

## SHORT COMMUNICATION

# Antilipid Peroxidative Property of Shilajit

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We have investigated the effect of shilajit on lipid peroxidation and glutathione content in rat liver homogenate. It inhibited lipid peroxidation induced by cumene hydroperoxide and ADP/Fe<sup>++</sup> complex in a dose dependent manner. It also reduced the rate of oxidation of reduced glutathione content and inhibited ongoing lipid peroxidation, induced by these agents immediately after its addition to the incubation system.

**Keywords:** Shilajit; lipid peroxidation; antioxidant.

### INTRODUCTION

Shilajit is a blackish brown exudation, consisting of organic substances, metal ions and minerals from steep rocks of different formations commonly found in the Himalayan belt (1000–3000 m) from Nepal to Kashmir. Chemically it possesses oxygenated biphenyls, oxygenated benzocumarins, several phenolic compounds, amino acids and triterpenes. It appears that latex and debris of several plants growing in those areas are responsible for the formation of shilajit.

Shilajit is in use for the management of bronchial asthma, diabetes, genitourinary infections, wound healing and stomach ulcer. It is also used in cold stress and as a general tonic. Shilajit shows a positive response on memory and anxiety. In the Ayurveda, it is also recommended as antiaging, as medhya rasayana, adaptogenic and mast cell stabilizing property (Ghosal *et al.*, 1989).

We report, for the first time, the antioxidant property of shilajit on a liver homogenate system, induced by cumene hydroperoxide and ADP-complexed iron. The results indicate its protective response on both enzymatic and non-enzymatic lipid peroxidation and glutathione content.

Besides the low molecular weight compounds, there are also several high molecular weight compounds that are present; such as humic acid (HA), fulvic acid (FA) and its derivatives, consisting of H-rich aromatic moieties. This suggests the involvement of humification in the formation of shilajit (Ghosal, 1988).

### MATERIALS AND METHODS

Shilajit, collected from Nepal, was powdered and dissolved in water. The soluble fraction was saved and dried to recover the pure shilajit. This process was repeated three times. Pure shilajit showed peaks in the ultraviolet range at 371 nm and 270 nm.

*In vitro* experiments were carried out on 5% rat liver homogenate in phosphate buffer saline (pH 7.4) as described earlier (Pandey *et al.*, 1994). In brief, the homogenate was preincubated for different time intervals

with different concentrations of shilajit and then cumene hydroperoxide (CHP, 1.5 mM) was added. At different time intervals, samples were collected and the concentration of malondialdehyde (MDA) and reduced glutathione was estimated by standard methods of Ohkawa *et al.*, (1979) and Ellman (1959) respectively as described earlier (Tripathi *et al.*, 1995; Sharma *et al.*, 1995). Similarly ADP-complexed iron (Hogeberg *et al.*, 1975), an example of enzymatic lipid peroxidation, was also added to the system and the above parameters were evaluated.

### RESULTS AND DISCUSSION

#### Protective effect of shilajit on induced lipid peroxidation

The homogenate was preincubated with different concentrations of shilajit for 20 min and then 1.5 mM cumene hydroperoxide and ADP-complexed iron were added in

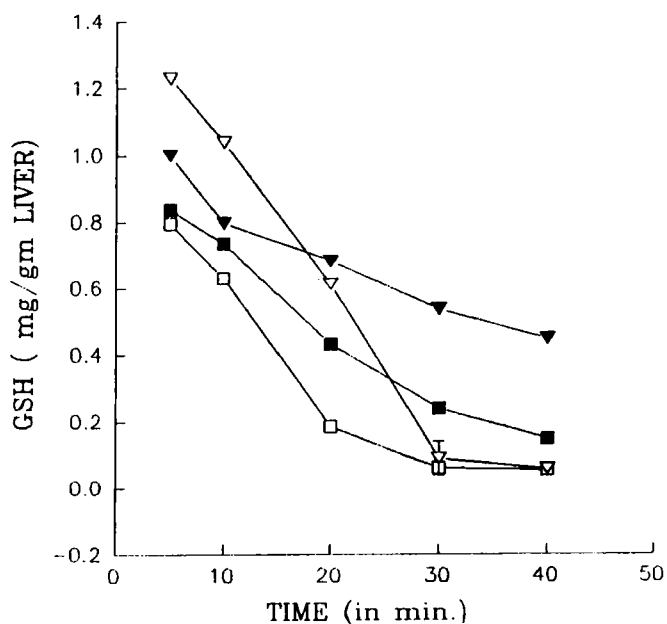
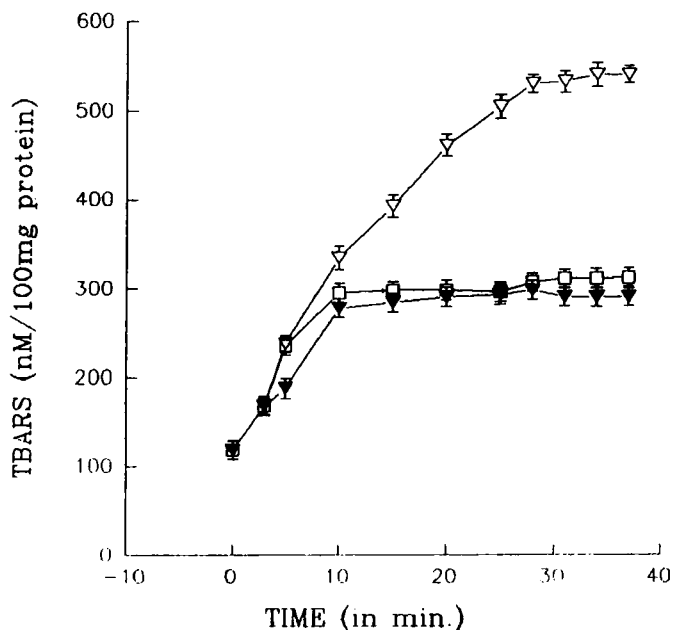


Figure 1. Effect of shilajit on CHP induced glutathione level in rat liver homogenate. Control (▽), shilajit (▼), CHP (□), shilajit+CHP (■).

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**Figure 2.** Curative effect of shilajit on CHP induced lipid peroxidation. No drug (▽), 5 min (▼), 10 min (□).

different plates to induce lipid peroxidation. Shilajit inhibited the lipid peroxidation in the dose dependent manner (Table 1) in both conditions.

#### Effect on reduced glutathione (GSH) content

In normal conditions, the glutathione content declined sharply and reached to the basal level in 50 min as a result of autooxidation, but in the presence of shilajit (1 mg/mL),

**Table 1.** Protective effect of shilajit on cumene hydroperoxide and ADP-complexed iron induced lipid peroxidation. (Values are mean  $\pm$ SD of six animals in each group)

Shilajit (mg/mL)	TBARS (nm/100 mg protein)	
	Cumene hydroperoxide	ADP-complexed iron
0.00	480.68 $\pm$ 11.50	477.82 $\pm$ 9.98
0.30	420.43 $\pm$ 10.93 <sup>a</sup>	438.78 $\pm$ 9.79 <sup>a</sup>
0.60	365.30 $\pm$ 11.25 <sup>a</sup>	393.82 $\pm$ 6.59 <sup>a</sup>
0.90	298.40 $\pm$ 10.23 <sup>a</sup>	301.64 $\pm$ 8.79 <sup>a</sup>
1.20	216.30 $\pm$ 10.83 <sup>a</sup>	225.30 $\pm$ 9.32 <sup>a</sup>
2.40	140.60 $\pm$ 11.23 <sup>a</sup>	186.25 $\pm$ 8.75 <sup>a</sup>

Level of significance <sup>a</sup> $p < 0.001$ .

Cumene hydroperoxide = 1.5 mM.

ADP-complexed iron = 1.6 mM ADP + 62  $\mu$ M FeCl<sub>3</sub>.

the rate of decline was significantly reduced (Fig. 1). Its normal level was maintained up to 60 min. This effect was seen even in the presence of 1.5 mM cumene hydroperoxide.

#### Curative response of shilajit

In a separate experiment the curative response of shilajit was investigated. Here, it was added to the incubation mixture after 4 min and 8 min of CHP addition. The results (Fig. 2) indicate a significant inhibition in the production of malondialdehyde.

It is now well established that free radicals are involved in several diseases (Cheeseman and Slater, 1993). The therapeutic use of shilajit in the cure of asthma, diabetes, wound healing, cold stress, ulcers (Tewari *et al.*, 1993; Jaiswal and Bhattacharya, 1992) and also the presence of several triterpenes, and phenolic compounds (Ghasal, 1988) led us to investigate the effect of shilajit on lipid peroxidation, because in these diseases, free radicals are involved and compounds of this chemical structure, have a free radical scavenging property. Interestingly, the water extract of shilajit also exhibits significant antioxidant property on both enzymatic and nonenzymatic lipid peroxidation. This property is not common with other antioxidants which are in common use and raises the therapeutic value of shilajit. On comparison with an established antioxidant of plant origin, i.e. *Rubia cordifolia*, we found that the activity of shilajit was more than the alcohol extract of *R. cordifolia* and comparatively less than parabenzquinone, a synthetic antioxidant (Tripathi *et al.*, 1995). The ED<sub>50</sub> ratio indicates that 1.2 mg of shilajit is equivalent to 2.3 mg of *R. cordifolia* extract and 0.06 mg parabenzquinone.

This study opens a ray of hope that although many plants described in ayurvedic literature are now extinct, they may be utilized therapeutically by using their exudate in the form of humous of that locality that has been formed over the centuries.

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