities in renal tissue of the rat.

The activity of cardiac GR was significantly decreased with ethanol treatment in the rats. (Mallikarjuna et al., 2007 [29] also reported similar decrease in GR activity with EtOH treatment (1.6 g/kg) in hepatic tissue of rats. Also Das and Vasudevan [30] in their alcohol dose dependent studies found a significant decrease in GR activity in the hepatic tissue of rats. The decrease in GR activity after ethanol intoxication reflects the impaired conversion of glutathione of oxidized form to reduced form [31] thus alters the GSH/GSSG ratio. The rats received *Bacopa monnieri* for a period of 4 weeks showed a remarkable elevation in cardiac GR activity. Similarly the decreased GR activity with ethanol treatment was also recovered with *Bacopa monnieri* supplementation. *Bacopa monnieri* contains a mixture of triterpenoid saponins designated as Bacosides A and B [32]. We speculate that these compounds may quench the alcohol-induced free radicals and improve the GR activity. The increased GSH concentration with *Bacopa monnieri*, which was also reported in this study, may further be responsible for the increased GR activity in the heart.

In our study, we observed significantly lower GSH levels in alcohol treated rats, which indicates increased oxidative stress in the cardiac tissue. Earlier reports also demonstrated the decreased GSH levels following alcohol administration in rats in different tissues [33]. This is presumably due to enhanced utilization of reduced glutathione by glutathione peroxidase in detoxification of H<sub>2</sub>O<sub>2</sub> generated by alcohol in reduced oxidative stress [34] or as a consequence of diminished glutathione reductase activity, which is crucial for maintaining reduced/oxidized ratio in the cell. Interestingly in our study, *Bacopa monnieri* supplementation to alcohol ingested rats exhibited increased GSH levels in the brain. Rohini et al., 2004 [35] explained that *Bacopa monnieri* exerts an antioxidant effects by



decreasing lipid peroxidation, increasing GSH level and maintaining normal levels of antioxidant enzymes. Furthermore, as reported in this study, the increased GPx and GR activities with *Bacopa monnieri* combination treatment may be responsible for the restoration of depleted GSH levels.

## Conclusion

The above findings show that alcohol induces an oxidative stress on the cardiac tissue by diminishing antioxidant status. *Bacopa monnieri* modulates alcohol induced peroxidative changes probably through its free radical scavenging and antioxidant activities in the cardiac tissue. Thus, the results of our investigation suggest that *Bacopa monnieri* can act as potent antioxidant against alcohol induced toxicity and hence may have useful in preventing cardiac damage caused by oxidative stress. These results suggest that *Bacopa monnieri* extract can be used to protect myocardial tissue damage.

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