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Impact of fermented polyherbal supplementation on hematobiochemical profile and growth parameters in broiler chickens

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ABSTRACT

Background: *Escherichia coli* (*E. coli*) infection leads to substantial economic losses in the global poultry industry and the growing issue of antibiotic resistance poses a serious threat to human health. The emergence of antimicrobial resistance due to excessive antibiotic use in poultry production has intensified the search for safer, natural alternatives to control bacterial infections. **Objective:** This study was conducted to evaluate the hematobiochemical safety profile of a fermented polyherbal formulation in broiler chickens. **Materials and Methods:** A total of 60 one day old Vencobb 400 broiler chicks were randomly divided into two groups, each comprising 30 birds. Group 1 served as the environmental control and was fed a basal diet without any additional treatment. Group 2 served as test group and received fermented polyherbal extract in drinking water @100 ml/litre. The study was carried out over a period of 42 days. **Results:** Fermented polyherbal extract demonstrated notable *in vitro* efficacy against *E. coli*, with an MIC of 12.5%. Statistical comparisons revealed no significant differences ($p > 0.05$) between G2 and G1 across all measured parameters: Haemoglobin (g/dl), Total Erythrocyte Count (TEC $\times 10^6$), Total Leucocyte Count (TLC $\times 10^3$), differential white blood cell counts (Heterophils %, Lymphocytes %, Monocytes %, Eosinophils %, Basophils %) and serum biochemical parameters: aspartate transaminase (U/L), alanine transaminase (U/L), total protein (g/dL), uric acid (mg/dL) and albumin (g/dL). All values remained consistent with the control group, suggesting a good safety margin from a hematobiochemical perspective. **Conclusion:** Findings from this 42-day study indicated that the fermented polyherbal extract is not associated with any observable hepatotoxic or nephrotoxic effects in boiler chickens. Based on the comprehensive analysis of key haematobiochemical parameters, the administration of the fermented polyherbal extract to the test group (G2) for 42 days demonstrated a favorable safety profile relative to the environmental control group (G1).

Keywords: Boiler chickens, Fermented polyherbal extract, Hematobiochemical, *Escherichia coli*.

INTRODUCTION

Consumption of some antibiotics have been prohibited by the European Union since 2006 because of the antibiotic resistance emergence in birds and their residual amounts existed in the carcass [1]. The ban on the use of antibiotic growth promoters (AGP) has prompted broiler chicken producers to look into substitutes for AGP, aiming to ensure the sustainability of broiler production. Various alternatives to antibiotic growth promoters (AGPs), including exogenous enzymes, herbal extracts, phytobiotics, prebiotics, probiotics, and symbiotics, have been shown to effectively improve digestive health in broiler chickens, thereby boosting production [2,3].

In recent years, herbal products have gained attention as effective and commonly used alternatives to AGPs in broiler farming. Active compounds in herbal products have been reported to enhance digestive tract morphology and function, improve physiological conditions, boost antioxidant status, and strengthen the immune response of broiler chickens [4,5]. For better growth, feed conversion ratio (FCR), and other purposes, several scientists used single or multiple herbs formulations [6,7]. But several literatures showed issue of palatability in poultry birds [8] and it can be resolved by using polyherbal formulations and fermented herbs. Various herbal ingredients for a polyherbal formulation were chosen based on existing scientific literature and the accessibility of these herbs in our immediate environment. There are many herbs that have an antibacterial, antioxidant and growth promoting impact based on *in vitro* finding. However, emphasis was placed on choosing widely recognized and easily accessible herbs to ensure practicality and convenience for poultry farmers at the field level. Botanical name, common name, parts and proportion of ingredients used for preparation of polyherbal formulation are narrated in table 1.

Biological fermentation effectively increases active substances and bioactive ingredients from raw

materials, improves feed palatability and makes it easy for animals to eat [9,10]. The bioavailability and bioactivity of active compounds in herbal products have been positively influenced by fermentation. In addition, several investigators assessed numerous attributes of fermented herbal formulations in broiler chickens for growth performance [11,12,13]. Considering the facts and scope of the experiment, current research was designed to assess the impact of fermented liquid polyherbal formulation on broiler chickens.

MATERIALS AND METHODS

Experimental birds

A total of 60 one day old broiler chicks (Vencobb 400) were purchased from a registered commercial hatchery. Upon arrival, they were weighed and randomly divided into 2 groups, each having 30 birds. Birds were kept at poultry unit, Department of livestock farm complex, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Navsari, Gujarat throughout the experimental period. This study was approved by Institutional Animal Ethics Committee (No. 128-VCN-VPT-2023).

General management of experimental birds

All birds were provided similar environmental and management condition during whole experimental period. Throughout the study, room temperature and humidity were monitored using a digital hygrometer. All birds were provided standard diet for various physiological phases (Pre-starter, Starter and Finisher) in order to meet their needs for protein and energy. Individual feeders and waterers were provided for each experimental bird housed in pens. All of the experimental birds were received *ad libitum* access to potable water during the experimental period. All birds were vaccinated with new castle disease virus vaccine on 7th and 21st day as well as infectious bursal disease virus vaccine on 14th day and 28th day. The general managemental procedures were conducted in accordance with the method described by Kamani *et al.* 2024^[14].

Experimental design

Experimental birds were divided into groups as below mention table. The fermented of herbal formulation was performed by inoculating *Lactic acid bacillus* (120 million spores, LACTOLUS DS, INTAS Pharmaceuticals Ltd.) along with 25 % sugar and 10 % corn starch. The fermentation was incubated at 35 °C to 37 °C for 3 days and preserved at 4 °C after filtration. This fermented extract was used as such without any further concentration or dilution. This experiment was carried out over a period of 42 days.

Groups	No. Birds	Treatment
Group I (Environmental Control)	30	Basal diet without any treatment
Group II (Test Group)	30	Fermented polyherbal supplement in drinking water @100 ml/litre

In vitro antimicrobial susceptibility test of fermented polyherbal extract

Polyherbal formulation without solvent reduction (100 %) was used for further serial dilution of polyherbal extract *i.e.* 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%. An overnight culture of *Escherichia coli* was prepared and standardized to a 0.5 McFarland turbidity, corresponding to approximately 1.5×10^8 CFU/mL. Turbidity was corrected using sterile broth or additional culture as needed. A working inoculum was prepared by diluting 100 µL of the standardized culture in 9.9 mL of sterile broth. For the broth dilution assay, serial dilutions of polyherbal extract (100%–0.78%) were prepared in sterile 96-well microtiter plates using nutrient broth. Vehicle control (sterile distilled water), positive control (enrofloxacin 250 µg/mL), growth control, and sterility control wells were included. All well received 100 µL of *E. coli* bacteria and except sterility

control, which received only broth. All tests were performed in triplicate. To confirm bacterial viability, 10 µL from the growth control well was serially diluted, plated on nutrient agar, and incubated at 37 °C for 18–20 hours. Post incubation, 30 µL of INT dye (Iodonitrotetrazolium chloride; 2 mg/mL) was added to each well and re-incubated for 30 minutes. A color change from yellow to red or pink indicated bacterial growth (Ellof, 2019) [15]. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration showing no color change. Colony-forming units (CFU/mL) were also correlated with McFarland standards.

Hematobiochemical investigation

On 42nd day, following blood collection hematology parameters *viz.* haemoglobin concentration (Hb), total leucocyte count (TLC), total erythrocyte count (TEC) and differential leukocyte count (DLC) were conducted manually. Serum was separated from blood samples collected in plain vials on 42nd day and stored at -20 °C till further biochemical estimations. The serum concentration of alanine transaminase (ALT), aspartate transaminase (AST), uric acid, albumin and total protein were estimated by using their respective commercial analytical kits (Q-Line Biotech Pvt. Ltd., New Delhi).

Voluntary feed intake

Feed was supplied in a fixed quantity every day to each of the two groups. The next morning, any residual feed was collected and weighed. The actual feed intake was calculated by subtracting the leftover feed from the amount initially provided. The daily procedure enabled weekly compilation of data to estimate the birds' voluntary feed intake. The average feed intake per bird per week (in grams) was determined by dividing the total feed consumed during the week by the number of birds in each group.

Body weight gain

On the day of their procurement individual body weight of the birds were recorded and subsequently at weekly intervals early in the morning prior to feeding throughout the entire experimental period. The difference between the body weight recorded in the current week and that of the preceding week was taken as weekly body weight. Within each group, the average weekly body weight gain and the cumulative body weight gain were computed for each replicate separately.

Feed conversion efficiency

Feed conversion efficiency is a critical parameter in the poultry industry, directly impacting profitability by measuring how effectively feed is converted into body weight gain. This efficiency is typically measured using the feed conversion ratio (FCR), which is calculated by dividing the total feed consumed by the bird's corresponding weight gain over a specific period. The formula used to determine FCR is expressed as:

$$\text{Feed conversion ratio (FCR)} = \text{Feed consumption} / \text{Body weight gain}$$

Statistical analysis

Data were analyzed using an independent samples t-test to compare the means of various hematobiochemical parameters between the two groups, using SPSS statistical software (version 20.0). Statistical significance was determined at $p < 0.05$.

RESULTS

In the current study the minimum inhibitory concentration (MIC) of polyherbal extracts were estimated with standard protocol of microbroth dilution technique against *E. coli*. The MIC against *Escherichia coli* was found at 12.5 % for fermented polyherbal extract, which is indicative of *in vitro* effect of current polyherbal

formulation. The presence of different phytochemicals from different plants is considered responsible for the antimicrobial activity of the formulation. Several studies have demonstrated the antimicrobial potential of herbal extracts against *Escherichia coli* i.e. Elmowalid *et al.* 2019, Hassan *et al.* 2016^[16,17].

The results of hematobiochemical parameters of the environmental control group (G1) and the test group (G2), which received fermented polyherbal extract for 42 days, are presented as mean \pm standard deviation in table 2. Based on the comprehensive analysis of key haematological markers, the administration of the fermented polyherbal extract to the test group (G2) for 42 days demonstrated a favorable safety profile relative to the environmental control group (G1). Statistical comparisons revealed no significant differences ($p > 0.05$) between G2 and G1 across all measured parameters. The haemoglobin level in the fermented polyherbal extract treated group (G2) was slightly lower (9.58 ± 0.32 g/dl) compared to the environmental control group (G1: 9.75 ± 0.28 g/dl). The TEC was marginally higher in the test group (G2: $4.46 \pm 0.16 \times 10^6$) than in the control group (G1: $4.37 \pm 0.18 \times 10^6$). This indicates that the polyherbal treatment did not negatively impact red blood cell production. The TLC was slightly elevated in the test group (G2: $14.16 \pm 1.01 \times 10^3$) compared to the control (G1: $13.00 \pm 0.57 \times 10^3$). This mild increase could suggest an immune modulatory effect of the herbal treatment, though the values remain within a normal physiological range. The DLC analysis revealed minor variations between the environment control (G1) and treated (G2) groups, with no significant abnormalities. The slightly lower heterophils and higher lymphocytes in G2 may suggest a mild anti-inflammatory or immunomodulatory effect of the fermented polyherbal extract. The aspartate aminotransferase (AST) levels were comparable between G1 (193.23 ± 42.98 U/L) and G2 (185.38 ± 23.58 U/L), indicating no significant liver or muscle damage due to the treatment. The total protein levels were slightly higher in G2 (5.68 ± 0.75 g/dL) than in G1 (5.45 ± 1.17 g/dL), suggesting no adverse effect on protein metabolism. Uric acid levels were similar in both groups (G1: 8.13 ± 1.13 mg/dL; G2: 7.93 ± 0.92 mg/dL), indicating no major disturbance in nitrogen metabolism or kidney function. Albumin levels were higher in G2 (3.28 ± 0.48 g/dL) than in G1 (2.81 ± 0.35 g/dL), which may suggest better protein synthesis or absorption in the treated birds. Overall, the fermented polyherbal treatment did not cause any adverse effects on the haematological or biochemical parameters in broiler birds. Some parameters, such as lymphocytes, monocytes and albumin, showed slight improvements, suggesting potential immune modulatory benefits. The results indicate that the treatment is safe and does not negatively impact the birds' physiological health.

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immunomodulatory effect of the fermented polyherbal extract. The aspartate aminotransferase (AST) levels were comparable between G1 (193.23 ± 42.98 U/L) and G2 (185.38 ± 23.58 U/L), indicating no significant liver or muscle damage due to the treatment. The total protein levels were slightly higher in G2 (5.68 ± 0.75 g/dL) than in G1 (5.45 ± 1.17 g/dL), suggesting no adverse effect on protein metabolism. Uric acid levels were similar in both groups (G1: 8.13 ± 1.13 mg/dL; G2: 7.93 ± 0.92 mg/dL), indicating no major disturbance in nitrogen metabolism or kidney function. Albumin levels were higher in G2 (3.28 ± 0.48 g/dL) than in G1 (2.81 ± 0.35 g/dL), which may suggest better protein synthesis or absorption in the treated birds. Overall, the fermented polyherbal treatment did not cause any adverse effects on the haematological or biochemical parameters in broiler birds. Some parameters, such as lymphocytes, monocytes and albumin, showed slight improvements, suggesting potential immune modulatory benefits. The results indicate that the treatment is safe and does not negatively impact the birds' physiological health.

The table 3 presents the weekly average individual feed intake (g/bird), body weight gain (g/bird) with mean \pm standard error (S.E.) and feed conversion ratio (FCR) for two treatment groups (G1 and G2) across different growth phases: pre-starter (week 1), starter (weeks 2–4) and finisher (weeks 5–6). Throughout the study period, both groups exhibited progressive increases in feed intake and body weight gain during the starter phase, which is typical of broiler growth patterns. However, in the finisher phase, a decline in weekly body weight gain was observed in both groups. Importantly, Group 2, which received the fermented polyherbal extracts, showed improved feed efficiency, as reflected by a lower cumulative feed conversion ratio (FCR) of 1.66 compared to 1.75 in Group 1. Notably, this improvement in growth performance and feed efficiency in Group 2 was achieved despite a lower total feed intake (3954 g/bird) compared to Group 1 (4121 g/bird).

Histopathological examination of liver and intestinal tissues from the Safety Group (G5), which received fermented polyherbal extract for 42 days, revealed normal histoarchitecture comparable to that observed in the Environmental Control group (G1). These findings confirm that administration of the fermented polyherbal extract for 42 days in broiler birds does not induce any histopathological alterations in liver and intestinal tissues.

DISCUSSION

Similar *in vitro* effects of various herbal formulations have been documented in earlier investigations. Elmowalid *et al.* (2019) determined *in vitro* antibacterial of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) aqueous extracts ^[16]. Both extracts showed concentration dependent antibacterial activities against *E. coli* O78. Micro well dilution assay revealed effective inhibition for *E. coli* with MIC values of 10 mg/mL. Hassan *et al.* (2016) studied antimicrobial effect of different plant extract (*Acacia arabica*, *Nymphaea lotus*, *Sphareranthus hirtus*, *Emblica officinalis*, *Cinchorium intybus* and *Cardus marianum*) against *E. coli* by micro broth dilution method ^[17]. They found MIC value of *Emblica officinalis* extract (hot water and 80% ethanolic extract) at 0.29 mg/mL against *E. coli*. These variations in values of MIC may arise from differences in extraction methods, solvent polarity and bacterial strains.

The current findings are in agreement with the study by Ao *et al.* (2011) ^[18], which evaluated the influence of fermented red ginseng extract (FRGE) on the growth and productivity of broilers and laying hens. The lymphocyte level in FRGE treatments (basal diet + 1 g/kg, 2 g/kg and 4 g/kg fermented red ginseng extract) was improved ($p < 0.05$) compared with control (CON). They also reported that no significant effects ($p > 0.05$) were observed in WBC, RBC, or total cholesterol levels in response to FRGE administration ^[14]. In a study by Pratama *et al.* (2021) ^[19], fermented *Averrhoa bilimbi* L. fruit filtrate was assessed for its influence on broiler growth, blood parameters, gut health, and carcass composition.

Table 1: Ingredients used for preparation of polyherbal formulation

S. No.	Botanical name of Plant	Common name	Part to be used	Proportion
1	<i>Glycyrrhiza glabra</i>	Jethi Madh	Dried roots & Rhizomes	4 parts
2	<i>Piper longum</i>	Pipper	Dried roots	2 parts
3	<i>Piper nigrum</i>	Black pepper	Dried fruit	2 parts
4	<i>Emblica officinalis</i>	Amla	Dried Fruit	2 parts
5	<i>Adhatoda vasica</i>	Ardusi	Dried leaves	1 part
6	<i>Ocimum sanctum</i>	Tulsi	Dried leaves	1 part
7	<i>Withania somnifera</i>	Ashwagandha	Dried roots	1 part
8	<i>Zingiber officinale</i>	Sunth	Dried roots	1 part
9	<i>Cinnamomum zeylanicum</i>	Dalchini	Dried bark	1 part
10	<i>Coriander sativum</i>	Coriander	Dried fruit	1 part
11	<i>Curcuma longa</i>	Haldar	Dried rhizomes	1 part
12	<i>Andrographis paniculata</i>	Kariyatu	Dried leaves	¼ part
13	Rock salt	-	-	¼ part

Table 2: Haematobiochemical parameters in environment control and fermented polyherbal treated broiler birds on 42nd day

Parameters	Treatment groups	
	G1 (Environment control)	G2 (Test group)
Haematological parameters		
Haemoglobin (g/dl)	9.75 ± 0.28	9.58 ± 0.32
TEC (×10 ⁶)	4.37 ± 0.18	4.46 ± 0.16
TLC (×10 ³)	13.00 ± 0.57	14.16 ± 1.01
Heterophils (%)	38.66 ± 39.16	37.18 ± 2.38
Lymphocytes (%)	51.83 ± 1.49	52.50 ± 1.66
Monocytes (%)	4.66 ± 0.49	5.50 ± 0.42
Eosinophils (%)	4.33 ± 0.42	4.16 ± 0.47
Basophils (%)	0.50 ± 0.22	0.33 ± 0.20
Serum biochemical parameters		
AST (U/L)	193.23 ± 42.98	185.38 ± 23.58
AST (U/L)	11.41 ± 2.52	11.02 ± 2.49
Total protein (g/dL)	5.45 ± 1.17	5.68 ± 0.75
Uric acid (mg/dL)	8.13 ± 1.13	7.93 ± 0.92
Albumin (g/dL)	2.81 ± 0.35	3.28 ± 0.48

Values bearing different superscripts (a, b) within a same column differ significantly from each other (P<0.05)

Table 3: Average weekly individual feed intake, average weekly body weight gain and feed conversion ratio (FCR) in broiler birds

Weeks	Average weekly individual feed intake (g/bird)		Average weekly body weight gain (g/bird) (Mean ± S.E.)		Feed conversion ratio (FCR)	
	G1	G2	G1	G2	G1	G2
First (Pre-starter)	144	134	102.50 ± 6.42	99.17 ± 5.83	1.40	1.35
Second (Starter)	408	314	255.00 ± 10.88	245.00 ± 17.84	1.60	1.28
Third (Starter)	716	650	472.50 ± 19.84	466.67 ± 29.74	1.52	1.39
Fourth (Starter)	908	898	610.83 ± 41.28	573.33 ± 36.58	1.49	1.57
Fifth (Finisher)	943	960	496.67 ± 47.45	535.00 ± 39.90	1.90	1.79
Sixth (Finisher)	1003	999	411.67 ± 44.15	468.33 ± 43.70	2.44	2.13
Total	4121	3954	-	-	1.75	1.66

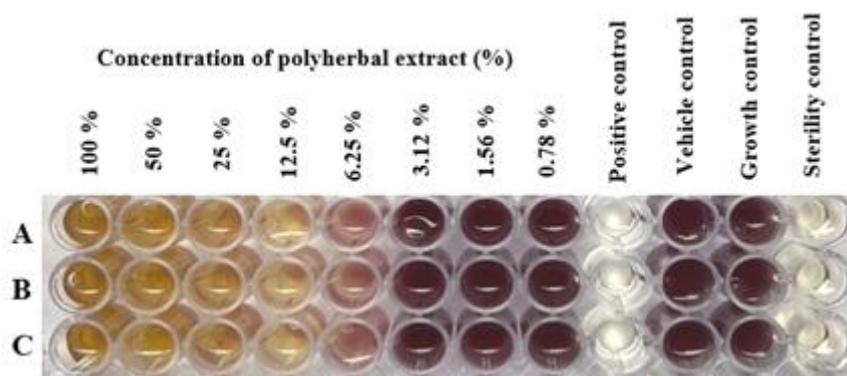


Figure 1: Minimum inhibitory concentration (MIC) of fermented polyherbal extract

They observed that on days 21 and 33, the numbers of thrombocytes decreased with the increased levels of fermented *A. bilimbi* L. fruit filtrate (0%, 0.5%, 1.0% and 2.0%) in the drinking water of experimental broilers [19]. As part of the innate effector cells, thrombocytes may take part in inflammation process in poultry. In this regard, any increase in thrombocyte concentrations may therefore be associated with the increased potential infection in broilers. Taken together, the decreased thrombocyte concentrations in the experimental birds treated with fermented *A. bilimbi* L. filtrate may therefore be associated with the reduced potential infections in these respective birds. On day 21, aspartate aminotransferase (AST) increased ($p < 0.05$) with the enhanced fermented filtrate concentrations. On day 33, alanine aminotransferase (ALT) increased ($p < 0.05$) following the increased fermented filtrate concentration in drinking water. Ellakany *et al.* (2017) investigated effect of fermented wheat germ extract (FWGE) on biochemical, physiological and performance parameters of broiler chickens [20]. They reported that FWGE only increased hemoglobin concentration significantly ($p \leq 0.05$) without alteration of red blood and white blood cells counts. They also observed that the dose of 1.5 g/kg feed decreased significantly ($p \leq 0.05$) AST level in blood of treated broiler chickens, from 20.655 mg/dL (control) to 17 mg/dL. The dose of 0.5 g/kg increased significantly ($p \leq 0.05$) the total protein level in blood of treated broiler chickens, from 4.950 mg/dL (control) to 5.45 mg/dL. But higher dose of 1.5 g/kg feed decreased the total protein level in blood significantly ($p \leq 0.05$) to 4.833 (mg/dL).

Both treatment groups demonstrated a typical growth pattern, with progressive increases in feed intake and body weight gain during the starter phase, followed by reduced weight gain during the finisher phase. The decline growth observed during the later weeks can be linked to the birds reaching physiological maturity and a natural decrease in growth rate as age progresses (Ravindran and Abdollahi, 2021) [21]. Incorporating fermented polyherbal extracts into the drinking water of broiler birds (Group 2) demonstrated a notable improvement in feed efficiency. Group 2 exhibited a lower cumulative FCR in comparison to Group 1, indicating improved feed conversion efficiency. This enhancement occurred even though Group 2 consumed slightly less total feed, suggesting that the polyherbal supplementation may have played a role in improving nutrient utilization, possibly by enhancing gut health and digestive efficiency. Similar observations regarding the efficacy of herbal and fermented plant extracts as natural growth enhancers have been reported by Jeong and Kim (2015) [22] and Bulu *et al.* (2020) [23]. The herbs used in the fermented extract are known to possess antioxidant, antimicrobial, and digestive stimulant properties, which may contribute to a more favorable gut environment, leading to better absorption of nutrients (Aladejana *et al.*, 2023) [24]. With respect to herbal products, fermentation has been shown to significantly enhance the concentration and activity of bioactive compounds. According to Hussain *et al.* (2016) [25], fermentation processes can increase the antioxidant potential of herbal formulations, improve their therapeutic efficacy, and contribute to better biological absorption of active constituents. This enhancement occurs due to the breakdown of

complex phytochemicals into more biologically active and absorbable forms during microbial fermentation.

CONCLUSION

Based on the findings of present study, it suggests that supplementation of fermented polyherbal extract at 100 ml/litre in drinking water up to 42 days can be used as a natural and safe alternate dietary substance without any ill effects on the health of broiler chickens. It can be concluded that fermented polyherbal extract is not associated with any observable hepatotoxic or nephrotoxic effects in boiler chickens.

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Conflict of interest

The authors declared no conflict of interest.

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