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Seasonal Variation in Hepatoprotective Activity of Titeypati (*Artemisia vulgaris* L.) Leaves on Antitubercular Drugs Induced Hepatotoxicity in Rats.

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Abstract

Plant Titeypati (*Artemisia vulgaris* L.), belongs to the family Asteraceae, is abundant in Sikkim and Darjeeling Himalayas in the middle and upper hill forest up to the height of 2000- 5000 ft. Titeypati is a medicinal plant traditionally used to treat a wide range of diseases. Hepatoprotective activity of Titeypati has been noted in hepatotoxicity in rats. Seasonal variation in this effect, if any, was studied. Results showed that Titeypati leaves during the period May – June had maximum protective effect on anti tubercular drugs induced hepatotoxicity in rats.

Keywords **:** Titeypati, *Artemisia vulgaris* L., hepatotoxicity, anti tubercular drugs .

Introduction

Plant Titeypati (*Artemisia vulgaris* L.) belongs to the family Asteraceae consists more than 500 species distributed globally. It is a perennial shrubby aromatic plant throughout the hills of India. The plant is abundant in Sikkim and Darjeeling Himalayas in the middle and upper hill forest up to the height of 2000- 5000 ft. The plant has different names : Titeypati in Nepali, Tuk – gnyel in Lepcha, Dhama naga in Tibetan, Dona in Hindi, Nagdamini in Bengali, Barha in Sanskrit and Indian worm wood in English. The whole plant has medicinal values. In Ayurvedic literature the plant is coined as appetizer, cures "kapha", asthma and itching, prevents convulsion [1]. The plant is traditionally used to treat a wide range of conditions, including circulatory problems, menopausal and menstrual complaints, gastrointestinal disorders, headaches, nose bleeds, muscle spasms, epilepsy, fever, rheumatism, asthma, gout, infertility, malaria and worm infestations, contact dermatitis, bacterial infections, inflammation etc. [2,3].

In modern research leaves of Titeypati has been demonstrated having anti microbial activity against different Gram-positive and Gram-negative bacteria [4-6]. This is due to the essential oil present in the leaves [7]. Twenty known flavonoids were isolated from *Artemisia vulgaris,* most of them are estrogenic in nature [8]. Water extract of the plant is good larvicide like kerosene. It has also feeble insecticidal property [9-10]. Hepatoprotective activity of aqueous-methanol extract of Artemisia vulgaris was noted by Gilani *et al*.[11]

Since medicinal values of plants vary with season [12-16], we were interested to note the seasonal variation, if any, in the hepatoprotective activity of the leaves of Titeypati (*Artemisia vulgaris* L.).

Materials and Methods

Titeypati leaves

Titeypati (*Artemisia vulgaris* L.) leaves were collected from the Medicinal Plants Garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India in January –

February, March- April, May - June, July - August, September - October, November - December, 2010. Leaves were identified by the experts of the department of Botany of the said University and a voucher specimen was kept in the Department of Biochemistry, North Bengal Medical College, Siliguri, West Bengal for future reference.

Fig. 1 : Leaves of Titeypati (*Artemisia vulgaris* L.)

Preparation of the Test Drug

Decoction from fresh tender leaves of Titeypati was prepared by the method of Pillai and Santhakumari [17]. The decoction was used as test drug in the dose of 1 ml/kg (1 g leaves per ml of decoction) orally through a feeding tube. Selection of the dose of the test drug was as per the method of Kate *et al.* [18].

Experimental animals

Wistar strain albino rats (180 - 200 g) of either sex were used for the study. Rats were housed in colony cages (5 rats / cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25 – 28 degree centigrade and

humidity 60 – 65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. 8 rats were used for each set of experiment. The animal experiment was approved by the ethics committee of the Institute.

Acute oral toxicity study

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh [19]. Test drug developed from the leaves of Titeypati leaves was given orally in doses of 1, 2, 3, 4 and 5 ml/kg to different groups of mice each group containing six animals. After administering the test drug, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

Chemicals and Drugs

Isoniazid, rifampicin (R), pyrazinamide (Z) were procured from Plethico Pharmaceuticals Ltd. Indore. All other chemicals were collected from Sigma Chemical Co., USA.

EXPERIMENTAL DESIGN

Rats were divided into following groups. In each group eight rats were employed.

Group 1: Vehicle control – gum acacia was given to rats orally for one month.

Group 2: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension was given to rats orally for one month. Doses are optimum to produce hepatotoxicity in rats [17].

Group 3: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of Titeypati of the month of January – February for one month.

Group 4: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of

Titeypati in the month of March – April for one month.

Group 5: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of Titeypati of the month of May – June for one month.

Group 6: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of Titeypati of the month of July – August for one month.

Group 7: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of Titeypati of the month of September – October for one month.

Group 8: Isoniazid (27 mg/kg/day) + rifampicin (54 mg/kg/day) + pyrazinamide (135 mg/kg/day) suspension and test drug (1 ml/kg) as obtained from the leaves of Titeypati of the month of November – December for one month.

Assessment of liver damage

To assess liver damage blood samples were collected from the rats by cardiac puncture under ether anesthesia on the 30th day. Serum total protein, serum bilirubin and serum alanine aminotransferase (ALT) were estimated by the methods as followed by Kate *et al*.[18].

Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 20th versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity studies

Acute toxicity studies revealed that the test drug (develop from the leaves of Titeypati did not produce any toxic symptoms when administered orally to mice in doses of 1, 2, 3, 4 and 5

ml/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment

Seasonal variation in hepatoprotective activity of the leaves of Titeypati is given in following table.

Table – 1: Showing seasonal variation in hepatoprotective activity of the leaves of Titeypati against antitubercular drugs induced hepatotoxicity in rats

Groups	Serum total protein (g/dl)	Serum bilirubin (mg/dl)	Serum ALT (Units/ml)
Group 1	7.2 ± 0.42	0.93 ± 0.07	36.34 ± 5.11
Group 2	5.0 ± 0.33**	2.16 ± 0.12**	189.56 ± 7.12**
Group 3	5.9 ± 0.43	1.86 ± 0.17	161.14 ± 7.01
Group 4	6.2 ± 0.31*	1.64 ± 0.12*	151.32 ± 6.55*
Group 5	6.8 ± 0.38**	0.98 ± 0.06**	38.94 ± 5.16**
Group 6	6.3 ± 0.33*	1.67 ± 0.13*	130.11 ± 6.32*
Group 7	5.8 ± 0.44	1.82 ± 0.15	161.34 ± 6.56
Group 8	5.5 ± 0.33	1.83 ± 0.16	170.72 ± 6.87

Values were mean ± SEM of eight animals in each group. * $p < 0.05$, ** $p < 0.001$.

Results showed that anti tubercular drugs in the given dose could produce hepatotoxicity in rats. Liver markers like serum bilirubin and serum ALT elevated and level of serum total protein decreased after administering the anti tubercular drugs. All changes were statistically significant. Leaves of Titeypati could protect the rats from hepatotoxicity. Maximum activity was noted by the leaves of May and June. Results were statistically significant up to the level of $p<0.001$.

Effect of leaves of Titeypati of different seasons on serum total protein in rats during

hepatoxicity as induced by antitubercular drugs is shown by the following figure (Fig 2).

Results showed that leaves of Titeypati of the period May to June could increase level of

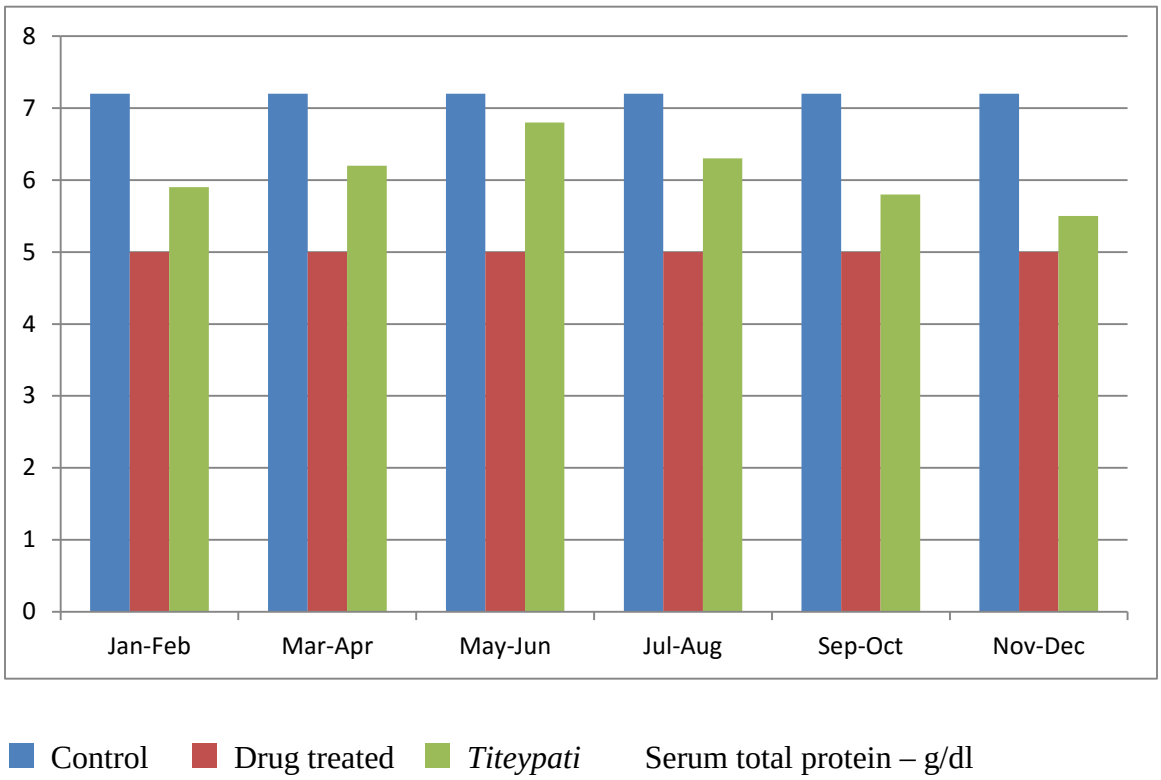

Fig 2 : Effect of leaves of Titeypati of different seasons on serum total protein in rats during hepatoxicity as induced by antitubercular drugs

serum total protein in rats almost to control value during hepatoxicity as induced by antitubercular drugs. In hepatotoxic rats serum total protein came 5.0 ± 0.33 g/dl while in Titeypati leaves (May – June) treated rats the value was 6.8 ± 0.38 g/dl (control value, 7.2 ± 0.42g/dl). Results were statistically significant up to the level of $p<0.001$. Leaves of Titeypati (for the months of March – April and July – August) could also increase serum protein level in rats during hepatotoxicity but the magnitude was less than that of the leaves of Titeypati for the months of May and June. Leaves of Titeypati for the months of September – October, November

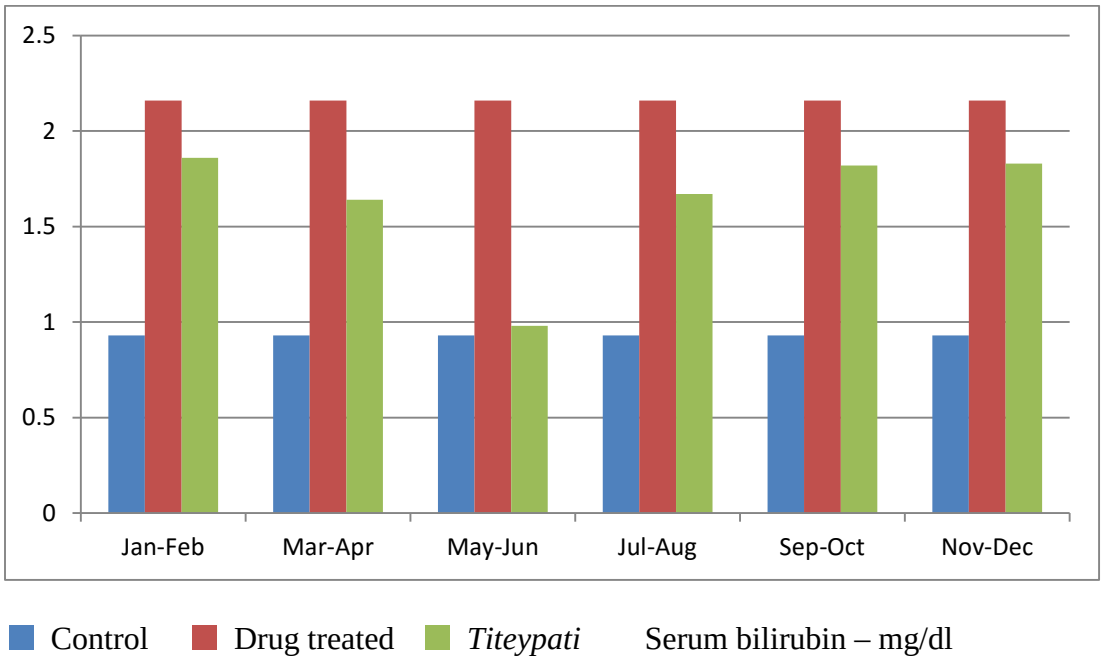

Fig 3 : Effect of leaves of Titeypati of different seasons on serum bilirubin in rats during hepatoxicity as induced by antitubercular drugs

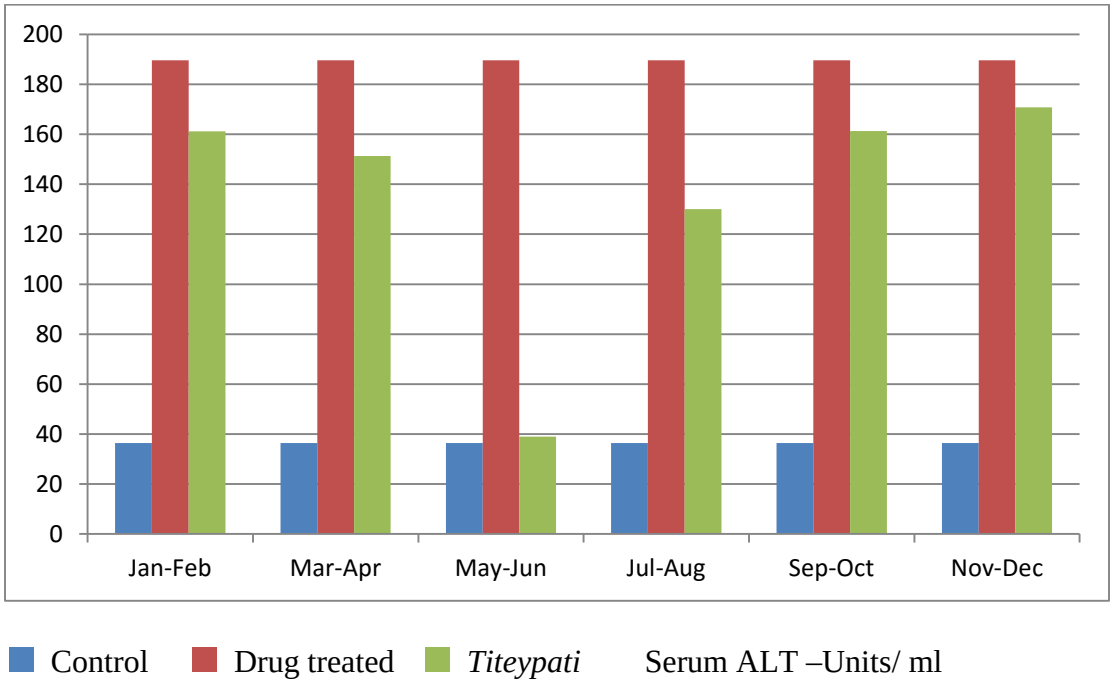

Fig 4 : Effect of leaves of Titeypati of different seasons on serum ALT in rats during hepatoxicity as induced by antitubercular drugs

– December and January – February , however, did not show any effect on serum total protein level in hepatotoxic rats. Same results were also found in case of serum bilirubin and serum ALT (Fig. 3 and 4) when the rats were treated with the leaves of Titeypati of the period May to June.

Figure 3 shows effect of leaves of Titeypati of different seasons on serum bilirubin in rats during hepatoxicity as induced by antitubercular drugs.

Results showed that antitubercular drugs could induce hepatotoxicity by increasing serum bilirubin in rats. Serum bilirubin level came 2.16 ± 0.12 mg/dl while in control group it was 0.93 ± 0.07 mg/dl. Treatment of these rats with leaves of Titeypati of the period May to June could decrease serum bilirubin level almost to control value. In this group level of serum bilirubin came 0.98 ± 0.06 mg/dl. Results were statistically significant up to the level of $p<0.001$. Leaves of Titeypati (for the months of March – April and July – August) could also decrease serum bilirubin level in rats during hepatotoxicity but the magnitude was less than that of the leaves of Titeypati for the months of May and June. Leaves of Titeypati of September – October, November – December and January – February , however, did not show any effect on serum bilirubin level in hepatotoxic rats.

Effect of leaves of Titeypati of different seasons on serum ALT in antitubercular drugs induced hepatoxicity in rats is shown in Figure 4.

It was noted that leaves of Titeypati of the period May to June could decrease serum ALT in rats almost to control value during hepatoxicity as induced by antitubercular drugs. In hepatotoxic rats serum ALT came 189.56 ± 7.12 U/mL while in Titeypati leaves (May – June) treated rats the value was 38.94 ± 5.16 U/mL (control value, 36.34 ± 5.11 U/mL). Results were statistically significant up to the level of $p<0.001$. Leaves of Titeypati (for the months of March – April and July – August) could also decrease serum ALT in rats during hepatotoxicity but the

magnitude was less than that of the leaves of Titeypati for the months of May and June. Leaves of Titeypati for the months of September – October, November – December and January – February , however, did not show any effect on serum ALT activity in hepatotoxic rats.

Gilani *et al*.[11] observed hepatoprotective activity of aqueous-methanol extract of *Artemisia vulgaris* during hepatoxicity in rats. Our study confirmed this observation.

Fluck and Pharm [20] showed influence of climate on the active principles in medicinal plants. Thereafter, series of experiments were conducted in this direction. Now a days numerous reports are available in literature which suggest that accumulation of chemical compounds in roots, stem and leaves of plants varies with season [12-16].

In the present study we also noted seasonal variation in hepatoprotective activity of the leaves of Titeypati. It was revealed that leaves of Titeypati of the period May to June had maximum hepatoprotective effect against antitubercular drugs induced hepatotoxicity in rats. This is probably due to maximum accumulation of some bioactive compound(s) in the leaves of Titeypati of that period responsible for hepatoprotective activity. We are now interested to isolate the bioactive compound(s) from the leaves of Titeypati of the period May to June. Work is in progress in this direction.

CONCLUSION

Titeypati leaves of the period May – June had maximum hepatoprotective activity on anti tubercular drugs induced hepatotoxicity in rats.

References

[1] Arya Vaidya sala (1994) *Indian medcinal plants*. orient Longman publisher, Chennai 202-5.

[2] Fetrow C, Avila J (2004) Professional's Handbook of Complementary & Alternative Medicines. Philadelphia, PA: Lippincott Williams & Wilkins.

[3] Gruenwald J: (2002) PDR for Herbal Medicines. 3rd edition. Montvale, NJ: Thomson

[4] Ghosh Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar (2013) Antibacterial activity of leaves of Titeypati (*Artemisia vulgaris* Linn), Bioscience Guardian, 3(1), 107-112.

[5] Mitra Prasanta Kumar (2014) Antibacterial activity of an isolated compound (AV-1) from the leaves of Titeypati (*Artemisia vulgaris* Linn.), World Journal of Pharmaceutical Research, 3(3): 4925 – 33.

[6] Mitra Prasanta Kumar (2014) Antibacterial activity of an isolated compound (AV – 2) from the leaves of Titeypati (*Artemisia vulgaris* Linn.). Mintage Journal of Pharmaceutical & Medical Sciences: 3(3): 12-14.

[7] Laxmi US, Rao JT (1991) Antimicrobial properties of the essential oils of *Artemisia pallens* and *Artemisia vulgaris.* Perfumerie und Kosmetik72:8:510-11.

[8] Lee SJ, Chung HY, Maier CGA, Wood AR, Dixon RA, Mabry TJ (1998) Estrogenic flavonoids from *Artemisia vulgaris* L. *J of Agri & food chem*. 46: 8: 3325- 29.

[9] Chopra Col Sir RN and Chopra IC. (1958) *Indigenous drugs of India*, U. N. Dhar and Sons Private Limted, Kolkata, 668.

[10] Gurung Bejoy (2002) In '*The medicinal plants of the Sikkim Himalaya'*, Pub. Gurung Jasmin Bejoy, Maples, chakung, West Sikkim, 51.

[11] Gilani AH[1], Yaeesh S, Jamal Q, Ghayur MN (2005) Hepatoprotective activity of aqueous-methanol extract of Artemisia vulgaris. Phytother Res. Feb;19(2):170-2

[12] Arambewela LSR and Ratnayake CK(1988) Vasicine contents and their seasonal variation in *Adhatoda vasica*. *Fitoterapia*. 59(2), 151-153.

[13] Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by
winter moth caterpillars. *Ecology*, 51, 565–581.

[14] Gupta PL (1977) Variation in morphological characters and active principle constituents of *Eclipta prostrata* Linn. under different seasonal and soil conditions. *JRIM* . 12(1). 80-84.

[15] Mauffette Y and Oechel WC (1989) Seasonal variation in leaf chemistry of the coast live oak *Quercus agrifolia* and implications for the California oak moth. *Phryganidia californica Oecologia*. 79, 439–445.

[16] Schultz JC, Nothnagle PJ and Baldwin IT (1982) Seasonal and individual variation in leaf quality of two northern hardwoods tree species. *American Journal of Botany* . 69, 753–759.

[17] Pillai NR and Santhakumari G (1981) Hypoglycaemic activity of *Melia azadirachta* Linn (Neem). *Indian J Med Res*. 74, 931-3.

[18] Kate BP, Kothekar MA, Tayade HP, Jaju JB and Mateenuddin (2003) Effect of aqueous extract of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drugs in rats. *Indian J Pharmacology*. 35, 177-180.

[19] Ghosh MN (2005) Toxicity studies in fundamentals of experimental pharmacology. Hilton and Company, Kolkata. 190-7.

[20] Fluck H and M Pharm**.** (1955) The influence of climate on the active principles in medicinal plants. *J. Pharm.Pharmacol*. **7**, 361-383.

Authors Column

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