

Full Length Research Paper

Ameliorative effects of melatonin on brain biochemical changes induced by subchronic co-administration of chlorpyrifos and cypermethrin in male Wistar rats

S. B. Idris^{1*}, S. F. Ambali² and M. M. Suleiman³

¹Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

²Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.

³Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

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The present study was aimed at evaluating the ameliorative effects of melatonin on chlorpyrifos and cypermethrin induced brain biochemical changes involving malonaldehyde (MDA), catalase, superoxide dismutase, glutathione peroxidase and acetyl cholinesterase in male Wistar rats. The fifty adult male Wistar rats used for this study were divided into 5 groups of 10 animals each. Group I was administered distilled water, group II was given soya oil (2 ml/kg), group III was given melatonin (0.5 mg/kg), group IV was given chlorpyrifos (7.94 mg/kg - 1/10th LD⁵⁰) and cypermethrin (29.6 mg/kg - 1/10th LD⁵⁰), and group V was pretreated with melatonin (0.5 mg/kg) and then 30 min later, chlorpyrifos (7.94 mg/kg-1/10th LD⁵⁰) and cypermethrin (29.6 mg/kg - 1/10th LD⁵⁰). The regimens were administered by gavage once daily for a period of 12 weeks. Increased MDA concentrations, decreased catalase, superoxide dismutase, glutathione peroxidase, and acetylcholinesterase activities were recorded in the group IV, however, these changes were ameliorated by melatonin. Therefore, it was concluded that melatonin mitigated chlorpyrifos and cypermethrin induced brain biochemical changes due to its antioxidant properties.

Key words: Chlorpyrifos, cypermethrin, melatonin, biochemical changes, oxidative stress.

INTRODUCTION

The use of newer chemical mixtures in agricultural pest control and public health is on the rise due to pest resistance to existing chemicals, these insecticidal mixtures have their own toxicological profile and dynamics, and this however, comes with its new challenges in terms of toxicity and environmental

pollution. Farmers need these formulations to boost food production, but these also have its associated side effects, some of which are unknown (Sekar et al., 2010). Pesticides containing organophosphates and pyrethroids is one of the most common formulations (Elhalwagy and Zaki, 2009). Application of both pesticides together is

*Corresponding author. E-mail: bankidris67@gmail.com. Tel: +238136103222.

economical since it translates to using minimal amounts as compared to when they are used singly, this is because the non-reversible inhibition of esterases by OPs results in slowing down the activity of enzymes responsible for cleavage of the ester bond in the pyrethroid (Latuszynska et al., 2001). Chlorpyrifos one of the most studied organophosphate, was synthesized by Dow chemical and released into the market in 1965 (USEPA, 2000). It acts mainly by inhibiting acetyl cholinesterase, though evidence suggests that chlorpyrifos induced oxidative stress in rat brain (Satinderpal et al., 2013). Cypermethrin, a type II pyrethroid with low mammalian toxicity have been reported to induce oxidative stress in rats brain (Belma et al., 2013). Antioxidants are substances which protect the system against free radical damage one of which is melatonin (Reiter et al., 2013). It functions as antioxidant via two levels first as a direct antioxidant which acts by stimulating glutathione, limiting the production of cytokines and inflammatory process and reduces greatly leak age of electrons from mitochondrial electron transport chain (Reiter et al., 2013), and secondly as an indirect antioxidant where it enhances the activity of other classical antioxidants with synergistic effects. This work was aimed at evaluating the brain biochemical changes induced by co-administration of chlorpyrifos and cypermethrin.

MATERIALS AND METHODS

Experimental animals

Fifty adult male Wistar rats weighing between 170 and 200 g sourced from the laboratory animal house of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria served as subjects. They were housed in cages in the Department of Veterinary Physiology and Pharmacology Students Teaching Laboratory, Ahmadu Bello University, Zaria. The rats were given access to pellets of food prepared from growers mash, maize bran and groundnut cake at the ratio of 4:2:1 and water was provided *ad libitum*. The rats were acclimatized for two weeks before the commencement of the experiment.

Chemicals

Commercial grade CPF (Sabero Organics Gujarat Limited, India) and cypermethrin (Jiangsu Yangnong Chemical Co. Limited, China) was obtained from reputable agrochemical stores in Zaria. They were reconstituted in soya oil (Grand Cereal and Oil Mills Limited, Jos, Nigeria) to appropriate working concentrations. Melatonin tablet (3 mg, Nature Made Nutritional Products, Mission Hills, USA) was dissolved in 6 ml of distilled water to make 0.5 mg/ml suspension daily before administration.

Subchronic toxicity study

The rats were divided at random into five groups of ten animals each. Rats in each group were weighed and marked on the tail with a marker for identification. Group I (DW) was administered distilled water and Group II (SO) was given soya oil at 2 ml/kg. Group III

(MEL) was administered with melatonin (0.5 mg/kg) (Gulterkin et al., 2006). Group IV (CC) was co-administered with CPF (1/10th LD₅₀) and cypermethrin (1/10th LD₅₀), and Group V (MCC) was pre-treated with melatonin 0.5 mg/kg then dosed with CPF (1/10th LD₅₀) and cypermethrin (1/10th LD₅₀). The regimens were administered once daily by oral gavage for 12 weeks. At the end of the dosing schedule, the rats were sacrificed and the brain tissue was evaluated for malonaldehyde (MDA) concentration, catalase, superoxide dismutase, glutathione peroxidase and AChE activities. Rats used in this work were handled in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Garber et al., 2011).

Assessment of brain MDA concentration

The principle of the method was based on spectrophotometric method of the colour, developed during the reaction of thiobarbituric acid (TBA) with MDA. The level of thiobarbituric acid-reactive substance (TBARS) and MDA is an index of lipid peroxidation. For determination of lipid peroxidation in the cerebral cortex and hippocampus, the method of Draper and Hardley (1990) as modified by Freitas et al. (2005) was used. Briefly, brain sample from each animal in all the groups immediately after decapitation was weighed and then homogenized in a known sample of ice-cold phosphate-buffer to obtain a 10% homogenate, which was centrifuged at 6000 × g for 10 min using a centrifuge, IEC HN (Damon/IEC Division, UK). 0.5 ml of the supernatant obtained following centrifuge was mixed with 1 ml of 10% trichloroacetic acid (TCA) solution and 1 ml of 0.67% TBA. The mixture was heated in boiling water bath for 15 min. Buta-2-ol (2:1 v/v) was added to the solution. After centrifugation (800 × g for 5 min), the MDA concentration was determined from the absorbance at 532 nm using a UV visible spectrophotometer (T180+ PG instrument Limited, United Kingdom). The MDA concentration in each sample was calculated from the absorbance coefficient of MDA-TBA complex (1.56 × 10⁵ cm/M) and expressed as nmol/mg of tissue protein. The concentration of protein in the brain homogenate was determined using the method of Lowry et al. (1952).

Brain biochemical analysis

The whole brain sample tissues were carefully collected and homogenized in 1% phosphate-buffered solution. Thereafter, catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured using commercial kits (Northwest Life Science Specialities, LLC, Vancouver, WA 98662) according to the manufacturer's instructions.

Effects of treatments on brain acetylcholinesterase (AChE) activity

AChE activity was determined by the method of Ellman et al. (1961) using acetylcholine iodide as substrate. Briefly, the brain was weighed using the Mettler weighing balance (Mettler^R P161, Mettler instrument AG, CH806, Geifensee Zurich, Switzerland) and then homogenized in cold (0 to 4°C) 20 mM phosphate-buffered saline (PBS), incubated with 0.01 M 5,5-dithio-bis-(2-nitro benzoic acid) in 0.1 M PBS, pH 7.0. Incubation was allowed to proceed at room temperature (26°C) for 10 min. Then acetylcholine iodide (0.075 M in 0.1 M, PBS, pH 8.0) was added to the tube and absorbance at 412 nm was measured with a Shimadzu UV spectrophotometer (Model UV 160; Kyoto, Japan). The changes in the absorbance were recorded for a period of 10 min at intervals of 2 min after the addition of acetylthiocholine (30 µl; final concentration = 0.5 mM) to the mixture. Thus, the change in the absorbance per minute was

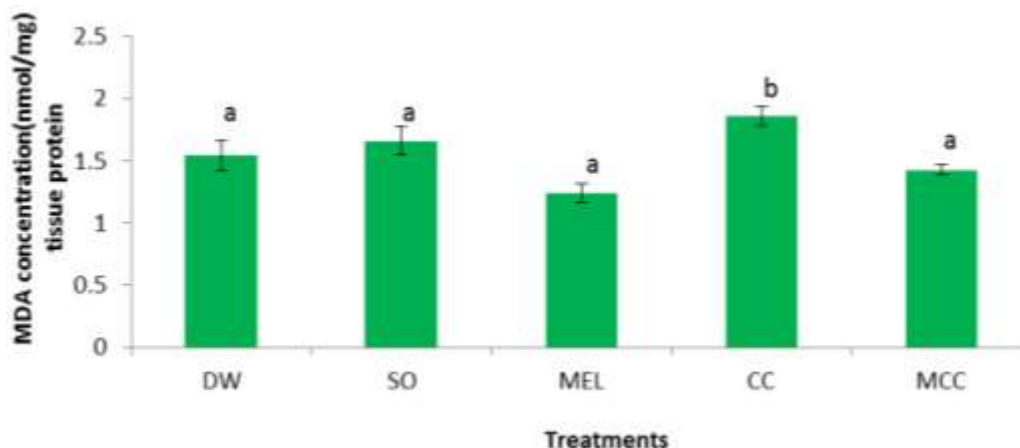


Figure 1. Effect of distilled water (DW), soya oil (SO), melatonin (MEL), chlorpyrifos + cypermethrin (CC) and melatonin + chlorpyrifos + cypermethrin (MCC) on brain malondialdehyde concentration in Wistar rats. ^{a,b}Values with different superscripts are significantly different.

determined.

The enzyme activity was calculated using the formula:

$$R = 5.74 \times 10^{-4} \times A / CO$$

where 5.74×10^{-4} is a dissociation coefficient, R = rate in moles of substrate hydrolyzed/min/g tissue, A = change in absorbance/min, and CO = original concentration of the brain tissues.

Statistical analysis

Data obtained were expressed as mean \pm standard error of mean (SEM). One way analysis of variance followed by Tukey's post hoc test was used to analyse biochemical parameters. Values of $P < 0.05$ were considered significant.

RESULTS

Effect of treatments on MDA concentration

There was a significant ($P < 0.05$) increase in the MDA concentration of the CC group when compared with that of the DW, MEL and MCC groups. There was no significant change ($P > 0.05$) in the MDA concentration of the MCC group compared to that of the DW, SO or MEL group (Figure 1).

Effect of treatments on brain superoxide dismutase activity

The effect of treatments on SOD activity is as shown in Figure 2. SOD activity was significantly ($P < 0.05$) lower in CC group compared to that of the DW, SO, MEL or MCC groups. There was no significant ($P > 0.05$) change in the MCC group compared to the MEL group, but there was a significant difference ($P < 0.05$) when compared

with the SO and DW groups.

Effects of treatments on brain catalase activity

The effect of treatments on brain catalase activity is as shown in Figure 3. There was significant decrease ($P < 0.05$) in the catalase activities obtained in the CC group compared to that of DW, SO, MEL and MCC groups. There was no significant change ($P > 0.05$) in the MCC group compared to the MEL group, but there was a significant ($P < 0.05$) difference in the MCC group compared to the DW and SO groups.

Effect of treatments on glutathione peroxidase activity

The effect of treatments on glutathione peroxidase activity is as shown in Figure 4. GPx activity was significantly ($P < 0.05$) lower in the CC group, compared to DW, SO, MEL or MCC group. There was no significant ($p > 0.05$) difference in the glutathione peroxidase activity of MCC group compared to those of DW, SO and MEL groups.

Effect of treatments on AChE activity

The effects of treatments on AChE activity is as shown in Figure 5. AChE activity was significantly decreased ($P < 0.05$) in CC group (24.70 ± 1.0) compared to that of the DW, SO, MEL or MCC group. The AChE activity in the MCC group was significantly ($P < 0.05$) higher compared to that of the CC group, but was not significantly ($P > 0.05$) changed relative to those of the DW, SO, and MCC groups.

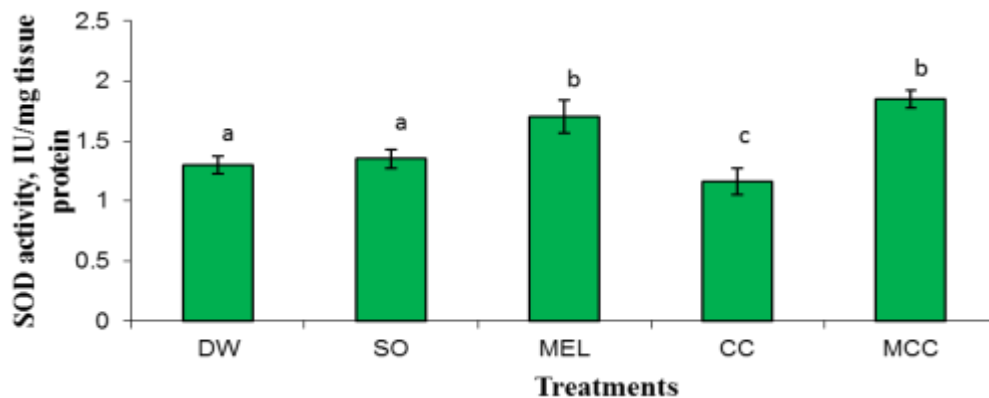


Figure 2. Effects of distilled water (DW), soya oil (SO), melatonin (MEL), chlorpyrifos + cypermethrin (CC) and melatonin + chlorpyrifos + cypermethrin (MCC) on brain superoxide dismutase activity in Wistar rats. ^{a,b,c}Values with different superscripts are significantly different.

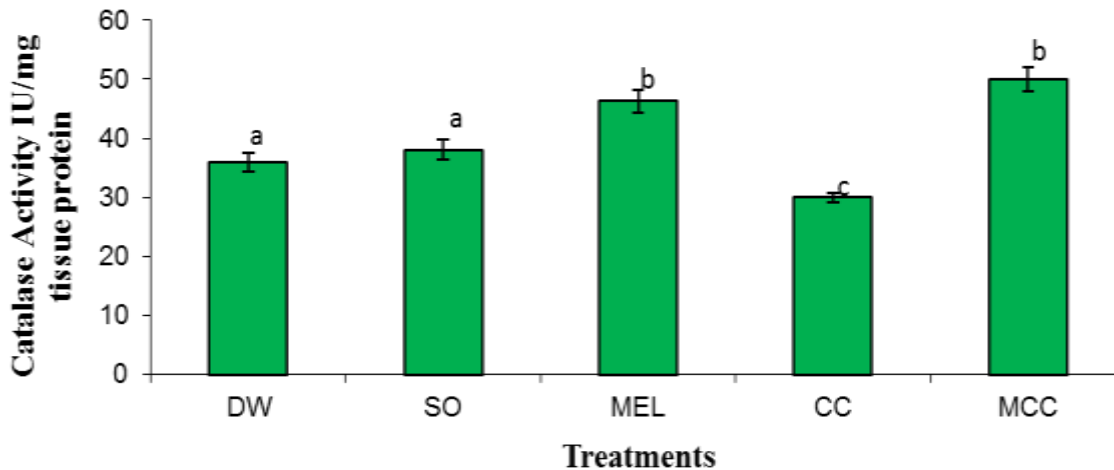


Figure 3. Effect of distilled water (DW), soya oil (SO), melatonin (MEL), chlorpyrifos + cypermethrin (CC) and melatonin + chlorpyrifos + cypermethrin (MCC) on brain catalase activity in Wistar rats. ^{a,b,c}Values with different superscripts are significantly different.

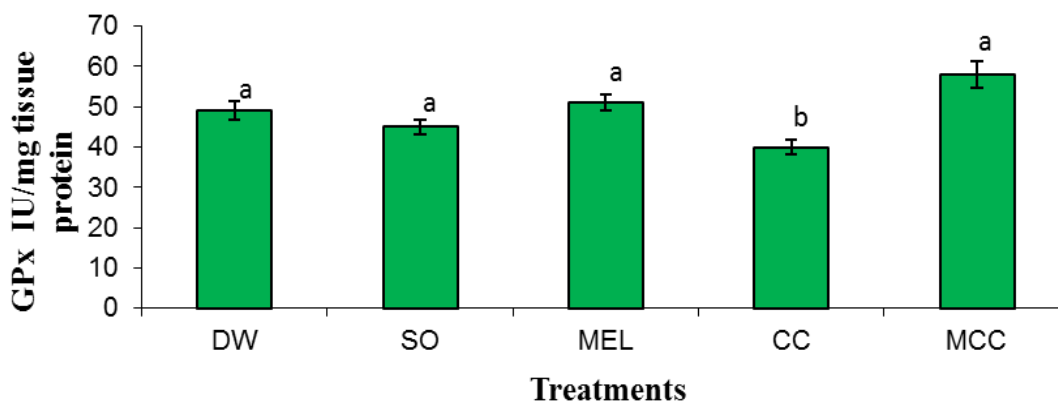


Figure 4. Effect of distilled water (DW), soya oil (SO), melatonin (MEL), chlorpyrifos + cypermethrin (CC) and melatonin + chlorpyrifos + cypermethrin (MCC) on brain glutathione peroxidase activity in Wistar rats. ^{a,b}Values with different superscripts are significantly different.

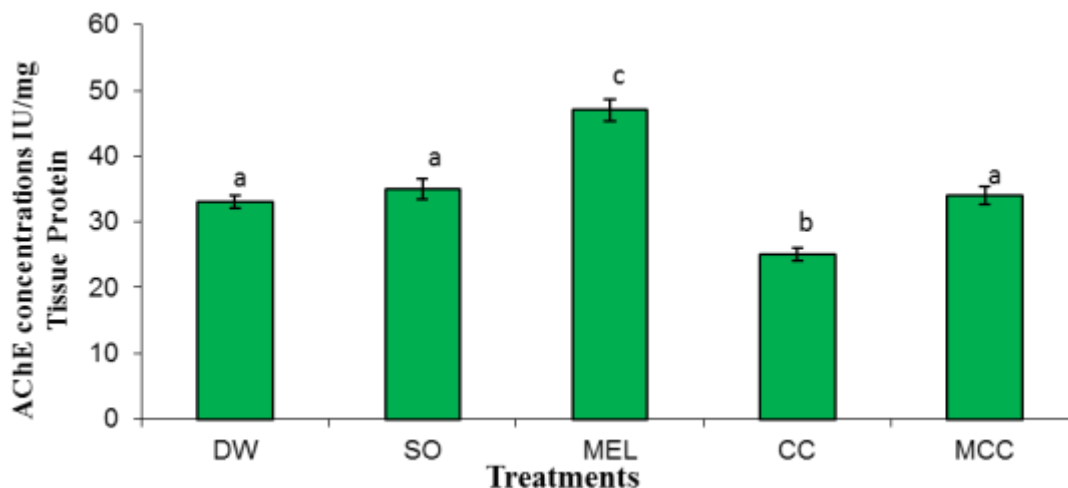


Figure 5. Effects of distilled water (DW), soya oil (SO), melatonin (MEL), chlorpyrifos+cypermethrin (CC) and melatonin + chlorpyrifos + cypermethrin (MCC) on brain acetylcholinesterase activity in Wistar rats. ^{a,b,c}Values with different superscripts are significantly different.

DISCUSSION

The higher MDA concentration recorded in the CC group agrees with the findings of Wielgomas and Krechniak (2007) following exposure to CPF and CYP. Similarly, Gultekin et al. (2006) and Ambali et al. (2010b) showed that MDA concentration in the brain was elevated following CPF exposure. MDA, a reactive aldehyde, is a product of lipid peroxidation which occurs when oxygen radicals react with polyunsaturated fatty acids residues in membrane phospholipids; this results in toxic stress in cells (Krishnanamoorthy et al., 2007). MDA is used as a biomarker to measure the level of lipid peroxidation in an organism (Ambali et al., 2012). The large amount of oxygen in the brain, high amounts of oxygen with fewer antioxidant systems compared to other tissues makes it vulnerable to oxidative stress (Ambali et al., 2012). The increase in MDA concentration in the present study, therefore, suggests the elevation of brain lipoperoxidation and that reactive oxygen species are involved in the mediation of these damages in the brain. Pretreatment with melatonin prevented a rise in the MDA concentration in the brain.

The decreased SOD activity in CC group suggests induction of oxidative stress by ROS. SOD, catalyses the dismutation of superoxide anion to H_2O_2 which is further catalysed to water, this is the first line antioxidant defence in the body (Tohr and Masuko, 2011). This decrease may be due to either a reduction in the synthesis or an increased degradation of SOD with increased lipoperoxidation induced by CPF and CYP. The higher SOD activity recorded in the MCC group suggests that melatonin caused an increase in intracellular scavenger activity against lipid peroxidation, thereby preventing oxidative stress. This increase in SOD activity of the

MCC group agrees with the findings of Bayır et al. (2011), they demonstrated the ability of melatonin as an antioxidant molecule to boost endogenous antioxidant reserves and, therefore, melatonin pretreatment may have protected SOD from the ravaging effects of CC-induced oxidative stress.

The decrease in catalase activity obtained in the CC group agreed with the findings of Wielgomas and Kriechnac (2007), the conversion of hydrogen peroxide to water and oxygen by catalase prevents tissue damage due to oxidative stress, furthermore, the reduced catalase activity may be due to the reduced conversion of superoxide anion to hydrogen peroxide by superoxide dismutase (Raina et al., 2009). The decreased catalase activity may also be due to the persistent long-term increased lipoperoxidation induced by CPF and CYP, which results in, increased catalase utilization and decreased synthesis due to high amounts of free radicals in the system. The increased catalase activity recorded in the melatonin pre-treated group showed that melatonin has the ability as an antioxidant molecule to boost the endogenous antioxidant reserve and accelerate the removal of ROS induced by CC. This agrees with the findings of Gulcin et al. (2009), they recorded increased catalase activities in erythrocytes of rainbow trouts following administration of melatonin.

The present study has shown that co-administration of CPF and CYP caused reduced GPx activity. This may be because GPx is known to decompose peroxides to water or alcohol while simultaneously oxidising GSH. Raina et al. (2009) demonstrated reduced GPx activity following cypermethrin exposure in rats, while CPF or CPF oxon has been shown to reduce GPx levels in the rat brain (Esparza et al., 2005). The higher GPx activity recorded in the MCC group showed that melatonin caused an

increase in intracellular scavenger activity against ROS-induced increase in lipid peroxidation, thereby, preventing oxidative stress. This agrees with the findings of Mauriz et al. (2007), they demonstrated that melatonin supplementation prevented the reduction in cytosolic and mitochondrial GPx in ageing rats.

The markedly decreased AChE activity in CC group agreed with the findings of Latuszynska et al. (2001) and Wielgomas and Krechniak (2007), they demonstrated that AChE activity in the brain markedly decreased in rats co-administered with CPF and CYP. Significant reduction in AChE activity in the rats' brain has been linked to oxidative stress (Tsakiris et al., 2000). Apart from the direct inhibition of AChE, oxidative stress has also been implicated in many pathologies caused by CPF (Uchendu et al., 2013). AChE is a membrane-bound enzyme and lipoperoxidation of the membrane causes a decrease in its activity (Uchendu et al., 2013). This may be responsible for the decrease in AChE activity seen in the CC group in the present study. Also, the decreased AChE activity may further explain the decrease in catalase, SOD and Gpx concentrations. The inhibition of AChE by CPF and CYP suggests an initiation of accumulation of free radical leading to lipid peroxidation which is responsible for the decrease in catalase, SOD and GPx activities. The increase in AChE activity recorded in melatonin treated group showed that melatonin exerted decreased lipid peroxidation and increased antioxidant status. The increased AChE activity in the MCC group showed that melatonin restored AChE activity following its inhibition by CPF and CYP.

In conclusion, this study demonstrated brain biochemical changes induced by CPF and CYP were ameliorated by melatonin.

Conflict of interests

The authors have not declared any conflict of interests.

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