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Uses of *Elaeis guineensis* oil for Stress Management during the Transportation of Catfish Fingerlings: A Dose-Dependent Outcome

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Cameroonian farmers used *Elaeis guineensis* oil (EGO) named usually palm oil to reduce stress and mortality during the transportation of catfish fingerlings. The present study is aimed to evaluate the uses of EGO for stress management during the transportation of catfish fingerlings. Antioxidant activities of EGO were assessed *in vitro*. 1500 fingerlings were transported from Douala (Littoral Region, Cameroon) to Yaoundé (Centre Region Cameroon). The transportation was for 7 h 55 min in black tins of 10 L which contain 8 L of water and 100 fingerlings each. The following treatment was administrated: commercial anti-stress, 2, 4, and 6 drops of EGO. Control received no

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treatment and all groups were triplicated. After 10 fingerlings were sacrificed by decapitation. Total protein, total bilirubin (TB), triglycerides level, and lactate deshydrogenase (LDH) activity were assessed in the liver as well as oxidant stress parameters. Brain and gills were fixed for histopathological analysis. Results showed that transportation of catfish fingerlings induced a significant increase of TB level and LDH activity in the liver. Indeed, it induced cerebellar and gills necrosis. Moreover, EGO exhibits antioxidant activities *in vitro* against DPPH, ABTS radicals, and possesses a ferric reducing antioxidant power of 6.31 mEAG/g. This observation was confirmed *in vivo* by the increase in a dose-dependent manner of GSH and nitrites levels in the liver compared to control. However, the administration of 6 drops of EGO increased significantly ($p < 0.05$) the activity of LDH in the liver compared to control. Thus, high dose induced anaerobic respiration which was confirmed by alveolar necrosis in gills and neurodegeneration although low dose of EGO (2-4 drops) prevented those alterations compared to control. Hence, low doses of *Elaeis guineensis* oil can prevent liver, cerebellar and gills impairment during artisanal transportation to reduce the effects of stress.

Keywords: Catfish; fingerlings; Cameroon; Elaeis guineensis oil; transportation.

1. INTRODUCTION

African catfish (*Clarias gariepinus*) appear as the major fish species cultured in sub-Saharan Africa. It is the second most commonly farmed species in Cameroon [1] and it is more resistant because of its bimodal-breathing [2]. Nevertheless, catfish fingerlings production is still perceived as a bottleneck in tropical countries. Synthetic hormones are often difficult to come by in Africa due to local socioeconomic conditions. Thus, the production of fingerlings had been strictly the prerogative of intensive farms [3]. Indeed, the scarcity of quality fingerlings at affordable prices is repeatedly mentioned as the key constraint faced by most farmers in Cameroon [4]. Many farmers have to transport fingerlings from recommended hatcheries since the fisheries sector is dominated by artisanal fisheries. During this transportation fingerlings sustained stress and hypoxia, thus generally lead to post-transportation high mortality [5,6]. It has been demonstrated that stress and hypoxia increased the production of Reactive Oxygen Species (ROS). High production of ROS in the Central Nervous System (CNS) and the liver initiate apoptosis which contributes significantly to cell death observed [7-9]. Furthermore, transportation of fingerlings leads them to anxiety, thus enhanced mortality. To alleviate fingerling death, many farmers use an artisanal protocol like put some drop of palm oil during transportation. Anti-oxidant properties of palm oil have been reported in several reviews [10-12]. The anti-oxidative defense system comprises several antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) [13] as well as non-enzymatic antioxidant systems such as bilirubin, glutathione (GSH), carotenoids, and vitamins C and E [14]. Numerous studies reported antioxidant effects of *Elaeis guineensis* usually named like palm oil tree [15-17]. Thus, the objective of this study is to evaluate if the antioxidant properties of palm oil can prevent hypoxia impairment due to transportation in catfish fingerlings. For that, the uses of EGO for stress management during the transportation of catfish fingerlings were evaluated. Specifically, antioxidants effects of palm oil were assessed *in vitro*, and activities of EGO at different doses were evaluated on neuronal loss, gill histopathological changes, and liver oxidative stress and failure.

2. MATERIALS AND METHODS

2.1 Solvents and Chemicals

The solvents and all the chemicals were of analytical grade. 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri (2-pyridyl) -s-triazine, potassium persulfate, and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 *In vitro* Antioxidants Activities of *Elaeis guineensis* Oil

Antioxidants properties of EGO were evaluated against ABTS and DPPH radicals. Furthermore, FRAP was also assessed.

2.2.1 Scavenging effects on DPPH

The antioxidant capacity of EGO was assessed like its ability to use the scavenging effect of

radicals on DPPH according to [18]. For that, 2 mL of DPPH (0.1mM in methanol) were mixed with 0.5mL of EGO in tween 80 (0.78-50mg/mL). The mix was homogenized for 5 min and dark-incubated for 60min at 20°C. For the control, methanol replaced EGO. Reference was ascorbic acid at 0.001 mg/mL to 0.25 mg/mL. Absorbances at 517nm were used to calculate radical scavenging activity (percentage of inhibition) with the formula.

$$\%\ Inhibition = \frac{(Control\ Abs\ -\ Sample\ Abs)}{Control\ Abs}\ x\ 100$$

2.2.2 ABTS cation decolorization assay

Coloration kinetic of ABTS ion was carried out according to Re et al. [19]. For that, 0.0384g of ABTS and 0.00662g of potassium persulfate ($K_2S_2O_8$) were mixed with 10mL of distilled water. The mix was homogenized for 5min and dark-incubated for 16 h at 20°C. ABTS+ solution was diluted with ethanol to obtain an absorbance of 1.30 ± 0.02 at 734nm and steady at 30°C (initial absorbance). Then, 1.8mL of this ABTS+ solution diluted was mixed with 0.2mL of EGO (100 mg/mL). Absorbance at 714nm and steady at ambient temperature while 1min was used to calculate radical scavenging activity (percentage of inhibition) with the formula:

$$\%\ Inhibition = \frac{(Control\ Abs\ -\ Sample\ Abs)}{Control\ Abs}\ x\ 100$$

2.2.3 Ferric reducing antioxidant assay

A standard curve was created by adding the FRAP reagent to a range of Fe^{2+} solutions of known concentrations which allow the Fe^{2+} concentration of the samples to be calculated thereby determining “antioxidant capacity”. TPTZ (2.4.6-tri (2-pyridyl) -s-triazine) solution was obtained by mixing TPTZ (10 mM) in 10 mL of HCl (400 mM diluted with distilled water) and 10 mL of iron chloride (FeCl3) in distilled water at 10 mM. 100 mL of acetate buffer (pH 3.6) was mixed with 10 mL of TPTZ and 10mL of iron chloride solution to obtain FRAP solution. After 2mL of FRAP solution were blended with 75µL of EGO, the mix was incubated for 15min. Absorbance was measured directly at 620 nm [20].

2.3 Experimental Animal

Healthy fingerlings (4-5 weeks old) weighing 5-11 g were supplied by the production facility of a reputed hatchery of Bonaberi, Douala (Cameroon). Before the transportation, they were fasted for 24 to micropellet Coppens (0.5 mm). The composition of animal diet was crude protein (55 %), Rohfett (16 %), Rofaser (1 %), Rohasche (9 %), vitamin A (25 000 IE7Kg), vitamin D (2000 IE7Kg), Vitamin E (400 mg/kg), Vitamin C (1000 mg/Kg). All experiments were conducted following the principles and procedures of the European Union on Animal Care (CEE Council 86/609) guidelines adopted by the Cameroon Institutional National Ethic Committee, Ministry of Scientific Research and Technology Innovation (Reg. number FWA-IRD 0001954).

2.4 Experimental Design

Fingerlings of *Clarias gariepinus*, mean weight 7.8 ± 0.2 g were transported from Douala (Littoral Region, Cameroon) to Yaoundé (Centre Region, Cameroon) by bus in black tins (measuring 60 cm x 30 cm x 30 cm) of 10 L containing 5 L of water. This transport takes place on the 3rd of July 2020 on 249.7 km of fully asphalted road. The conditions were Douala: temperature 30 °C, wind SW 5 km/h, humidity 91 %, UV index 7/10 and Yaoundé: temperature 31 °C, wind SW 5 km/h, humidity 79 %, UV index 8/10. Each tin contains 100 fingerlings. The following treatment was administrated: CA (commercial anti-stress: vitamin A, D3, E, K3, B1, B2, B12, B6, C, Niacin D Capan, NaCl, KCl, MgCl, and essential amino acid), EGO2 (2 drops of EGO), EGO4 (4 drops of EGO), EGO6 (6 drops of EGO). Control received no treatment and all treatments were made in triplicate.

A group of 10 fingerlings were sacrificed before the transportation (BTRANS). The transportation spent 7h55min. When fingerlings arrived at the laboratory, 10 of each triplicate was sacrificed by decapitation (n = 10). Gills and brain were fixed in formaldehyde 10 % buffered for histopathological analysis. A 10 % homogenate of the liver was realized after weighted. Homogenates were centrifuged at 3500 rpm (15 min at 4° C) to obtain supernatant samples which were kept at -15° C for the determination of oxidative stress parameters like Malondialdehyde (MAD), reduced glutathione (GSH), nitrites, superoxide dismutase (SOD) and catalase (CAT) activities or levels were assessed in a 10 % homogenate of the liver (in Tris buffer 50 mM ; pH 7,4). Furthermore, lactate dehydrogenase (LDH), total proteins (TP), total bilirubin (TB), and triglycerides (TG) activities or levels were also assessed in the same homogenate.

2.5 Determination of Relative Weight of Liver

The relative fresh weight of liver was calculated using the following formula according to Akhtar et al. [21].

Liver weight ratio = (Liver weight (g) / Body weight (g)) × 100

2.6 Biochemical Assays

Lactate deshydrogenase (LDH) activity, triglycerides (TG), and total bilirubin (TB) were assessed using the commercial kit Biolabo indications. They have been determinated according respectively to Henry et al. [22] methods, Fossati and Prencipe [23] coupled with Trinder reaction [24] methods, and Malloy-Evelyn [25] modified by Martha et al. [26] methods. Malondialdehyde (MDA) and reduced glutathione (GSH) levels in liver homogenate were determined using the procedure described by Wilbur et al. [27] and Ellman [28] respectively while the nitrites content was determined using the method describe by Green et al. [29]. SOD and catalase activities were assessed respectively by Misra and Fridovish [30] and Sinha [31] methods.

2.7 Histopathological Analysis of Brain and Gills

Gills and brain after a fixation of 2 weeks in formaldehyde 10% buffered were trimmed and dehydrated in alcohol of croissant gradient (70 %, 80 %, 90 %, and 100 % (3 baths)). After tissues were clarified in 2 baths of xylene (1h30min per bath) and impregnated in molten paraffin at 60°C (for 5h). Histopathological analysis were carried on 7µm sections of paraffin-embedded and haematoxylin eosin stained for brain and gills respectively. Microphotographies were obtained using a light microscope (Leitz wetzlar Germany 513) connected with a digital camera celestron 44421 linked to a computer where images were transferred.

2.8 Statistical Analysis

Data were expressed as mean ± Standard Error on Mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test using GraphPad Prism 7.00. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 *In vitro* Antioxidant Activities of *Elaeis guineensis* Oil

According to the results obtained in Table 1, vitamin C exhibits greater anti-free radical activity than that of EGO. It had a low IC_{50} against the DPPH and ABTS radicals. The inhibitory concentration 50 of vitamin C is 24.56 µg/mL while the one of EGO is 3612.00 µg/mL for the DPPH radical. Vitamin C IC_{50} is 37.75 µg/mL against radical ABTS and EGO inhibited ABTS radical at 1213.00 µg/mL. The concentration of FRAP is 6.31 mEAG/g.

3.2 Effects of *Elaeis guineensis* Oil on Liver

Elaeis guineensis oil's effects on liver's weight, biochemical parameters (TP, TB, LDH, and TG), and antioxidants status (SOD, CAT, GSH, MDA) are resumed in Table 2. It appears that EGO has no significant effects on the liver's relative weight, total protein (TP), total bilirubin (TB), triglycerides (TG), malondialdehyde (MDA) compared to control. The administration of palm oil increased in a dose-dependent manner GSH level in the liver compared to control. The increase of GSH level was significant ($p < 0.05$) in the liver of fingerlings that received 6 drops of palm oil. Besides EGO increase significantly ($p < 0.05$) activities of superoxide dismutase and catalase in the liver at the dose of 2 drops compared to control. Besides, 6 drops of *E. guineensis* increase significantly ($p < 0.05$) the activity of lactate deshydrogenase and nitrites level in the liver of fingerlings compared to control.

Table 1. Inhibitory concentration 50 of palm oil against DPPH and ABTS radicals and FRAP concentration

Parameters Substances	FRAP (µEAG/g)	IC_{50} of DPPH (µg/mL)	IC_{50} of ABTS (µg/mL)
Vitamin C	-	24.56	37.75
Palm oil	6310.68	3612.00	1213.00

Table 2. *Elaeis guineensis* oil effects on liver's weight, biochemical parameters and antioxidants status

	BTRANS	Control	AS	EGO2	EGO4	EGO6
Liver weight ratio	14.05 ± 0.20	14.36 ± 0.29	14.40 ± 0.25	14.65 ± 0.26	13.09 ± 0.24	13.90 ± 0.23
Total protein (mg/mL)	0.077 ± 0.31	0.078 ± 0.24	0.071 ± 0.20	0.079 ± 0.32	0.076 ± 0.39	0.076 ± 0.39
Total bilirubin (µmol/L)	3.45 ± 0.34	5.92 ± 0.52^{x}	5.96 ± 0.62^{x}	5.95 ± 0.55^{x}	5.96 ± 0.55^{x}	5.93 ± 0.55^{x}
Lactate deshydrogenase (µKat/L)	4.85 ± 0.49	6.05 ± 0.32	6.62 ± 0.50	7.14 ± 0.89	8.12 ± 0.32^{x}	11.14 ± 0.31^{ay}
Triglycerids (mmol//L)	1.87 ± 0.43	1.79 ± 0.57	1.52 ± 0.30	1.62 ± 0.54	1.48 ± 0.35	1.74 ± 0.33
Superoxide dismutase (unit of SOD/g of liver)	200.45 ± 0.78	196.72 ± 1.09	236.47 ± 0.67	266.02 ± 1.67^{a}	201.65 ± 0.56	204.28 ± 0.51
Catalase (mM of H_2O_2/min/g of liver)	88.50 ± 0.36	61.86 ± 0.59	95.47 ± 0.64	123.65 ± 0.55^{a}	92.98 ±0.84	125.81 ± 0.57^{a}
Reduced glutathione (mol/g of liver)	0.00018 ± 0.50	0.00014 ± 0.52	0.00012 ± 0.47^{x}	0.00014 ± 0.41	0.00015 ± 0.47	0.00017 ± 0.46$^{\alpha}$
Malondialdehyde (mol/g of liver)	0.37 ± 1.01	0.065 ± 1.64^{x}	0.066 ± 1.76^{x}	0.051 ± 1.55	0.058 ± 1.94	0.045 ± 1.69
Nitrites (µmol/mg of liver)	0.0018 ± 0.61	0.0013 ± 0.77	0.0013 ± 0.55	0.0014 ± 0.59	0.0018 ± 0.52	0.0021 ± 0.63$^{a\alpha}$

Values are mean ± SEM. ^{x}p <0.05; ^{y}p <0.01: significant difference compared to BTRANS group; $^{\alpha}p$ <0.05: significant difference compared to the group of fingerlings treated with the commercial anti-stress. BTRANS: group of fingerlings before transportation; Control: group of fingerlings which received no treatment; AS: group of fingerlings treated with the commercial anti-stress, EGO2, EGO4 and EGO6: group of fingerlings treated with palm oil at 2, 4 and 6 drops respectively

3.3 Effects of *Elaeis guineensis* Oil on Neuronal Loss

As shown by Fig. 1, acute stress due to transportation induces a neuronal loss in the cerebellum of fingerlings. The administration of commercial anti-stress and low doses (2 and 4 drops) of palm oil reduced neuronal loss in the grey matter of the cerebellum of fingerlings compared to control. Nevertheless, the administration of 6 drops of oil has not effect on the neurodegeneration in the medulla part of the cerebellum of fingerlings.

3.4 Effects of *Elaeis guineensis* Oil on Gills

According to figure 2, stress due to transportation induces alveolar cell necrosis in both primary and secondary lamellae of fingerling's gills compared to group BTRANS. Palm oil at 2 and 4 drops like the commercial anti-stress prevent alveolar cell necrosis in the gill of fingerlings of catfish due to transportation stress compared to control. Like in the cerebellum 6 drops of palm do not reduce alteration due to stress in gills of fingerlings compared to control.

4. DISCUSSION

In Africa, approximately 200 million people derive high-quality and low-cost proteins from fish [32]. African catfish (*Clarias gariepinus*) is the main fish species cultured in sub-Saharan Africa and the second most commonly farmed species in Cameroon [1]. There is strong input from authorities to increase the national production of fish. Fish provide high protein foods to more than 11 million Cameroonians and also generate jobs for thousands of them [33]. During this transportation fingerlings sustained stress and hypoxia, thus generally lead to high mortality [5,6]. The present study is aimed to evaluate the efficacy and innocuousness of an artisanal method of fingerlings transportation. Therefore, *Elaeis guineensis* oil (EGO) usually named palm oil uses was evaluated for stress management during the transportation of catfish fingerlings. For that, two sets of experiments were carried out. Results of the present study confirmed that transportation is a stressful experience for fingerlings [5,6]. Indeed, catfish fingerlings exhibit after transportation a high total bilirubin level and necrosis in both cerebellar and gills tissues. Firstly, the antioxidant potential of

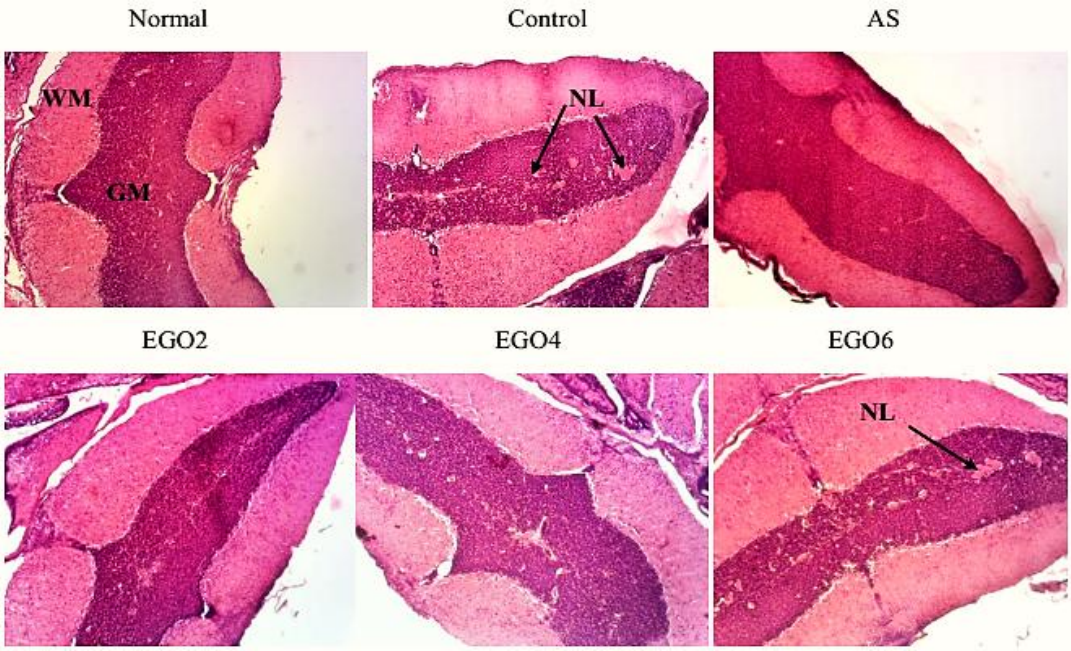

Fig. 1. Effect of EGO on corpus cerebellum of catfish fingerlings exposed to stress. (Formaldehyde's; H&E; X100) BTRANS: group of fingerlings before transportation; Control: group of fingerlings which received no treatment; AS: group of fingerlings treated with the commercial anti-stress, EGO2, EGO4, EGO6: group of fingerlings treated with palm oil at 2, 4 and 6 drops respectively. NL: neuronal loss, GM: grey matter; WM: white matter

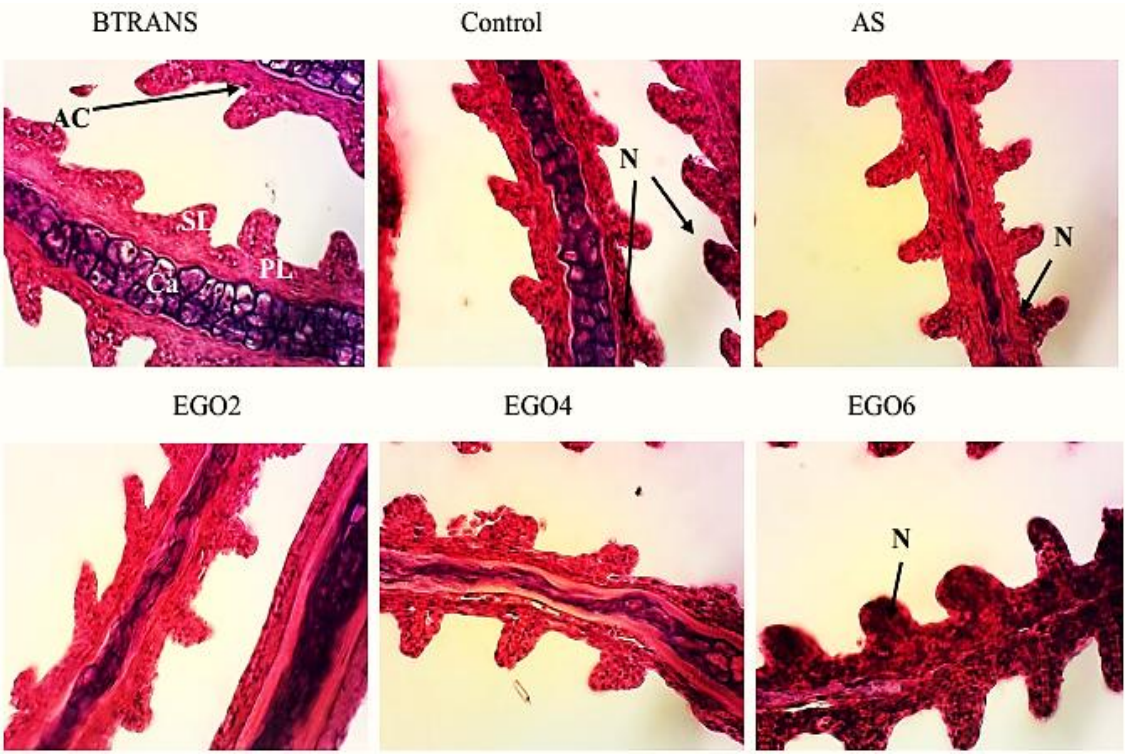

Fig. 2. Effects of EGO on gill epithelium of catfish fingerlings exposed to stress. (Formaldehyde's; H&E; X100). BTRANS: group of fingerlings before transportation; Control: group of fingerlings which received no treatment; AS: group of fingerlings treated with the commercial anti-stress, EGO2, EGO4, EGO6: group of fingerlings treated with palm oil at 2, 4 and 6 drops respectively. Ca: cartilaginous tissue; AC: alveolar cell; SL: secondary lamellae; PL: primary lamellae; N: necrosis

EGO was assessed *in vitro*. Secondly, *E. guineensis* oil's effects on the liver, gills, and cerebellum were assessed in fingerlings after the transportation of catfish fingerlings in a tin. As result, EGO exhibited a good antioxidant potential against DPPH and ABTS. This antioxidant activity is confirmed *in vivo* by the increase of antioxidant enzymes (superoxide dismutase and catalase), reduced glutathione and nitrites levels in the liver of fingerlings transported with EGO. Indeed, on the first hand, during artisanal transportation fingerlings are exposed to stress and hypoxia which are known to alter redox [34,35]. On the other hand, palm oil contains α-tocopherol a powerful antioxidant that can improved redox balance and finally led to the increase of GSH [12].

Catfish survival and growth like the other fish depend on dissolved oxygen conditions [36,37]. Besides, oxygen deficit induces a severe impairment of pulmonary tissue function. In the present study, *E. guineensis* oil at low doses (2 and 4 drops) prevents alveolar cell necrosis in the gill. This can probably reduce post-transportation mortalities. Nevertheless, palm oil at 6 drops increases pulmonary impairment in catfish gill. This result suggests that high doses of EGO promote hypoxia. This result is confirmed by observing the cerebellar region of catfish fingerlings. As a matter of fact, while low doses (2 and 4 drops) reduced neuronal impairment, high dose (6 drops) promoted neuronal loss. Besides, exposure of animals to hypoxia has been reported to induce several changes in the organism, including alteration of alveolar permeability [36], and "high doses" of EGO are probably increase oxidation in water like reported by [38].

5. CONCLUSION AND RECOMMENDATIONS

The artisanal transportation induced stress which impaired among others liver, gills and brain functions. The administration of low doses of

Elaeis guineensis oil (2 drops for 10 L of water) prevent significantly gills and brain impairment. In view of aforesaid facts the following recommendations are forwarded:

- Artisanal transportation of catfish fingerlings must be carried out for reduced time and the water must be change frequently;
- Palm oil can be uses at low doses (less than 4 drops) during artisanal transportation of catfish fingerlings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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