



EVALUATION OF DIMETHOATE-INDUCED TOXICITY ON BLOOD PHYSIOLOGY, LIPID METABOLISM, BIOCHEMICAL MARKERS, AND THYROID HORMONAL BALANCE IN NILE TILAPIA

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ABSTRACT

Present experiment was performed to assess the toxicological impacts of pesticide dimethoate on haematological alterations in fish, Nile tilapia. Nile tilapia is a section feeder, omnivorous fish and mostly refined fish in South Asia. Fish were divided in three groups. One group was treated as control while second and third groups were exposed to 4 mg/L and 8 mg/L dimethoate respectively for 28 days. The most haematological change was severe microcytic hypochromic anemia. Results revealed that the value of HGB (control 5.53±0.40) (low 4.53±0.44) (high 3.10±0.51) (g/dl), RBC (control 2.47±0.35), (low 1.78±0.98), (high 1.44±1.4) (x106/ μL), HCT (control 17.6±1.30), (low 13.6±1.45), (high 10.0±0.90) (%), MCV (control 111±1.34) (low 129±2.98) (high 135±3.6) (fL), MCH (control 33.2±1.13) (low 40.4±1.56) (high 43.6±1.91) (pg), MCHC (control 28.4±1.13) (low 36.5±1.72) (high 41.0±2.26) (g/dl), RDW-SD (control 76.4±2.52) (low 70.6±1.91) (high 60.5±1.40) (%), PDW (control 11.1±1.1) (low 8.62±0.83) (high 4.63±0.69) (%) and PCT (control 0.650±0.07) (low 1.02±0.22) (high 1.58±1.02) (%) were significantly reduced. WBC (control 20.9±1.10) (low 24.9±1.80) (high 29.9±3.22) (x103/μL), RDW (control 16.5±0.78) (low 19.5±1.22) (high 22.3±1.47), PLT (control 207±2.00) (low 199±3.06) (high 168±1.23) (x103/μL), MPV (control 9.27±0.88) (low 11.4±1.1) (high 19.2±1.78) (fl), neutrophils (control 17.2±0.862) (low 19.7±1.04) (high 27.9±1.84) (%), lymphocytes (control 82.1±1.06) (low 92.3±1.12) (high 4110±2.08) (%), monocytes (control 2.24±0.07) (low 3.11±0.25) (high 5.04±1.12) (%) and eosinophils (control 1.18±1.60) (low 1.89±1.87) (high 3.33±2.33) (%) were increased respectively as compared to pesticide free group. After the obtained results, it indicates that dimethoate has caused widespread toxic effects on aquatic organisms specially fish.

INTRODUCTION

Morphometric studies currently, there is a lot of worry over the harmful ecological effects that come with the careless use of pesticides in agriculture. Agrochemicals called pesticides are frequently employed in agriculture to manage a variety of pests, including fungi, insects, undesirable weeds, and parasitic nematodes (Tudi et al., 2021). In order to improve food production and quality, they are widely utilized in both wealthy and developing nations. Consequently, pesticides and our nation's advanced agricultural achievements have a strong and unbreakable relationship. India ranks fourth in the world for exports of pesticides and is the largest state in Asia, ranking twelfth in terms of production and consumption (Devi et al., 2017; Khan et al., 2025).

The harmful possessions of toxic pesticides on the ecosystem and biodiversity have been the subject of several studies conducted over the past few decades. These chemicals, in particular the persistent ones, cause residues that pose a variety of health risks as well as affect the natural ecological equilibrium. Just a tiny ratio of all pesticides are really successful in excluding or managing the intended pest, and the common is discharged into the atmosphere, especially the aquatic ecosystem, wherever they negatively affect non-target species (Özkara et al., 2016; Tudi et al., 2021).

Pesticides negatively impact aquatic ecosystems, as demonstrated by a wealth of research. They contaminate water by letting runoff and erosion from treated areas, spilling agrochemical formulations accidentally, encompassing spread crops to the control of bodies of water, and causing air pollution

from agrochemical industrial effluents. They also poison water by clearing excess origination into rivers, ponds, and seas and by driving the washing water of scattering equipment into water bulks (Barlas, 1999).

Numerous interdependent aquatic life forms are killed by water pollution, which also contributes to the bio magnification of persistent pesticides. Fish mortality and environmental effects may arise locally from this. Fish are very susceptible to changes in the biological and physicochemical properties of aquatic environments. Research has shown that fish are at risk of dying from exposure to harmful substances in aquatic environments (Caldas et al., 1999).

Fish are therefore thought to be extremely sensitive biological indicators of any unfavourable alteration to the aquatic ecosystem. Additionally, there have been instances of widespread aquatic animal deaths due to pesticide overdoses in many regions of the world. There is documented fish mortality as a result of pesticides such as the aldrin (organochlorine pesticide) and malathion (*organophosphorus pesticide*) groups (Jayaraj et al., 2016).

Profound alterations in the internal environment, such as biochemical traits, tissue integrity, immunological state, and blood parameters in fish, can be useful indicators of stress, exposure to toxins or pesticides, the beginning of a disease, or any detrimental change in the quality of the water. Well-established biomarkers that have been investigated in a variation of fish species include tissue biochemistry, hormones, hematology, and histopathology (Tufail et al, 2025; Akram et al., 2025). The least intrusive of them is the study of hematological

parameters as biomarkers because it seldom necessitates killing fish in order to collect blood samples. Blood can give precise information about how pesticides affect their exposure in the environment, and hematological parameters typically give a strong first warning sign of a fish's altered physiological condition brought on by stress, pollution, pesticide exposure, or infection (Joseph & Raj, 2011).

Important hematological parameters for fish include total leucocyte count (TLC), coagulation time, packed cell volume (PCV), erythrocyte sedimentation rate (ESR), absolute values, total erythrocyte count (TEC), and hemoglobin content (Singh and Srivastava, 2010). Fish variations in these metrics make it simple to evaluate changes in the quality of the aquatic environment. Because of this, it's a really helpful tool for studying the toxicological effects of pesticides on fish, and you have access to a lot of research in this field (Le Goff & Giraud, 2019; Javed et al., 2025).

Fish contains glucose in their blood which remains in the liver in the form of glycogen and is converted to glucose when needed (ÇELİK et al., 2008). Their conversion is done by two processes one is glycogenolysis and the other one is gluconeogenesis (Aslan, 2009). Dimethoate treated fish shows a significant increase in the level of glucose because Dimethoate causes stress in fish, and due to stress the level of glucose increases (Barton, 2002; Carballo et al., 2005).

Objectives

To comprehensively evaluate the toxicological effects of Dimethoate exposure

in Nile Tilapia (*Oreochromis niloticus*) by assessing alterations in hematological parameters, lipid profile, serum enzyme activities, and thyroid hormone levels, thereby determining its impact on physiological, biochemical, and endocrine functions.

MATERIALS AND METHODS

Specimen collection and acclimatization

A total of 15 randomly selected fingerlings of the fresh water fish Nile talapia of body length 10-15cm and body weight 20-30g were purchased from the Head Baloki fish pond, and brought to the fisheries laboratory located inside the Zoology Department of the University of Okara, Renala Khurd on the island of Okara. All of the fish were examined to see whether or not they had any infectious diseases before being moved to the aquarium. Following the transfer of the fish, they were placed in glass aquariums that contained water from the tap. Utilizing a recirculation-aerated system (RAS) that included a water renewal system that changed the water on a daily basis to eliminate food leftovers and excrement of fish, fish were acclimated to the laboratory environment for a period of ten days prior to the trial. Oxygen concentration of 7.25 ± 0.23 mg/L, temperature 24.5 ± 2.7 c and pH of 7.46 ± 0.28 of water were upheld throughout the experimental duration of 28 days.

Experimental design

Experiment was designed for 28 days and fish were kept in aquariums with 40-liter water capacity. The fishes were fed with commercial feed one time in 24 hours during the experimental duration. It was preferred that fish of similar weight should be placed in

the same group. Two treatments were performed depending based on the concentration of Dimethoate. The first group was control with no exposure to Dimethoate group 2 was given 4mg/L Dimethoate and group 3 was given 8mg/L Dimethoate. All the protocols for handling of fish were implied after having approval from ethical committee of department of zoology at University of Okara, Renala Khurd.

Hematological study

Following tests were performed in this means.

White Blood Cells (WBC)

These are abbreviated as WBCs and are the cells of immune system, which function as protecting body against foreign microorganisms and infectious diseases. All the white blood cells are produced from multi potent cells called hematopoietic stem cells present in bone marrow. Leukocytes are present in whole body of organism involving lymphatic and blood system.

$WBC (10^9/L) = \text{Average of cells} \times \text{correction for dilution} \times \text{No. of squares} \times \text{Volume of one square}$

Hemoglobin (Hb)

The quantitative measurement of Hb was calculated by MICRO-LAB 3000 (Merck, Germany). The wavelength of instrument was set to 540nm. 5ml solution of Hb was taken and then mixed this solution with 20 μ l of whole blood and left this mixture for five minutes at room temperature and then Hb concentration was calculated in g/dl.

Total Red Blood Cells Count (TRBC)

Using improved Neubaur Ruling Hemacytometer total red blood cell count was determined. The blood was diluted at ratio of 1:200 as 4ml of diluting solution was taken

and mixed with 20 μ l of blood with the help of micropipette and then left for five minutes. Little amount of this fluid is then inserted into counting chamber. RBCs were counted in 5 small squares with large central squares. Red blood cell number was calculated using standard formula which is given below.

$RBC \% = 50 \text{ volume factor} \times 200 \text{ Dilution Factor} \times \text{Number of cells counted} / 100$

Mean Corpuscular Volume (MCV)

It is abbreviated as MCV is the mean of volume of red blood cells in a sample. It increases or decreases directly proportional to mean of red blood cells. Standard of mean corpuscular volume shows normocytic low MCV illustrate microcytic while high MCV shows macrocytic.

Conventional formula which is given below is used to calculate Mean Corpuscular Volume (MCV).

$MCV(\text{femto liters}) = \text{Hematocrit} \% \times 10 / \text{RBC count (million}/\mu\text{l})$

Mean Cell Hemoglobin

Mean cell hemoglobin is abbreviated as MCH. Concentration of MCH referred to mean of hemoglobin present in red cell in organism body. Hemoglobin is necessary protein present in RBCs which aid red blood cells to carry oxygen to tissues and other body cells.

It was calculated with the help of conventional formula.

$MCH(\text{pico grams}) = \text{hemoglobin gdl} \times 10 / \text{RBC count (millions}/\mu\text{L})$

Mean Cell Hemoglobin Concentration (MCHC)

It is abbreviated as MCHC. It is the estimation of mean concentration hemoglobin found in red cells. MCHC is a parameter of complete blood count abbreviated as CBC.

The concentration of mean cell hemoglobin concentration was calculated by using following formula and written as g/dl.

Mean cell hemoglobin concentration (g/dl) = hemoglobin gdl \times 100PCV

Platelets Counting

These are small cells of blood that helps body to form cots to stop bleeding from injury. If any vessel of body gets damaged due to injury, it sends signals to which platelets responds. Then platelets move to site of injury and form clot to repair the injured site. It is spreads across overall surface of damaged vessels to inhibit bleeding from this site and this process is known as adhesion. The story behind this phenomenon is that when platelets move to damaged sit they develop sticky tentacles that help these platelets to stick over the damaged vessels. They also keep sending chemical signals to call more platelets to site of injury and this process is known as aggregation.

Assessment of biochemical parameters

We used the gold standard method for calculating VLDL and LDL levels that was laid forth by Zaahkouk, Helal et al. (1996). Using the approach described by Hadie, Ghani et al. (2013) and standard kits, the T3, T4, and TSH levels were evaluated.

Statistical analysis

Utilizing the analysis of variance (ANOVA) tool, the statistical analysis was performed., with a significance threshold of $p < 0.05$. In order to create graphical representations, GraphPad Prism version 9.4.7 was used.

RESULTS

Hematological studies

After the study period, the hematological data indicated that the concentrations of hemoglobin (HGB), red blood cells (RBC), hematocrit (HCT), red cell distribution width standard deviation (RDW-SD), platelet distribution width (PDW), and PLT in the fish that had been treatment with dimethoate were noticeably decreased than those in the group that those in the group that had not been exposed to any pesticides. while, white blood cells, platelets, mean corpuscular volume (MCV), MCH, MCHC, RDW, MPV, PCT, neutrophils, monocytes, and were shown to have significantly increased. Additionally, the exposure to Dimethoate as a consequence, the number increased of neutrophils, lymphocytes, monocytes.

The information is shown in Table 1. Nile's haematological profile at the time. In this experiment, tilapia was subjected to varying doses of Dimethoate. The data are stated as the average value with the addition or subtraction of the standard deviation. Asterisk (*) is used to indicate that there are notable distinctions between the two ($P < 0.05$) when compared with the group that did not include dimethoate carrying data. NS stands for "not significant".

Table 1: hematological parameters of Nile tilapia

Parameters	Control	Low	High
HGB (g/dl)	5.53 \pm 0.4 0	4.53 \pm 0. 44	3.10 \pm 0. 51
WBC ($\times 10^3/\mu\text{L}$)	20.9 \pm 1.1 0	24.9 \pm 1. 80	29.9 \pm 3. 22
RBC ($\times 10^6/\mu\text{L}$)	2.47 \pm 0.3 5	1.78 \pm 0. 98	1.44 \pm 1. 4
HCT (%)	17.6 \pm 1.3	13.6 \pm 1.	10.0 \pm 0.

	0	45	90
MCV (FL)	111±1.34	129±2.98	135±3.68
MCH (pg)	33.2±11.3	40.4±1.56	43.6±1.91
MCHC (g/dl)	28.4±1.13	36.5±1.72	41.0±2.26
RDW %	16.5±0.78	19.5±1.22	22.3±1.47
RDW-SD (%)	76.4±2.52	70.6±1.91	60.5±1.40
PLT (x10 ³ /μL)	207±2.00	199±3.06	168±1.23
MPV (fl)	9.27±0.88	11.4±1.1	19.2±1.78
PDW%	11.1±1.1	8.62±0.83	4.63±0.69
PCT%	0.650±0.07	1.02±0.22	1.58±1.02
Neutrophils %	17.2±0.862	19.7±1.04	27.9±1.84
Lymphocytes %	82.1±1.06	92.3±1.12	110±2.08
Monocytes %	2.24±0.07	3.11±0.25	5.04±1.12
Eosinophils %	1.18±1.60	1.89±1.87	3.33±2.33

Biochemical Analysis

After the study period, the biochemical data indicated that the concentrations of Cholesterol, Triglycerides, LDL, VLDL, ALT, ALP, AST, Blood glucose, TSH, blood urea, BUN, and creatinine in the fish that had been treatment Dimethoate were noticeably increase than those in the group that those in the group that had not been exposed to any pesticides. while, HDL, Protein, Albumin, Globulin, T3, and T4 were

shown to have significantly decline.

The information is shown in Table 2. Nile's biochemical profile at the time. In this experiment, tilapia was subjected to varying doses of Dimethoate. The data are stated as the average value with the addition or subtraction of the standard deviation. A sterisk (*) is used to indicate that there are notable distinctions between the two ($P < 0.05$) when compared with the group that did not include dimethoate carrying data. NS stands for "not significant."

Table 2: Biochemical parameters of freshwater fish Nile tilapia

Parameters	Control	Low	High
Cholesterol (mg/dl)	135±1.83	155±2.70	181±4.08
Triglycerides (mg/dl)	168±3.99	191±4.67	215±4.95
HDL (mg/dl)	35.6±1.95	28.4±1.52	14.4±0.92
LDL (mg/dl)	67.5±1.33	79.9±1.39	99.9±2.08
VLDL (mg/dl)	33.8±0.73	38.5±1.67	47.8±1.98
ALP (U/L)	296±1.35	338±1.84	427±3.95
ALT (U/L)	39.6±0.52	46.7±0.98	66.6±1.45
AST (U/L)	249±1.00	324±1.53	443±3.61
Serum Protein (g/dl)	3.63±0.45	2.88±0.33	1.77±0.11
Albumin (g/dl)	1.80±0.33	1.52±0.12	0.880±0.06
Globulin (g/dl)	1.77±0.52	1.57±0.42	0.960±0.12
Blood glucose (mg/dl)	56.0±1.00	72.3±1.11	99.7±2.23
T3 (nmol/L)	1.42±0.08	1.24±0.6	0.693±0.3
T4 (nmol/L)	75.3±1.53	54.0±1.47	23.0±1.01
TSH (nmol/L)	0.570±0.10	0.933±0.2	1.53±0.54
Urea	13.5±0.5	20.7±0.87	41.0±1.5

(mg/dl)			
BUN (mg/dl)	6.87±0.70	9.67±0.85	19.6±1.26
Creatinine (mg/dl)	1.02±0.03	1.38±0.55	2.67±1.0

DISCUSSION

Pesticides, which function as xenobiotics and environmental pollutants, pose significant health risks and have extensive biological effects. Scientific evidence demonstrates that teratogenic disorders, genotoxicity, embryonic toxicity, and histological damage are all caused by carbamates, organophosphates, and organochlorines, which make up major pesticide categories (Fetoui et al., 2010). Aquatic organisms, along with other forms of wildlife, suffer high sensitivity to contaminants such as pesticides and heavy metals and their associated carcinogenic properties, as well as mutagenic properties and neurotoxic and cytotoxic effects (Akhtar et al., 2021). Runoff from farms and factories into bodies of water contains harmful chemicals that cause bioaccumulation effects and physiological disturbances in aquatic organisms (Naz et al., 2021). Pesticide stockpiling within the liver substantially affects how cells perform their functions because this organ oversees detoxification processes. Research focused on ecological toxicity needs to occur in order to determine exposure hazards for aquatic species as well as humans (Kim & Kang, 2016). When fish species come into contact with pesticides, they show multiple detrimental effects on their physiology and biochemical processes as well as tissue anatomy with evidence of DNA damage. The organophosphate pesticide dimethoate is commonly used to get rid of

pests in agriculture, and it is toxic to tilapia fish and other fish species that are exposed to it (Al-Saeed et al., 2023; Silva et al., 2020). Research investigators examined the effect dimethoate had on producing changes throughout tilapia fish blood and biological chemistry.

Haematological Alterations

According to Mahmood et al. (2023), haematological changes provide crucial evidence about the physiological issues fish experience when exposed to xenobiotics. This evaluation technique remains a standard procedure to determine health status across different types of vertebrates, from birds (Akmal et al., 2023) to mammals (Ahmad et al., 2023). The research uncovered substantial decreases in dimethoate-exposed fish Hb concentration as well as RBC and HCT levels, RDW-SD measurements, and platelet and PDW parameters when compared to untreated fish. Dimethoate exposure led to equivalent haematological changes in the blood of *Cyprinus carpio*, according to Ghelichpour et al. (2019). Previous studies that evaluated albino rats under dimethoate treatment observed related anomalies in their blood indices (Shalby, 2006).

This study detected decreased parameters of Hb, HCT, and platelets, together with PDW, after fish were exposed to dimethoate for 28 days. The results that Naseem et al. (2022) found when they treated Nile tilapia with dimethoate were similar to those that Jamil et al. (2024) found when they studied different fish species that were exposed to pesticides. Pesticides lower the number of red blood cells and haemoglobin because they stop erythropoiesis, which could be caused by damage to haemopoietic tissue

(Nithiyanandam et al., 2007). The poor oxygen transport function of low haemoglobin levels blocks tissue supply and reduces metabolic rates alongside decreased energy production. Severe haemoglobin decrease creates anaemia that occurs from the breakdown of red blood cells, as reported by Kumari and Subisha (2010). The damaging effect of pesticides on liver and kidney tissues causes a reduction in HCT values. An anaemic condition develops when both HCT decreases and MCV increases.

These cell components play a vital role in both coagulation processes and haemostasis by originating from bone marrow tissues. The blood-clotting disorder thrombocytopenia results from low platelet levels, whereas it represents a major source of bleeding abnormalities (Akram et al., 2021). Environmental scientists believe that dimethoate exposure disrupted local bone marrow tissues, which led to reduced blood components during the study period, according to Al-Layl (2004). Dimethoate pesticides appear to destroy red blood cells directly while simultaneously damaging haemopoietic tissue.

When fish were exposed to dimethoate, there was a big rise in the number of white blood cells (WBC), mean corpuscular haemoglobin (MCH), mean platelet volume (MPV), plateletcrit (PCT), neutrophils, lymphocytes, monocytes, and eosinophils. The higher levels of WBCs and related markers found in fish are the same as what was reported in Shalby (2006) and Jamil et al. (2024). The human body produces more white blood cells when it activates immune responses because of toxic stressors (Rogers et al., 2013). The clear rises in neutrophils,

lymphocytes, eosinophils, and WBC counts show that the body is responding to pesticide damage in two ways: immunologically and inflammatoryly (Zeinab Mohamed et al., 202). The levels of MCH molecules in the blood of exposed fish point to a condition named macrocytic anaemia, which affects their red blood cell size (Saravanan et al., 2011). High MPV levels led to more activated platelets, which made people more likely to get heart diseases and thromboembolic events (Karabacak et al., 2014). The measurements of RBCs and RDW-SD with MPV, PCT, and neutrophils experienced minimal modification. The dimethoate exposure failed to produce any substantial effects on MCHC and RDW levels. All blood test results changed significantly when exposed to all of the substances because liver and kidney problems hurt bone marrow production (Bihari et al., 2016).

Biochemical Alterations

Blood biochemical analyses can evaluate fish health status and the effects of environmental contaminants (Sachar & Raina, 2014). The evaluation of toxic pollutants through serum biochemical tests serves as an important method to analyse how environmental pollutants affect aquatic organisms (M. Hussein et al., 2021). The research showed that blood glucose levels were elevated in dimethoate-treated fish as a result of stress, which affected their metabolism. The stress-induced elevation of glucose results from the liver's glycogen mobilisation process, which functions as a protective mechanism during prolonged stress events and creates gluconeogenesis through lactate and amino acids (Barton, 2002). The research results matched previous data from

fish experiments using dimethoate, which were conducted on *Cyprinus carpio* by Ghelichpour et al. (2019) and Nile tilapia by Naseem et al. (2022).

The liver serves as the main regulator of triglyceride and cholesterol metabolic processes (Umar et al., 2025). The toxicants disrupt cholesterol and triglyceride levels because they cause liver damage and impede absorption, thus impacting lipoprotein synthesis (Hoseini et al., 2014). The research findings showed both increased cholesterols together with low-density lipoproteins (LDL), triglycerides and very low-density lipoproteins (VLDL) and reduced high-density lipoprotein (HDL) levels. Previous studies about pesticide effects on lipid profiles confirm the research findings of Jamil et al. (2024) and Ghelichpour et al. (2020) as well as Naseem et al. (2022). The elevated levels of triglycerides and cholesterol relate to catecholamine-stimulated lipolysis that leads to increased fatty acid synthesis (Rai et al., 2009).

The exposure to pesticides led to noticeable increases in liver function biomarkers, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT). Hepatic tissue damage caused the reduction of serum total protein and globulin levels (Mossalem et al., 2013; Iftikhar et al., 2025). Biochemical studies have shown such changes occur in fish exposed to pesticides, according to Naseem et al. (2022), Ibrahim et al. (2018), and Jamil et al. (2024). Plasma proteins serve as indicators of immune function and organ health. Declines in plasma proteins following pesticide exposure may reflect impaired protein synthesis due to liver

damage (Shahzadi et al., 2024). The reduction in globulin levels may compromise immune defenses, making fish more susceptible to infections (Zaccaroni et al., 2009). Furthermore, pesticide exposure led to increased blood urea nitrogen (BUN) and creatinine levels, indicating nephrotoxicity (Shahzadi et al., 2024).

Thyroid function was also affected by dimethoate exposure, with reductions in triiodothyronine (T3) and thyroxine (T4) levels, while thyroid-stimulating hormone (TSH) levels increased significantly. These findings suggest disruptions in the hypothalamic-pituitary-thyroid axis (Weiss et al., 2003; Bilal^{a,b}, 2021), as previously documented in pesticide toxicity studies (Hadie et al., 2013; Waldbillig, 1998).

CONCLUSION

Present investigation confirmed that Dimethoate, an organophosphate is the primary factor that causes significant changes in blood of fish in aquatic ecosystem. Harmful toxicological alterations in haematological and biochemical parameters were reported after 28 days of exposure in Nile tilapia. The current observation indicates that this pesticide poses a significant threat to aquatic life. To minimize the toxic effects of pesticide, their use should be reduced and more environmentally friendly organophosphate pesticide should be developed with faster degradation ability.

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