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Adaptogenic potential of Poorna Chandrodayam Chendooram (PCM-Metallic Drug) on free radical and antioxidants levels in brain, liver and kidney of rats

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Abstract
We evaluated the Adaptogenic potential of Poorna Chandrodayam Chendooram (PCC - Metallic Drug) on Free radicals, Antioxidative and Metallothionein (MT) status in Brain, Liver and Kidney of Normal rats compared to Drug Treated rats. The concentrations of Lipid Peroxidation (LPO), Protein carbonyls (PCO), activities of Xantione oxidase (XO), Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), Glutathione (GSH), vitamin C and vitamin E and Metallothionein (MT) were used as biomarkers. In the Brain, Liver and Kidney of young animals, enhanced oxidative stress was accompanied by compromised antioxidant defenses. Administration of Poorna Chandrodayam Chendooram (PCC - Metallic Drug) effectively modulated oxidative stress and enhanced antioxidant status in the Brain, Liver and Kidney of rats. The results of the present study demonstrate that Poorna Chandrodayam Chendooram (PCC - Metallic Drug) inhibits the Free radicals and increased antioxidant, Metallothionine which might be due to bioactive compound present in the Drug.

Keywords: PCC, MT, LPO, PCO, XO, SOD, CAT, GSH, Vitamin C and E

Introduction
Siddha is a unique system of Indian medicine, is confined to Tamil Nadu, the land of Siddhars, the sage people who had originated this system of medicine. Siddha system not only describes the medicines for ailments and also the way of living like yoga, asanas, breathing exercise and day to day life manners. In Siddha systems of Medicine, Siddhars were advanced the knowledge of chemistry and they were well acquainted with alchemy, the science of conversion of base element into gold. The principal aim of Siddhars in using metals and minerals in the formulation of medicine was to evolve drugs that would arrest the decay of the body. These drugs will promote longevity. Siddhars not only used the herbals but also the minerals, animal products and poisons as medicines. The poisons usually get therapeutic value after proper purification and preparation methods had been done properly [1]. During the process of mercury it is either triturated or boiled with different types of herbal juices and organic acids over fire. Modern scientific reports available that carboxylic acids and amino acids such as citric acid, malic acid and histidine are potential ligands for heavy metals and so could play a role in tolerance and detoxificationp [2].

The modern terminology of adaptogen has been termed as Kayakalpa in the traditional Siddha system of medicine which is being explained as any compound or drug or diet that could protect body from disease, decay and that promote longevity with youthfulness. The physiological changes occur during chronological ageing is being sustained by the Kayakalpa, i.e. it promotes functional well being of individual. It is prepared from the unique and marvelous acquainted knowledge of Siddhars through the processing and preservation technique. Kayakalpa does not mean restoring older to youth; it means the defense of youth without mere lethargy both physically and mentally. Kayakalpa prevents and brings us to a state that the body could not only be healthy but also fulfills the purpose of being healthy, becoming a person meaningful and very much in harmony with the nature [3].

Among Kayakalpa drugs Poorna Chandrodhaya Chenduram get prime importance and are prescribed to promote longevity and used as adaptogenic. According to Siddhars, any metal of their compounds which were ground with the juice of plants, vinegar, and etc.

and then dried and calcined or burnt sufficiently well or heated in a furnace, till it is converted into red powder is known as chenduram. This is unique formulation made up of 3 important constituents, mercury, gold and sulphur, which were medicinally purified by means of traditional method. In medicinal purification the object aimed at are (a) elimination of harmful matter from the drug (b) modification of undesirable physical property of the drug (c) conversion of some of the characteristic of the drugs an (d) the enhancement of the therapeutic action thereby potentiating the drug. It is very effective when administered in the following condition such as emaciation/consumption/TB/Bronco spasm or Broncho pneumonia, constipation, diarrheoa of sprue syndrome/ malabsorption syndrome, jaundice, myalgia, gastritis, fever, gonorrhea, colic, excruciating pain, Obesity and excusive unwanted over growth [4]. PCC as a care for all types of disease, as a blood purifier, nervous stimulant, immunomodulant, will power and for longevity.

PCC is commonly used for treating tuberculosis, jaundice, fevers and bronchitis. It is as well used in rat bite, cancerous ulcers and myalgia. It is administered to patients along with honey [5]. Potency of various types of Siddha drug varies according to their efficacy and drug preparation method. The drug potency varies from 3hrs to more than 100 years. Poorna Chandrodaya Chenduram is one of the chenduram type of drug and it has immense potentiality up to 75 years. Various methods of preparation of Poorna Chandrodaya Chenduram are available as the combination of gold (1 part), mercury (8 parts) and sulphur (16) parts is used commonly). In the present study we investigated the Adaptogenic effect of Poorna Chandrodayam Chendooram (PCC-Metallic Drug) on Free radicals, antioxidant and Metallothionine defense in the Brain, liver and kidney of Normal young rats compared with Treated young rats.

Materials and Methods

Collection of samples

On completion of the experimental period, animals were sacrificed with ether anaesthesia. Organs such as Brain, Liver and kidney tissues were excised immediately and immersed in physiological saline and blotted with filter paper and used for 10% homogenate preparation using Tris-Hcl buffer (pH 7.5). Organ specimens were fixed in 10% buffered formalin for histopathological studies.

Selection of animal

Healthy and pure strain Male Wistar rats, Rattus norvegicus, varying from the body weight of 120-150 g were procured from the Venkateshwara Enterprises, Bangalore and maintained in the Central Animal Home, Department of Siddha Medicine, Tamil University, and Thanjavur. Experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Tamil University, Thanjavur. The animals were kept on standard diet (Kamadhenu Agencies, Bangalore) and water was given ad libitum.

Drug Preparation

The Poorna Chandrodayam Chendooram drug obtained from the SKM Siddha and Ayurvedic Medicine's India Private Limited, Saminathapuram, mudakurichi, Erode- 638104. Tamilnadu, India. The drug (Poorna Chandrodayam Chendooram) is not soluble in water therefore a suspension of gum acacia is made for oral administration. The 10 gm. Of gum acacia dissolved in 100 ml of distilled water by gradual trituration in a mortar. Then well prepared solution was taken and added Poorna chandrodayam chendooram at the dose of 3 mg/ml/100 gm.

Experimental Design

After acclimatization, the rats were divided into 2 groups, each having 8 rats. Group I: Untreated control were received water only. Group II: Young rats were treated with Poorna chandrodaya chendooram (3.0 mg/kg body wt. calculated from human dose) with honey for 7 weeks (orally administered).

Assay of oxidation products

The activity of NADPH Oxidase was determined by following the method of Styne-parve and Benert [6]. The activity of xanthione oxidase was assayed by the method of Stripe and Della Corte [7], Lipid peroxidation was assessed by determining the level of malondialdehyde (MDA) in the Brain,liver and Kidney homogenates by spectrophotometric method of Beuge and Aust [8] ; the results were expressed as nmoles of MDA formed/mg protein using 1,1,3,3-tetraethoxypropane as standard. The protein carbonyl (PCO) content was analyzed using 2,4- dinitrophenylhydrazine (DNPH) as described by Levine [9].

Assay of enzymatic antioxidants

The Superoxide dismutase (SOD) activity was measured by the method of Kakker [10] using NADH-PMS-NBT. Catalase (CAT) activity was measured by the method of Sinha [11] in which disappearance of peroxide was followed spectrophotometrically at 620 nm nm; one unit of activity is equal to the µmol of H2O2 degraded min-1. Glutathione peroxidase (GPx) was assessed by the method of Rotruck [12].

Estimation of non-enzymatic antioxidants

Reduced glutathione (GSH) was measured as described by Ellman [13] using 5, 5-dithiobis- (2-nitrobenzoic acid) (DTNB) reagent. Ascorbic acid (vitamin C) and α-tocopherol (vitamin E) contents were evaluated according to Omaye [14] and Desai [15] respectively.

Estimation of Metallothionein (MT)

Metallothionein (MT) in different tissues of rats were determined according to Virengo [16] using the Ellman reagent DTNB (5, 5"Dithiobis 2- Nitro Benzoic acid).

Tissues were homegenised in a 20 mM Tris-HCl buffer, pH 8.6 supplemented with 0.5M sucrose, 0.01% β-mercapto ethanol, and a protease inhibitor cocktail. After centrifugation at 30000xg for 20 mins, the supernatant was suspended in 49%ethanol plus 3.7% chloroform and recentrifged at 6000xg for 10 mins. Acidified cold 87% ethanol was added to the supernatant and the mixture was stored at -20oC for 1 hour. After centrifugation at 6000xg for 10 mins the pellet was resuspended in 20 mM Tris-HCl buffer, pH 8.6 containing 87% ethanol, 1% chloroform and centrifuged at 6000xg for 10 mins. The pellet dried under a nitrogen gas stream and resuspended in a solution containing 0.16 Nacl, 0.5 N HCl and 2mM ethylenediamine tetra acetic acid, 0.3 ml aliquots were then added with 4.2 ml of 0.43 M DTNB dissolved in 0.2 M phosphate buffer, pH 8.0 containing 2M NaCl. Sample were centrifuged at 3000xg for 5 mins at room temperature and absorbance of a clear supernatant determined at 412 nm using reduced glutathione as the reference standard.

Statistical Analysis

Values are expressed as mean ± SD for 8 rats in each group and significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey"s test for posthoc multiple comparison tests. GRAPHPAD INSTAT Statistical Software package was used for analysis and $p<0.05$ was considered to be significant [17].

Results

Table 1 details the levels of Brain, liver and kidney NADPH Oxidase, Xanthine oxidase, LPO, and PCO activity in Normal and PCM treated young rats. The activity of NADPH oxidase were minimised in Poorna Chandrothaya Chendooram treated rats compared with untreated control rats, with the decrease of 38% for liver, 42% for brain and 32% for kidney tissues in Poorna Chandrothaya Chendooram treated rats. The activity of XO was decrease level was 56% for liver, 81% for brain and 85% for kidney of Poorna Chandrothaya Chendooram treated rats. The level of LPO was found to be decreased in Liver, Brain and Kidney tissues of rats compared with untreated young rats.The decrease LPO levels was 64% for Liver, 49% for Brain and 50% for kidney in Poorna Chandrothaya Chendooram treated rats respectively. The Poorna Chandrothaya Chendooram treated rats showed decreased PCO content when compared with untreated young rats. The decreased level was 30% for liver, 18% for brain and 33% for kidney in PCC treated rats respectively.

The activities of Brain, liver and kidney mitochondrial MnS OD, CAT, GPx, and GST in Normal control and PCM treated young rats arepresented in Table 2. The activity of SOD (20% for liver and 15% for Kidney) significantly increased in liver, and kidney of PCC treated rats compared to untreated young rats. In the brain tissues SOD was found increased (57%) during the PCC supplementation when compared to normal young rats. The CAT activity was found to be significantly increased in liver, brain and kidney of PCC treatment to the normal young rats compared to untreated young rats, with the increase was 20% for liver, 09% for brain and 14% for kidney tissues of rats respectively. The activity of GPx in liver, brain and kidney was found to be significantly increased in PCC treated rats was 44% for liver, 22% for brain and 32% for kidney tissues.

Table 3 shows the level of Non-enzymatic antioxidants in brain, liver and kidney of untreated normal and PCC treated rats. The levels of GSH, VIT C and VIT E were found to be increased in Liver, Brain and Kidney tissues of PCC administered rats was found increased when compared with untreated normal young rats. The increase levels being 33%, 46% and 43% for GSH, 84%, 22% and 52% -for VIT C and 30%, 34%and 44% for Vit E in Liver, Brain and Kidney of PCC treated rats respectively.

Table 4 expressed the level of MT in brain, liver and kidney Tissues of Untreated young rats and PCC treated young rats. Significant increase in the content of MT was observed in PCC treated young rats when compared to untreated young rats. The MT content was found to be significantly increased in kidney (66%) of PCC treated rats. The MT content of Liver and Brain tissues was found to be 33% and 20%.

Discussion

Antioxidants are molecules that can neutralize free radicals by accepting or donating electrons to eradicate the unpaired form of the radical, breaking the chain of oxidation consequences [18]. Many studies have shown that natural antioxidants not only play a major role against reactive oxygen species (ROS), but also trigger lipid peroxidation [19-21] They are believed to play a role in preventing the development of chronic diseases like cancer, heart disease, stroke, AD, RA and cataracts [22].

NADPH Oxidase has been suggested as an important source for the formation of free radicals. The NADPH Oxidase transfer electron from NADPH at the cytosolic side of the membrane to molecule O2 at the other side of the membrane [61]. In our present study the decrease in the activities of NADPH Oxidase observed in PCC treated young rats" tissues indicates the protective effective of the drug against the formation of ROS by inhibiting superoxide generating enzyme NADPH Oxidase as reported earlier [24]. It is also reported that flavonoids are not only free radical scavenger but also inhibitors of enzymes like NADPH Oxidase in human neutrophil [25].

XO is an enzyme involved in aerobic purine degradation. It catalyses the oxidation of hypoxanthine to xanthine resulting in the formation of superoxide radicals and H_2O_2 (Parks and Granger 1986). XO derived from xanthine dehydrogenase is an initial translation product, by proteolysis [26] (Amaya *et al*., 1990). Administration of PCC to young rats decrease the activity of tissues XO through the scavenging of superoxide, hydroxyl radical and hydrogen peroxide as evidenced. Cos [27] described that flavonoids inhibit xanthine oxidase activity. The herbo mineral complexation with Flavonoids, Poly phenols and VitC might suggest the possibility of inhibitory action on XO as demonstrated by Changn [28].

Malondialdehyde (MDA), a generally used biomarker of lipid peroxidation, is synthesized from the breakdown of lipid peroxyl radicals during oxidative stress. Measured level of MDA is considered a direct index of oxidative injuries associated with lipid peroxidation [29]. This compound is a reactive aldehyde, and is one of the several reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products. The production of this aldehyde is used as a biomarker to assess the level of oxidative stress in an organism [30]. Administration of Poorna Chandrothaya Chendooramto young rats had shown reduction in lipid peroxide levels, which is measured in the terms of MDA. The reduction of LPO level may be due to the H+ donating capacity of organometallic components of Poorna Chandrothaya Chendooram, which might be evidenced to the termination of LPO chain reaction based their reducing power.

ROS are known to convert amino group of protein to carbonyl moieties [60]. Modification of different intra cellular proteins including key enzymes and structural protein have been demonstrated to lead to the neurofibrillary degradation of neurons in Alzheimer"s disease (AD) [32]. Oxidative modification of protein could also contribute to secondary impairment of biomolecules,for instance inactivation of DNA polymerase in replicating DNA and the development of new antigen provoking autoimmune response [33]. The decreased level of PCO in supplementation with PCC may reduce the levels of oxidation to protein aging. Administration of free radical scavengers such as flavonoids and polyphenolic acids scavenges free radicals and prevent oxidation of lipids and protein [34]. Subathra [35] has reported that herbal drugs reduced in the PCO in rats. The observation of reduction in the PCO contents in the functional organs such as brain, liver and kidney evidences the antioxidant potential of PCC. Thus the finding evidences protective efficacy in the cells during supplementation of PCC.

SOD is protective against deleterious effect of oxy radicals in the cells by catalyzing the dismutation of superodie radicals [36]. Su [37] had reported that polyphenolic extract from herbal drug increased the levels of SOD *in vivo*. Zhang [38] has reported the significance elevation in the SOD by flavonoid extract had inhibited oxidative stress in rat tissues PCC Supplementation increased the level of MnSOD to near that seen in younger treated animals, but had no effect on MnSOD activity in young rats. Our finding with the increased availability of endogeneous SOD during PCC treatment could justify, that the drug could boost endogenous enzymes through genes. Further, SOD increment in the tissues of PCC treatment to normal young male rats may suggest the possibility of adaptive mechanism to be defensive against free radical induced functional changes in the body and also in supportive of earlier possible evidences.

Catalase (CAT) is an enzymatic antioxidant generally distributed in all animal tissues. It decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [39]. Catalase activity varies greatly from tissue to tissue, the highest activity is found in liver and kidney, whereas the lowest activity is seen in the connective tissue [40]. Inhibition of this enzyme may improve sensitivity to free radical-induced cellular damage. Therefore reduction in the activity of CAT may leads to deleterious effects as a result of superoxide and hydrogen peroxide assimilation. Our results with the increment of CAT activity during PCC treatment might suggest the complete scavenging of peroxide oxidants. Suggesting the protective efficacy of functional organs against free radical accumulation.

GPx is a seleno enzyme, hunts the highly reactive lipid hydroperoxide in the aqueous phase of cell membranes. Glutathione peroxidase catalyzes the reduction of hydroperoxides, with glutathione (GSH) being oxidized to glutathione disulfide (GSSG). The latter is converted to glutathione by glutathione reductase in the presence of NADPH [41]. In our present observation, increase in tissue GPx activity may be due to the enhancement of GSH during treatment of PCC and further supported by the finding flavonoids stimulated the GSH synthesis in cells [42]. Suchalatha and Shyamaladevi [43] reported that administration of herbal drug increase the GPx activity in isoprotenol induced oxidative stress in rats. Maharishi Amrit Kalash an herbal formulation which also increased in the GPx activity in rats [44].

GSH is an vital intracellular defense against damage by ROS. The diminished form of glutathione is necessary to maintain the normal reduced state of cells so as to alleviate all the injurious effects of oxidative stress [45]. The reason of glutathione (GSH) depletion in diabetic rats could be explained by the increased rate of reduced glutathione oxidation to oxidized glutathione as a result of enhanced production of free radicals [46]. The elevated level of GSH was observed in our present study during PCC treatment could possibily may be due to the presence of organic (flavonoid and polyphenols) compound present in PCC [47]. Extracts of plants have been stated to potentiate the activity and level of SOD and GSH, respectively [48].

Vitamin C is a potent antioxidant, and acts as a scavenger of ROS to inhibit, or at least alleviate, the deleterious effects caused by ROS [49]. It works synergistically with vitamin E to quench free radicals and also regenerates the reduced form of vitamin E [50-51]. Ascorbic acid protects metabolic processes against H2O2 and other toxic derivatives of oxygen, acting as a chain breaking antioxidant, it impairs with the formation of free radicals in the process of formation of intercellular substances through the body, including collagen, bone matrix and tooth dentine [52]. The supplementation of PCC to rats improved Vit C compared to normal young rats which may be correlated with increase of GSH in PCC treated rats improves the recycling of Vit C. This finding justifies the antioxidative capacity of PCC.

Vitamin E prevents lipid peroxidation and maintains GSH and ascorbic acid levels in damaged tissues by inhibiting free radicals formation [53]. Rana [54] reported that vitamin E has a protective effect against mercury induced toxicity. Vitamin E inhibits oxidative damage caused by metals intoxication. Administration of PCC enhanced the level of Vit E in PCC treated in the tissues of rats. Phenolic and some flavonoids owing to their intermediate redox potential and physiochemical characteristics, can possibly act an interface between ascorbate and tocopherol [55]. Hence PCC brought the increase in the level of Vit E by improving the levels of Vit C as mentioned earlier. Thus, it is clear from the present study that the increase in the vitamin E levels by the PCC proves its defensive antioxidant potential.

Metallothioneins (MTs) belong to the group of intracellular cysteinerich, metal-binding unique proteins involved in diverse intracellular functions [56], in the detoxification of heavy metals and in the maintaining of essential metal ion homeostasis [57]. Early studies suggested that MT –I/II which are virtually expressed in all tissues could have significant antioxidant capacity and also suggested to provide cellular protection against a variety of DNA damages induced by chemicals or radiation [58] The elevated of MT s in the liver, brain and kidney tissue of present study may suggest the possible cellular protective efficacy of PCC against reactive oxygen speceies. The determination of MT in the brain may also support the possible relevance to MT III and this may evidence neuroprotective role against many neurological diseases [59]. Our finding with increase in MT content during PCC supplement may evidence its antioxidant capacity of the prepared medicine by eliciting preventive efficacy on the free radicals formation and its causative degenerative disease due to oxidative stress.

Table 1: Effect of Poorna Chandhrothaya Chendooram on oxidation products in control and treated rats

Organs	Normal Control	PCC Treated
Brain		
NADPH Oxidase	11.40±0.05	6.64±0.04***
XO	0.047±0.002	0.009±0.005***
LPO	3.500±0.52	1.827±0.10***
PCO	7.74±0.36	6.36±0.18***
Liver		
NADPH Oxidase	23.53±0.73	15.13±0.37***
XO	1.240±0.007	0.550±0.005***
LPO	2.652±0.26	0.975±0.37***
PCO	5.95±0.01	4.16±0.05***
Kidney		
NADPH Oxidase	19.26±0.12	12.40±0.04***
XO	0.853±0.005	0.133±0.007***
LPO	3.294±0.31	1.634±0.08***
PCO	6.16±0.08	4.10±0.05***

Each values are expressed as mean ± SD for eight rats in each group, NADPH, µm of NADPH oxidizes/mins/mg protein, XO, mg of uric acid formed/min/mg protein, MDA, µ Moles of MDA formed / mg Protein, PCO, nmol of DNPH incorporated/mg protein; *As compared with Normal Control,*$p< 0.05$, **$p< 0.01$, ***$p< 0.001$

Table 2: Effect of Poorna Chandrothaya Chendooram on enzymatic antioxidants in control and treated rats

Organs	Normal Control	PCC Treated
Brain		
SOD	7.285±0.46	11.927±0.43***
CAT	45.493±0.64	49.447±0.33***
GPx	0.131±0.05	0.541±0.05***
Liver		
SOD	13.859±0.62	16.807±0.34***
CAT	76.491±0.64	81.552±0.19***
GPx	0.187±0.01	0.491±0.03***
Kidney		
SOD	12.729±0.80	14.039±0.52***
CAT	31.712±0.86	36.735±0.28***
GPx	0.136±0.01	0.0356±0.01***

Each values are expressed as mean ± SD for eight rats in each group; SOD, 50% reduction of NBT/min/mg protein; CAT, mmol H_2O_2 consumed/min/mg protein; GPx, mmole GSH utilized/min/mg protein;*As compared with Normal Control, *p<0.05, **p<0.01, ***p<0.001

Table 3: Effect of Poorna Chandrothaya Chendooram on non - enzymatic antioxidants in control and treated rats

Organs	Normal Control	PCC Treated
Brain		
GSH	30.588±.0.93	34.012±0.70***
VIT-C	0.10±0.07	0.89±0.03***
VIT-E	6.12±0.15	6.78±0.15***
Liver		
GSH	45.153±0.43	50.362±0.45***
VIT-C	2.52±0.31	4.57±0.5***
VIT-E	6.18±0.40	7.93±0.46***
Kidney		
GSH	27.690±0.37	30.815±0.53***
VIT-C	1.28±0.06	1.65±0.02***
VIT-E	4.49±0.44	7.22±0.49***

Each values are expressed as mean ± SD for eight rats in each group: GSH, µg/mg Protein; Vit-C, µg/mg Protein; Vit-E, µg/mg Protein,*As compared with Normal Control, *p< 0.05, **p< 0.01, ***p< 0.001

Table 4: Effect of Poorna Chandrothaya Chendooram on Metallothionein level in control and treated rats

Organs	**Normal Control**	**PCC Treated**
Brain	3.04 ±0.01	5.23±0.05***
Liver	9.26±0.02	11.90±0.07***
Kidney	9.68±0.07	12.25±0.10***

Each value are expressed as mean ± SD for eight rats in each group; Metallothionein, µg/gm tissues *As compared with Normal Control, *p< 0.05, **p< 0.01, ***p< 0.001

Conclusion

Overall, the study concluded that supplementation of poorna chandhrothaya chendooram supplementation to rats significantly increased antioxidant and decreased the free radicals induced oxidative products. The functional significance of PCC is due to the adaptogenic property that could improve the well being of a normal life span free of ailments. Boosting the endogenous availability of antioxidants will be more rewarding and through it effect as antistress, adaptogenic and antiaging activity. It is evidenced that Poorna chandrothaya chendooram administration to the normal male rats could elicits adaptogenic functional ability by its cytoprotective and antioxidative activity further with defense detoxifying potential. This study therefore confirms local claims on the efficacy of the drug and may provide effective intervention for free radical mediated diseases.

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