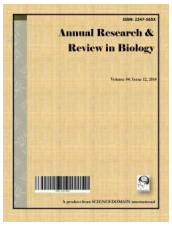

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Effect of Dimethoate on Prolactin Cells of Freshwater Catfish *Heteropneustes fossilis* after Short-term and Long-term Exposure

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The effects of dimethoate on pituitary prolactin cells of *Heteropneustes fossilis* was investigated in this study. The fish *Heteropneustes fossilis* were subjected to sub-lethal concentration of dimethoate - 2.24 mg/l i.e. 75% of 96 h LC_{50} for short-term (24, 48, 72 and 96 h), and 1.00 mg/l i.e. 25% of 96 h LC_{50} for long-term (6, 12, 24 and 36 d) exposure. Pituitary was removed from anaesthetised fish and fixed for histological examinations. *H. fossilis* exposed for short-term (96 h) at sub-lethal dimethoate concentration, exhibited marked changes in structure and staining properties of prolactin cells. PRL cells did not show much histological alterations after 24 h, however, after 96 h exposure exhibited severe vacuolization and nuclear pycnosis, indentation and deformity of nuclear boundaries. In long-term dimethoate exposure the PRL cells of *Heteropneustes fossilis*, exhibited low staining response invisible vacuolization and distinct nuclear boundaries after 6 day. However, severe vacuolization, pycnosis and the nuclear diameter reduction in PRL cells were noticed after 24 d and 36 d exposures. The study concluded that dimethoate affects calcium regulating prolactin (PRL) cells and disturb the calcium homeostasis of fish which is important for fish health and their survival.

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1. INTRODUCTION

Calcium homeostasis in mammals is achieved by hyper and hypocalcaemic hormones [1]. The parathyroid hormone (PTH) and vitamin D_3 metabolites are well established as hypercalcaemic; whereas, calcitonin is a hypocalcemic hormone. Fish have adopted similar strategy to maintain plasma calcium level. However, the hormones involved in Ca^{2+} regulation in fish are different from those in higher vertebrates. Parathyroid gland which secretes hypercalcaemic PTH, is absent in fish. Instead, prolactin (PRL) cells located in rostral pars distalis (RPD) of pituitary secrete a hypercalcaemic factor - the prolactin [2]. The hypocalcaemic factors in fish are calcitonin and stanniocalcin. In most of teleosts including *Heteropneustes fossilis*, the PRL cells extend ventrally and laterally around the proximal pars Distalis of pituitary.

In fish, PRL plays important role in freshwater osmoregulation, preventing both the loss of ions and the uptake of water [3]. The role of pituitary gland in calcium regulation was first shown in the killifish, *Fundulus heteroclitus* [4]. The PRL cell activity and level of calcium in the external medium show inverse relationship that is, decrease in external calcium concentration stimulates PRL cell activity while increased calcium level reduces the activity [5]. However, there are few reports that contradict these findings [6,7].

Pesticides, metals and other environmental pollutants interfere with the osmotic and ionic regulation of organisms and thereby affect the physiological processes in the body [8-12]. Prolactin cells show hyperactivity in fishes when exposed to pesticides or other harmful chemicals that interfere with calcium homeostasis in the body. Dimethoate (O,O-dimethyl-S-(N-methylcarborylmethyl)-phosphoro-dithioate) is a systemic organophosphorous insecticide, widely used against household and agricultural insect pests. The pesticide persists in soil and atmosphere and enters water bodies through rain water runoff affecting non-target organisms such as fish. It is one of the most preferred insecticides used in India. The present study focuses on effect of dimethoate on pituitary prolactin cells which plays important role in calcium homeostasis of the fish.

2. MATERIALS AND METHODS

Fish, *H. Fossilis* (both sexes; length: 17.4 ± 1.1 cm and weight: 27.1 ±2.0 g), were procured from local ponds and safely brought to laboratory and transferred to 500 L capacity plastic tanks containing tap water. The experiment was conducted during the month of August. The physicochemical properties of water during bioassay were – temperature 24 ±1℃, pH 7.2 ± 0.15, dissolved oxygen 7.8 ± 0.76 mg/l and hardness as CaCO3 115.34 ± 1.45 mg/l. No mortality was recorded in control and experimental group. Fish were daily fed with a mixture (about 0.1 g/fish) of wheat flour, mustard cake, dried prawn powder, and soybean in a ratio of 3:1:1:1. Dimethoate 30% effective concentration technical grade (Rallis India Pvt. Ltd., Mumbai, India) was used. Glass aquaria (30 L capacity), filled with 25 L tap water, and were used for the experiments.

LC_{50} values were determined in earlier study of Pandey et al. [13] and fish were exposed to dimethoate for examinations of histological changes in PRL cells. Fish were subjected to sub-lethal concentration of dimethoate 2.24 mg/l (75% of 96 h LC_{50}) for short term (24, 48, 72 and 96 h), and 25% of 96 h LC_{50} (1.00 mg/l) for long term (6, 12, 24 and 36 d) exposure. A set of control was run concurrently. Fish (exposed and control), were randomly selected from each duration and anaesthetized with 0.03% MS-222 (tricaine methane sulfonate). Pituitary, attached together with brain was carefully dissected out and fixed in Bouin's fluid for 24 h. Before embedding, the tissue (brain) was washed thoroughly to remove traces of the Bouin's fluid. After procedural dehydration and embedding, serial sagittal (vertical longitudinal) sections were cut at 5-6 µm and stained following Cleveland Wolfe trichrome procedure after the modification of Ruijter et al. [14]. To determine the average nuclear diameter of the gland cells, 50 nuclei were randomly selected from every fourth section of the gland and their diameter was measured with the help of oculometer under oil immersion (x1000). In total, over 200 nuclei were always measured for each gland. The nuclear volume (NV) was calculated by the formula- $NV= 4/3\ \pi\ a.b^2$ (Where, 'a' is the major nuclear axis and 'b' represents the minor nuclear axis).

3. RESULTS

3.1 Cellular Changes

In *Heteropneustes fossilis*, the prolactin cells (PRL) are located in rostral pars distalis (RPD) and may be identified by erythrosinophilic response of cytoplasm showing red granules. These cells demonstrate positive staining property to azocarmine, erythrosin and acid fuchsin (Fig. 1). The PRL cells in control form a compact mass around the neurohypophysis (NH), extending ventrally and laterally for a distance around the proximal pars distalis. The PRL cells have indistinct cellular boundary with distinct rounded nuclei containing one or more nucleoli (Fig. 2).

The histological examination of the prolactin cells of dimethoate exposed fishes for different durations, showed marked changes in their structure and staining properties. In short-term experiment the PRL cells exhibited hypertrophic nuclei, distinct nuclear boundary and apparent increase in nuclear diameter after 24 h of dimethoate exposure when compared to control (Fig. 3). Following 48 h exposure the PRL cells showed an increase in granulation, staining response and vascularization (Fig. 4). The nuclei exhibit reduction in nuclear diameter as compared to 24 h value. There was further reduction in nuclear diameter after 72 h and the nuclei at some places became indented and their cell boundaries were deformed. PRL cells exhibited vacuolization at random places and showed increased staining property (Fig. 5). After 96 h of exposure the PRL cells exhibited severe vacuolization and pycnosis of the nuclei. The nuclear boundaries were indented and highly deformed. The nuclear diameter and staining response was further reduced (Fig. 6).

In long-term dimethoate exposure the PRL cells of *Heteropneustes fossilis*, exhibited low staining response and no vacuolization after 6 d and the nuclei were generally oval or spherical with distinct nuclear boundaries (Fig. 7). After 12d of dimethoate exposure the cellular vacuolization was increased and the staining property of cells reduced. The nucleus showed pycnosis together with deformed and indented nuclear boundary and diameter of nuclei got reduced (Fig. 8). The PRL cells following 24 d exposure exhibited reduced staining response, severe cellular vacuolization, nuclear pycnosis and reduction in nuclear diameter. There was an increase in vascularization (Fig. 9). After 36 d of pesticide exposure there was severe vacuolization in PRL cells. The nuclei exhibited pycnosis and the nuclear diameter recorded further reduction and cells showed poor staining response and increased vascularization (Fig. 10).

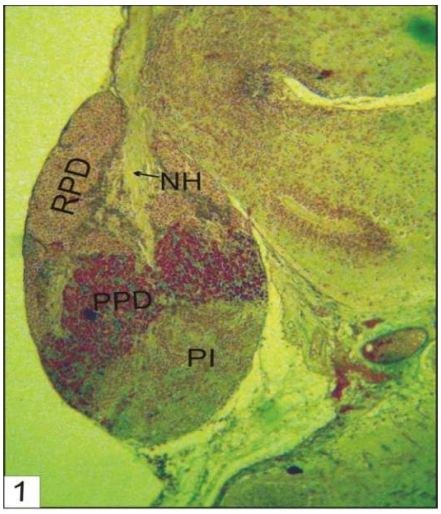

Fig. 1. Saggittal section of fish (*Heteropneustes fossilis*) brain showing pituitary and its different regions: Rostral Pars Distalis (RPD), Proximal Pars Distalis (PPD), Neurohypophysis (NH) and Pars Intermedia (PI). [Cleveland Wolfe trichrome, X100]

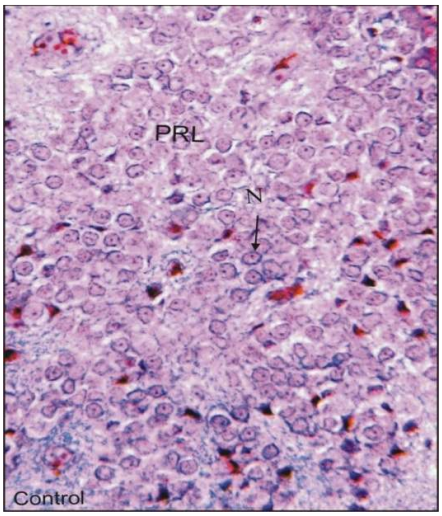

Fig. 2. Pituitary of untreated Control showing PRL cells with indistinct boundary and distinct oval nuclei (N). [Cleveland Wolfe trichrome, X1000]

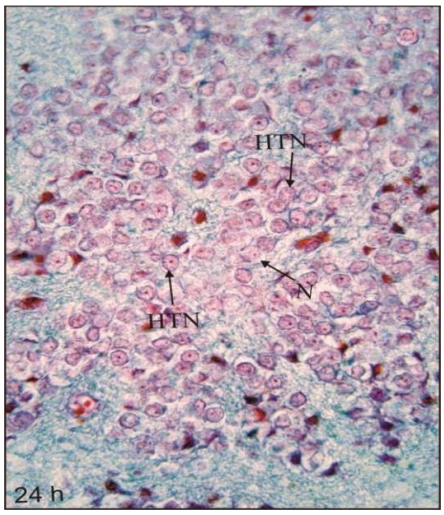

Fig. 3. 24 h dimethoate treated pituitary PRL cells exhibiting hypertrophic nuclei (HTN) and distinct nuclear boundaries (N). [Cleveland Wolfe trichrome, X1000]

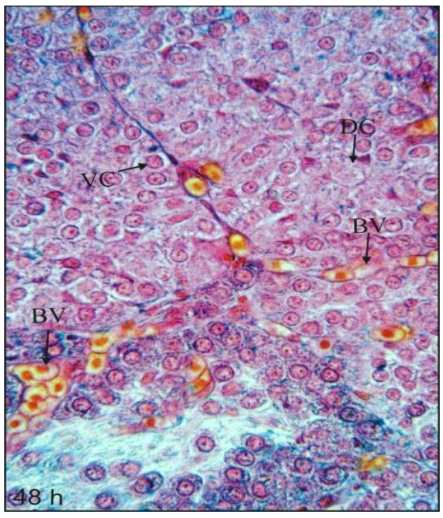

Fig. 4. 48 h dimethoate treated pituitary PRL cells showing increased granulation and staining response; vacuolization of cells (VC); cellular degeneration (DC); rounded or oval nuclei exhibiting hypertrophy; decrease in nuclear diameter and increased vascularization (BV). [Cleveland Wolfe trichrome, X1000]

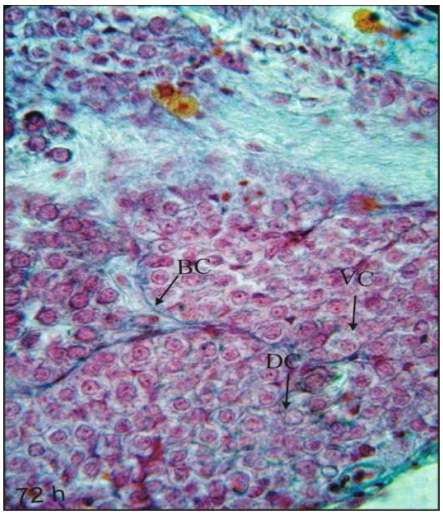

Fig. 5. 72 h dimethoate treated pituitary PRL cells exhibiting reduction in nuclear diameter; indented and deformed nuclear boundaries (DC); vacuolization (VC); and increased staining property. [Cleveland Wolfe trichrome, X1000]

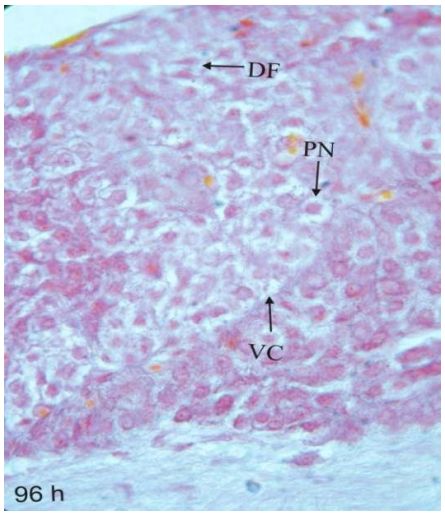

**Fig. 6. 96 h dimethoate treated pituitary PRL cells exhibiting severe vacuolization (VC) and pycnotic nuclei (PN). The nuclear boundaries are indented and highly deformed (DF). The nuclear diameter is reduced.
[Cleveland Wolfe trichrome, X1000]**

3.2 Changes in Nuclear Volume

The pituitary prolactin cells of fish *H. fossilis* exhibited altered nuclear activity when subjected to sub-lethal dose of dimethoate for short-term (96 h) and long-term (36 d) exposure. In this study the prolactin cells underwent hypertrophy with a significant increase in nuclear diameter after 24 h of dimethoate exposure. However,

following 48 h exposure the nuclear diameter decreased and the reduction continued up to 96 h. Vacuolization and degeneration was observed following 72 h and 96 h of exposure (Fig. 11). In long-term experiment the PRL cells did not show any remarkable change in their activity after 6 d exposure. After 12 d, the PRL cells exhibited reduced nuclear diameter, vacuolization and low staining property which progressively increased till 36 d (Fig. 11).

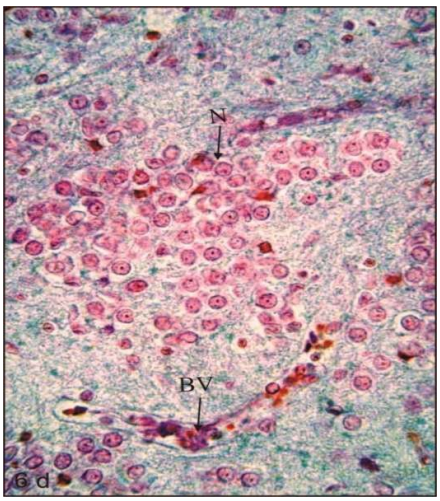

Fig. 7. 6 d dimethoate treated pituitary PRL cells exhibit low staining response and absence of vacuolization; generally oval with distinct boundaries (N). [Cleveland Wolfe trichrome, X1000]

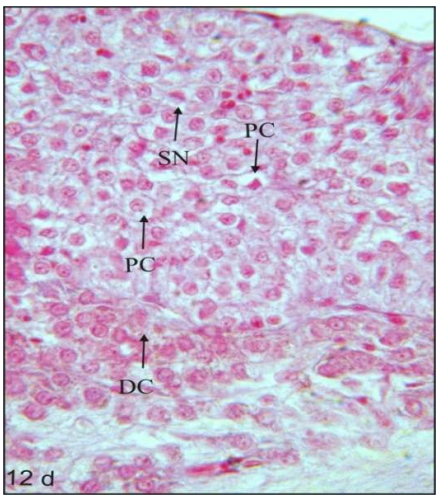

Fig. 8. 12 d dimethoate treated pituitary PRL cells showing reduced staining property; increased vacuolization; pycnotic cells (PC); deformed and indented nuclear boundary; nuclear shrinkage (SN) and cellular degeneration (DC). [Cleveland Wolfe trichrome, X1000]

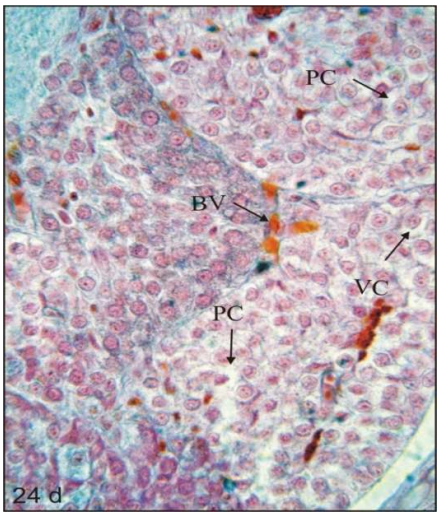

Fig. 9. 24 d dimethoate treated pituitary PRL cells exhibiting reduced staining response, severe cellular vacuolization (VC), nuclear pycnosis (PC), reduced nuclear diameter and increased vascularization of the gland (BV). [Cleveland Wolfe trichrome, X1000]

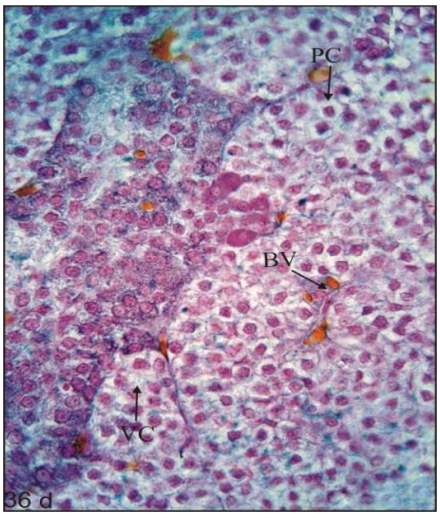

Fig. 10. 36 d dimethoate treated pituitary PRL cells showing severe vacuolization (VC); pycnosis (PC), reduction in nuclear diameter, poor staining response and increased vascularization of gland (BV). [Cleveland Wolfe trichrome, X1000]

4. DISCUSSION

The pituitary prolactin cell hyperactivity indicates release of hormone prolactin, one of the main osmoregulatory hormones in the fish. It was observed in this study that dimethoate exposure caused hypocalcemia which probably induced the activity of PRL cells. However, following 48 h exposure the nuclear diameter decreased and the reduction continued up to 96 h. Hypertrophy with a significant increase in nuclear diameter after 24 h dimethoate exposure in PRL cells, supports the earlier observation of Das et al. [2] who reported hypercalcemia during initial (24 h) exposure and declined serum Ca^{2+} level after 48 h. Similarly in long-term experiment, hypercalcemia was reported during early exposure followed by hypocalcemia after 12 d [2,12]. These observations may be corroborated by histo-morphological changes in nuclear volume and size of PRL cells. The study derives support also from the observations of other reports on altered prolactin cell activity under the influence of various toxic materials. Hypocalcemia was observed in *Oreochromis mossambicus* after cadmium exposure and marked morphological and ultra-structural changes in PRL cells indicating enhanced release of prolactin hormone [15]. Contrary to it, James and Wigham did not find any consistent effect on prolactin cell activity in *Salmo gairdneri* when exposed to cadmium [16]. The inhibitory effects of kepone on prolactin synthesis in freshwater eel, Amphipnous gachua have also been reported [17]. Similarly, Larsson and Haux report disturbance in serum prolactin levels in DDT exposed freshwater eel, Anguilla anguilla [18]. In Sarotherodon mossambicus, an increase in serum prolactin level after sub-lethal exposure of dimecron was observed [19]. The sub-lethal exposure of ziram causes significant reduction in prolactin levels after 24, 48 and 72 h while the combined exposure of sub-lethal dimecron-ziram causes significant decline in serum prolactin levels [20]. In another report, Thangavel et al. have observed inconsistency in prolactin levels of Sarotherodon mossambicus exposed to endosulfan. They observed an elevation (193%) in prolactin levels following 12 h of exposure and thereafter, a drop (-9%) following 24 h and again significant elevation (2%) following 5 days [21]. The increase in plasma prolactin levels in fishes exposed to DDT has been reported [22]. Hyperactivity of prolactin cells marked by increased nuclear volume in the catfish Prolactin cells of a teleost *Heteropneustes fossilis* after exposure to metacid-50 and cypermethrin [23,24]. However, no histological change in the prolactin cells on short-term deltamethrin exposure, but in long-term exposure they found an increase in nuclear volume and granulation of prolactin cells after 14 d exposure and degeneration of cells following 28 d was observed [25].

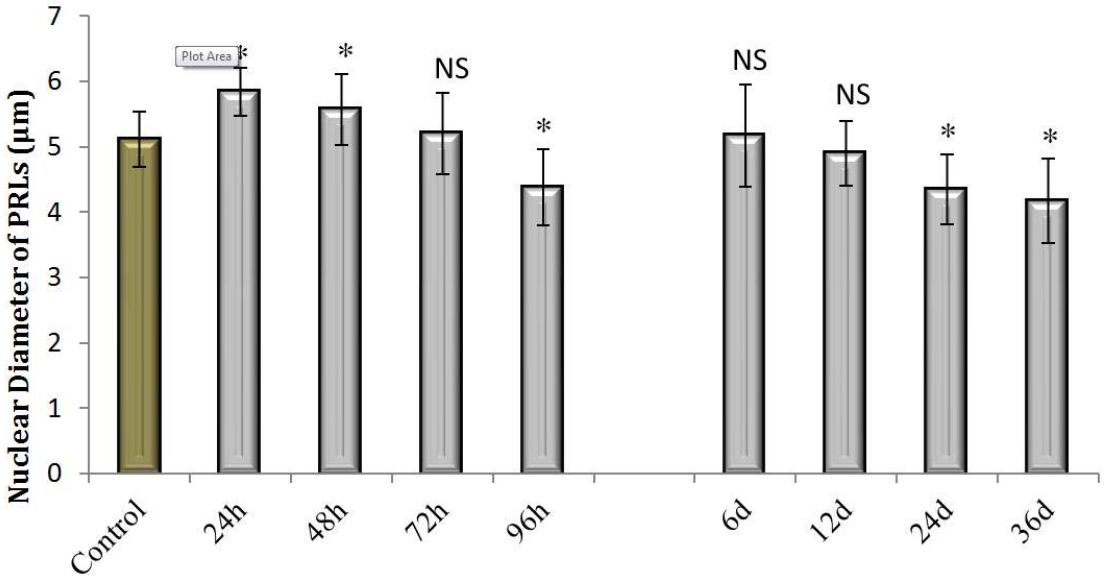

Fig. 11. Nuclear diameter of prolactin cells after dimethoate exposure in *Heteropneustes fossilis*

***** *Significant (P< 0.05, Student's t-test), NS = Not significant*

Prolactin is considered responsible for the maintenance of plasma electrolyte level, mainly by controlling permeability of the gill epithelium [26]. Moreover, in freshwater fishes prolactin has a specific action on calcium metabolism. In various fishes it promotes hypercalcemia, mainly by the increasing the rate of calcium uptake through the gills [27]. Prolonged exposure of fish to pesticides cause severe damage to the gill epithelium [28] therefore, prolactin action to promote calcium uptake from the gills is hampered and the serum Ca2+ level is further reduced. Under the aggravating hypocalcemic conditions during prolonged exposure, the PRL cells become exhausted and exhibit severe vacuolization, degeneration and reduced staining properties (Figs. 1-10).

5. CONCLUSION

From this study it may be concluded that exposure of dimethoate causes histological changes in pituitary gland and severely affects calcium regulating prolactin (PRL) cells and disturb the calcium homeostasis of fish which is important for overall fish health and their survival.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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