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Antipyretic and antioxidant activities of *Eleucine indica*

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ABSTRACT

Eleucine indica is a medicinal plant used by the Ibibios of southern Nigeria in the treatment of malaria fever and also as a tonic. This study was to evaluate the antipyretic activities of the ethanol extract and the antioxidant activities of the extract, n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions of the whole plant. Basal rectal temperatures of adult albino rats of both sexes were recorded and the animals fasted for 24 h but allowed access to water ad libitum. They were then treated with DNP (10 mg/kg) and amphetamine (5 mg/kg) intraperitoneally. Within 30 min following the administration of amphetamine, animals with increased temperature of 1◦C were selected and randomized into five groups of six animals each. Group 1 received 10 ml/kg of distilled water orally. Group's 2 - 4 animals were administered 200 – 600 mg/kg of the extract intraperitoneally respectively. Group 5 animals received 100 mg/kg of acetyl salicylic acid orally. Yeast-induced pyrexia was achieved using 10 ml/kg of Brewer's yeast suspension injected subcutaneously in the back below the neck. Rectal temperatures were then obtained at 0.5 h and thereafter hourly for 5h. Superoxide dismutase, reduced glutathione, catalase, free radical scavenging with 1, 2-diphenyl-2-picrylhydrazyl, lipid peroxidation and methaemoglobin were measured in rats using standard methods. The result showed a significant ($p < 0.05 – 0.001$) and dose-dependent reduction in the elevated body temperature in rats pre-treated with the extract compared to control. There were also significantly high levels of superoxide dismutase and increased levels of reduced glutathione, catalase, free radical scavenging activity with DPPH, lipid peroxidation and methaemoglobin in both serum samples and liver homogenates of rats relative to control. These results corroborate with the ethno botanical use of the plant as antipyretic and depicts that the plant has both antioxidant ad pro-oxidant properties.

Keywords: Antipyretic, Antioxidant, *Eleucine indica*.

INTRODUCTION

Medicinal plants are widely used as source of alternative therapeutic tools for the prevention or treatment of many diseases. Pyrexia or fever is defined as an elevation of body temperature and is a response to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF- α) are formed in large amount under this condition, which increase PGE_2 which in turn triggers hypothalamus to elevate body temperature [1]. Fever is associated with symptoms of sickness behavior which consist of lethargy, depression, anorexia, sleepiness, and inability to concentrate. This increase in set point triggers increased muscle tone and shivering [2].

Antipyretics are drugs which can reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus which regulate the set point of body temperature [3]. Most of the antipyretic drugs inhibit Cox-2 expression to reduce the elevated body temperature by inhibiting prostaglandin E2 (PGE2) biosynthesis. Moreso, these synthetic agents irreversibly inhibit Cox-2 with high selectivity but are toxic to the cortex of brain, hepatic cells, glomeruli and heart muscles, whereas the natural Cox-2 inhibitors are known to have lower selectivity with fewer side effects. Therefore, a herbal antipyretic agent with reduced or no toxicity is in great demand [4, 5].

The antioxidant activities of medicinal plants are mainly attributed to phenolic compounds such as flavonoids, phenolic acids and tannins amongst others [6, 7]. The accumulation of free radicals is capable of causing pathological conditions such as ischemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's disease, mongolism, ageing process and dementia [8]. Some of these free radicals are reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and nitric oxide (NO) which are known to be directly or indirectly involved in DNA damage leading to mutations [9]. Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds such as ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione [10].

Therefore, there is a potential need for natural antioxidants with no or minimal side effects

Eleucine indica, L. Gaertn (Poaceae) is called nkimenang (Ibibios), and crowsfoot or goose grass (English). It is an annual growing to 0.45m and is considered as an adventitious species that is native in the tropics and subtropical regions [11]. *Eleucine indica* leaves have been used as an antihelminthic and for the treatment of dysentery [12]. It is one of the medicinal plants used in the treatment of malaria fever among the Ibibios of Southern Nigeria. The whole plant decoction is used to treat joint and malarial pains and to restore menstruation in females suffering from ammenorrhoea. The whole plant especially the root is depurative, diuretic, febrifuge and laxative. It is also used in the treatment of influenza, hypertension, oliguria and urinary retention as well as kidney problems in Trinidad and Tobago [13]. The plant has been reported to have phytochemical content of sterol glucoside forms [14] and C-glycosyl-flavone possessing anti-inflammatory activities [15] antibacterial, antioxidant and non-cytotoxic properties [16].

This study was embarked upon because there is little or no scientific literature on the antipyretic action of this plant and the antioxidant reports are not extensive. It is hoped that this will help to corroborate the scientific bases and standardize its use as antipyretic and antioxidant in general medical practice.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid and 1, 2-diphenyl-2-picrylhydrazyl were purchased from Sigma (USA) and 5-5'-dithiobis-2-nitrobenzoic acid was procured from E-Merk (Darmsradt, Germany). All other chemicals used were of analytical grade and obtained from reliable commercial sources.

Plant materials

The plant material (*Eleucine indica*) was collected in April, 2009 from Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey (a plant Taxonomist) in the Department of Botany and Ecological Studies, University of Uyo, where a voucher specimen (UUH1409) was deposited.

Preparation of extracts

The plant material was air-dried and then oven-dried at reduced temperature 35± 2°C . It was thereafter ground into powder and cold-macerated in 70% ethanol for 72h, and filtered. The filtrate was dried *in vacuo* using the rotary evaporator. 30g of the dried extract was partitioned using various solvents such as n-hexane, chloroform, ethyl acetate, butanol and water to obtain their respective fractions. The crude extract and the fractions were stored in a refrigerator at – 4°C until required for use.

Preliminary Phytochemical Investigation

The extract was screened for bioactive ingredients such as saponins, alkaloids, tannins, phlobotannins, flavonoids, anthraquinones, cardiac glycosides and sugar [17, 18].

Animal Stock

Adult albino rats and mice were obtained from the Animal House of the University of Jos, Jos, Plateau State and were maintained in the University of Uyo Animal House and fed with growers pellet Feed (Bendel Feeds and Flour mills Ltd, Edo State) with water given *ad libitum*. Approval for the use of animals for the study was obtained from the Animal Ethics Committee, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Preparation of Animal Samples for Oxidation Studies

The animals (rats) were allowed to acclimatize for 7 days. They were fed with 100mg/ml of extract and its fractions orally for 7 days. Only healthy animals were used. At the end of the 7th day, the animals were sacrificed and the blood was collected into plain bottles, allowed to clot and centrifuged to remove the serum. The liver of the animals were also collected, rinsed in buffered saline. About 1 g of the liver was homogenized with 9.0 ml of 0.4 mM phosphate buffer pH 8.0. The analysis carried out in both the serum and the liver homogenate include Superoxide Dismutase (SOD), Reduced Glutathione (GSH), Catalase (CAT) and Malondialdehyde (MDA) [19].

Evaluation of Antipyretic Activities of Extract

Yeast-induced Pyrexia in Rats

Adult albino rats of both sexes were used for this experiment. Their rectal temperatures were taken using digital clinical thermometer. The rats were then injected subcutaneously with 10 ml/kg of Brewer's yeast suspension in the back below the neck. Immediately after yeast administration, food was withdrawn. 18 h post administration, the rise in temperature was recorded. Only animals with a body temperature increasing by 1°C were used and separated into five groups of six animals per group. Group 1 animals received 10 ml/kg of distilled water orally, and served as the control group. Groups 2 - 4 animals were administered 200 – 600 mg/kg of extract intraperitoneally respectively. Group 5 animals received acetylsalicylic acid (100 mg/kg; p.o). Rectal temperatures were recorded at 0.5 h and then hourly for 5 h. The differences between the actual values and the starting values were registered for each time interval [20].

D-amphetamine–induced Pyrexia in Rats

Adult albino rats of both sexes were fasted for 24 h but allowed access to water *ad libitum.* Basal rectal temperatures of these animals were then obtained and 5 mg/kg of Amphetamine was administered. Within 30 min following the administration of amphetamine animals with increased temperature of 1°C were selected and randomized into five groups of six animals per group. Group 1 received 10 ml/kg of distilled water orally. Groups 2 - 4 animals were administered 200 – 600 mg/kg of the extract intraperitoneally respectively. Group 5 animals received 100 mg/kg of acetyl salicylic acid orally. Rectal temperatures were thereafter obtained at 0.5 h and thereafter hourly for 5 h [21, 22].

2, 4- Dinitrophenol (DNP) – Induced Pyrexia in Rats

Adult albino rats of both sexes were fasted for 24 h but allowed access to water *ad libitum.* Thereafter, their basal rectal temperatures were taken. DNP (10 mg/kg) was then administered to all the animals intraperitoneally (i.p). Hyperthermia was expected to develop within 30 min following the administration of DNP. Animals with increase of temperature by 1°C were selected and randomized into different groups of six rats per group. Group 1 served as control and received 10 m/kg of distilled water orally. Groups 2 - 4 were administered 200 – 600 mg/kg of the extract intraperitoneally (i.p) respectively. Group 5 animals received acetyl salicylic acid 100 mg/kg orally. Rectal temperatures of all the groups were then recorded at an hourly interval for 5 h [21, 22].

Oxidant and Anti-oxidant Effects of Extract

Effect of Extract on Antioxidant Enzymes

Assay for Superoxide Dismutase Activity (SOD): Superoxide dismutase activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 3 °C. One unit of SOD activity is the amount of SOD necessary to cause 50 % inhibition of epinephrine auto-oxidation. The assay was performed in 3.0 ml of 50 mM Na_2CO_3 buffer to which 0.02 ml of the sample was added. About 0.03 ml of the epinephrine stock solution was then added to the above before taking absorbance readings at 480nm for 3-5 min [19].

***Estimation of Reduced Glutathione*:** Reduced glutathione (GSH) activity was determined by the method of Bhattacharya and Pallab [19]. To the homogenate was added 10 % tricarboxylic acid (TCA) and centrifuged. To 1.0 ml of the supernatant was added 0.5 ml of Ellman's reagent {(19.8 mg of 5, 5-dithiobisnitrobenzoc acid (DTNB) in 100 ml of 0.1 % Sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M; pH 8.0)}. The absorbance was read at 412 nm.

***Estimation of Catalase Activity*:** Catalase (CAT) activity was assayed colorimetrically at 620 nm and expressed as moles of H_2O_2 consumed/min/mg/protein as described Bhattacharya and Pallab [19]. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M pH 7.0 phosphate buffer, 0.1 ml of dichromate-acetic acid reagent (5 % potassium dichromate and glacial acetic acid were mixed in 1 : 3 ratio).

Effect of Extract on 1, 2-diphenyl-2-picrylhydrazyl (DPPH)

The free radical scavenging ability of the extract against DPPH free radical was evaluated [23]. Briefly, 1 ml aliquot (0.05 g of the extract was dissolved in 20 ml methanol) was mixed with 1 ml, 0.4 mM methanolic solution consisting DPPH radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

Estimation of Lipid Peroxidation Product Malondialdehyde (MDA)

Lipid peroxidation as evident by the formation of thiobarbituric acid (TBARS) was measured by the method of Jiang, Hunt and Wolff [24]. To 0.1 ml of tissue homogenate was added 2 ml of (1 : 1 : 1 ratio) TBA – TCA – HCI reagent (thiobarbituric acid 0.37 %, 0.25 N HCl and 15 % TCA) and placed in water bath for 15 min, cooled and centrifuged at room temperature for 10 min and the absorbance read at 535 nm.

Methaemoglobin Generation of the Extract and its Fractions

Methaemoglobin generation of the extract and its fractions were measured by adding 0.1 ml of each extract/fraction (1 mg/ml) to 1 ml of whole blood and left to stand for 1 h. Methaemoglobin level was determined by spectrophotometric method of Harvey [25]. About 0.5 ml of the extract-whole blood mixture was diluted with 25 ml of phosphate buffer in test tube. The absorbance of the diluted extract with the whole mixture (5 ml) was read first against blank (water) at 630 nm and designated A. About 0.1 ml of neutral sodium cyanide was then added to the whole blood mixture and the absorbance, B, was read again. About 0.1 ml potassium ferricyanide was added to another 5 ml of the diluted whole blood and the absorbance, C, was read after which 0.1 ml of sodium cyanide was added to the mixture and read as absorbance, D.

Methaemoglobin was calculated as:

$$\text{Methaemoglobin} \quad (\%) = \frac{A - B}{C - D} \times 100$$

Statistical Analysis

Results were expressed as multiple comparisons of Mean ± SEM. Significance was determined using One-way Analysis of Variance (ANOVA) followed by Tukey-Kramer multiple comparison post test. A probability level of less than 5% was considered significant.

RESULTS

The study showed that subcutaneous injection of yeast suspension markedly increased the rectal temperature 8 h following the administration. The extract decreased the rectal temperature in a dose- and time-dependent manner. This decrease was statistically significant ($p < 0.01 – 0.001$) as shown in Table 1. In amphetamine-induced pyrexia, the extract showed significant ($p < 0.05 – 0.001$) and dose-dependent reduction in the elevated body temperature as shown in Table 2. The highest dose of the extract (600 mg/kg) compared well with acetyl salicylic acid. Also, in Dinitrophenol (DNP) – induced pyrexia, there was a dose- and time-related decrease in temperature compared to control. This decrease was statistically significant ($p < 0.05 – 0.001$) as shown in Table 3.

On the effect of extract and its fractions on antioxidant enzymes, the crude extract and its fractions exhibited significantly high levels of Superoxide dismutase in both serum sample and liver homogenate of albino rats respectively, relative to control (Figures 1 and 2). A high level of reduced Glutathione enzyme was observed in animals pretreated with extract and its fractions in both serum and liver homogenate of albino rats when compared to control, as shown in Figures 3 and 4. **S**imilarly**,** a high level of catalase enzyme was noted in both serum and liver homogenate of albino rats relative to control (Figures 5 and 6). Various degrees of antioxidant activity were observed with DPPH. The aqueous fraction showed the highest free radical scavenging activity depicted by percentage inhibition of DPPH absorption (79.0%) followed by n-hexane (77.3%), crude (69%), butanol (63.1%) and chloroform (51.3%). The fraction with the least free radical scavenging activity was ethyl acetate with 39.7% DPPH absorption as shown in Figure7.The level of MDA in both serum sample and liver homogenate of the crude extract and its fractions respectively were high when compared to control as shown in Figures 8 and 9. High levels of methaemoglobin generation were observed with the crude extract and its fractions when compared to control, the highest being n-hexane fraction (Figure 10).

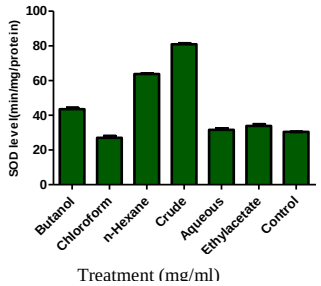

Figure 1: Effect of extract and its fractions on serum Superoxide dismutase

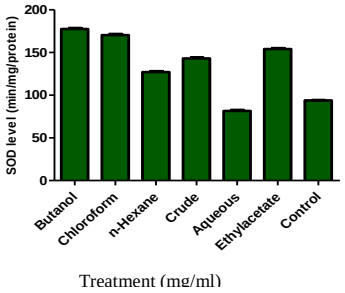

Figure 2: Effect of extract and its fractions on Superoxide dismutase in liver homogenate

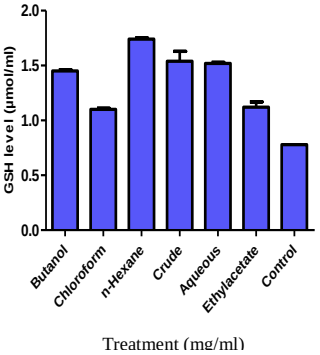

Figure 3: Effect of extract and its fractions on serum reduced Glutathione

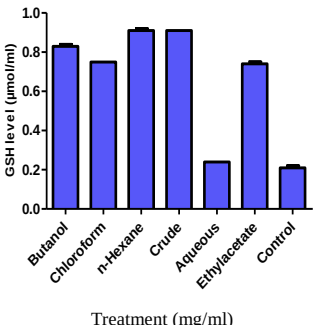

Figure 4: Effect of extract and its fractions on reduced Glutathione in liver homogenate

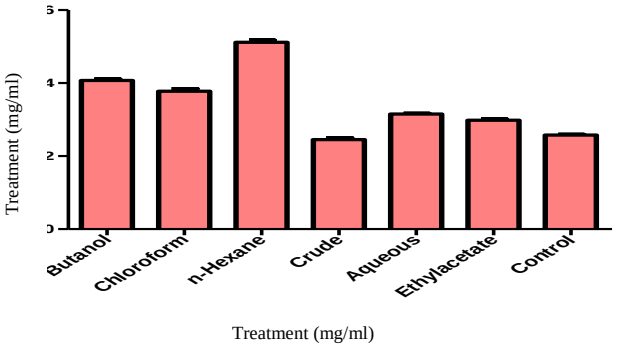

Figure 5: Effect of extract and its fractions on serum Catalase

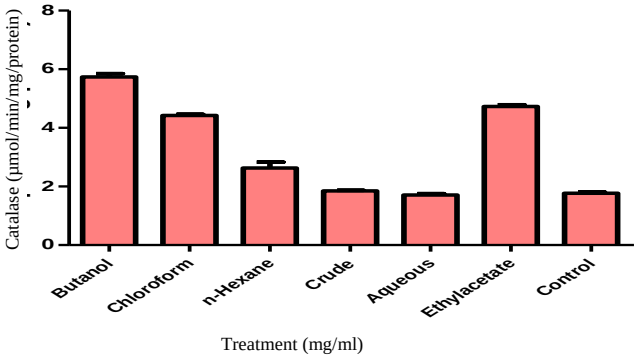

Figure 6: Effect of extract and its fractions on Catalase in liver homogenate

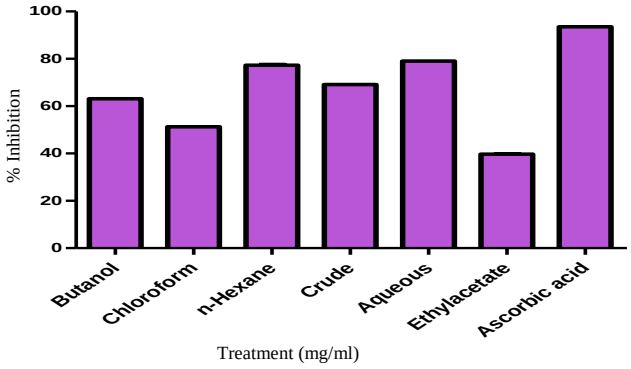

Figure 7: Effect of extract and its fractions on 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

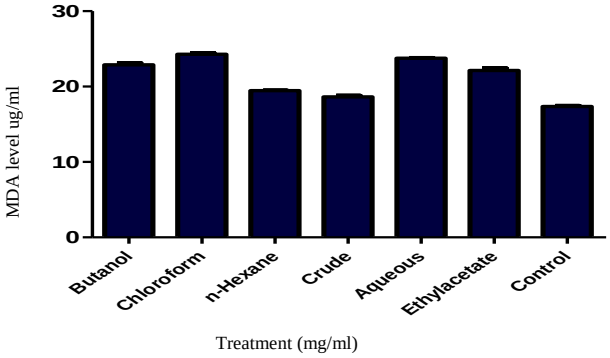

Figure 8: Effect of extract and its fractions on serum lipid peroxidation

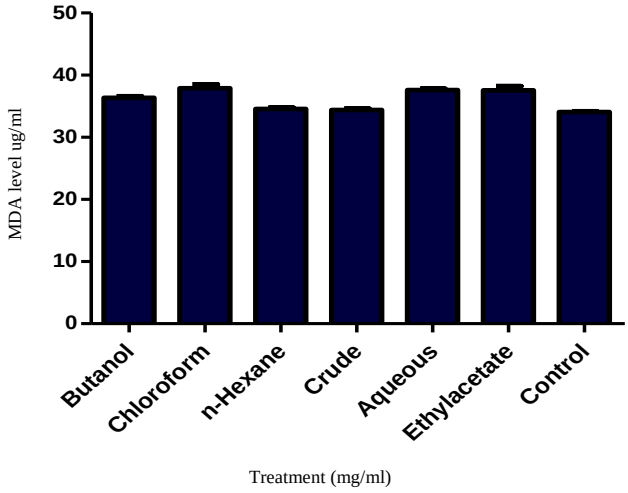

Figure 9: Effect of extract and its fractions on lipid peroxidation in liver homogenate

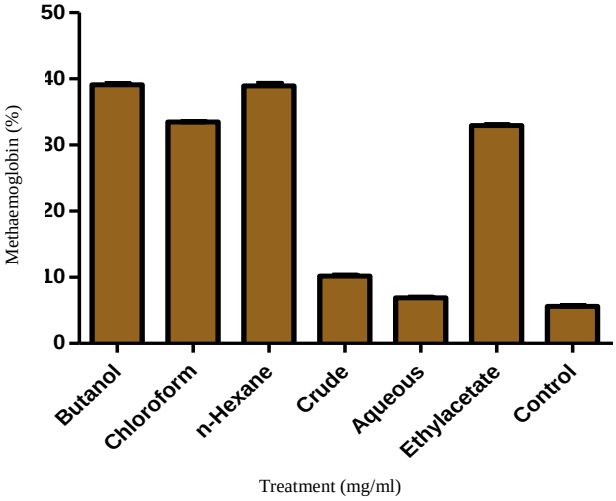

Figure 10: Effect of extract and its fractions on methaemoglobin

Table 1: Effect of *E. indica* extract on yeast-indued pyrexia in rats

Treatment /Dose (mg/kg)	Time (hours)							
	0	18	0.5	1	2	3	4	5
Control	36.43 ± 0.22	38.07 ± 0.05	37.97 ± 0.22	38.20 ± 0.22	38.37 ± 0.21	38.53 ± 0.22	38.77 ± 0.17	38.62 ± 0.17
Extract 200	36.63 ± 0.11	38.22 ± 0.13	37.63 ± 0.13	37.42 ± 0.07[a]	37.20 ± 0.08[b]	36.75 ± 0.08[b]	36.52 ± 0.11[b]	36.40 ± 0.10[b]
400	36.62 ± 0.10	38.12 ± 0.11	37.53 ± 0.11	37.28 ± 0.13[b]	37.11± 0.11[b]	36.62[b] ± 0.10[b]	36.40 ± 0.11[b]	36.35[b] ± 0.10[b]
600	36.80 ± 0.06	38.03 ± 0.09	37.52 ± 0.11	37.00 ± 0.11[b]	36.47 ± 0.08[b]	36.23 ± 0.08[b]	36.17 ± 0.08[b]	36.07 ± 0.09[b]
ASA 100	36.25 ± 0.13	38.28 ± 0.11	37.40 ± 0.10	37.15 ± 0.10[b]	36.83 ± 0.09[b]	36.33 ± 0.09[b]	36.57 ± 0.08[b]	36.35 ± 0.10[b]

Values represent Mean ± SEM. Significant [a]p <0.001; [b]p <0.001 (n=6)

Table 2: Effect of *E. indica* on amphetamine-induced pyrexia in rats

	Treatment /Dose (mg/kg)	Time (hours)							
		BT	0	0.5	1	2	3	4	5
Control	-	36.10 ± 0.18	37.40 ± 0.08	37.68 ± 0.09	37.86 ± 0.07	38.40 ± 0.10	38.68 ± 0.09	38.73± 0.13	38.58 ± 0.06
Extract	200	36.47 ± 0.15	37.98 ± 0.09[b]	37.62 ± 0.09	37.33 ± 0.07[b]	37.02 ± 0.10[b]	36.82 ± 0.09[b]	36.68 ± 0.08[b]	36.53 ± 0.09[b]
	400	36.62±0.08[a]	37.67 ± 0.08	37.27 ± 0.09[a]	36.82 ± 0.10[b]	36.65 ± 0.10[b]	36.42 ± 0.06[b]	36.40 ± 0.06[b]	36.30 ± 0.04[b]
	600	36.13 ± 0.09	37.72 ± 0.06	37.05 ± 0.08[b]	36.53 ± 0.07[b]	36.43 ± 0.05[b]	36.33 ± 0.09[b]	36.25 ± 0.08[b]	36.03 ± 0.07[b]
ASA	100	36.60 ± 0.07	37.45 ± 0.08	37.05 ± 0.08[b]	36.70 ± 0.06[b]	36.50 ± 0.10[b]	36.43 ± 0.04[b]	36.33 ± 0.06[b]	36.20 ± 0.06[b]

Values represent Mean ± Significant [a]p<0.05; [b]p<0.001 (n=6).

Table 3: Effect of *E. indica* extract on 2, 4 dinitrophenol (DNP)-induced pyrexia in rats.

Group	Weight (g)	Time (hours)							
		B.T	0	0.5	1	2	3	4	5
Control	_	36.62±0.06	37.87 ±0.08	38.03 ± 0.08	38.25±0.08	38.45±0.08	38.58 ± 0.05	38.30±0.11	37.85 ± 0.09
Extract	200	36.30 ±0.11	37.53 ±0.07[a]	37.59 ± 0.04[b]	37.58±0.03[b]	37.37 ± 0.05[b]	36.10 ± 0.07[b]	36.85±0.04[b]	36.63 ± 0.02[b]
	400	36.47±0.12	37.63± 0.07	37.40± 0.10[b]	37.22± 0.05[b]	36.92± 0.10[b]	36.70± 0.06[b]	36.60 ±0.05[b]	36.42± 0.05[b]
	600	36.52± 0.04	37.58± 0.03	37.33± 0.05[b]	37.12± 0.04[b]	36.70± 0.06[b]	36.58 ±0.05[b]	36.30± 0.04[b]	36.22± 0.04[b]
ASA	100	36.40± 0.06	37.68± 0.05	37.55± 0.04[b]	37.35± 0.04[b]	36.87± 0.08[b]	36.52± 0.06[b]	36.43± 0.05[b]	36.20± 0.07[b]

Values represent Mean ± SEM. Significant [a] $p<0.05$; [b] $p<0.001$ (n=6)

DISCUSSION

Pyrexia results from secondary impact of infection, tissue damage, graft rejection, inflammation, malignancy or other disease conditions. The tissue so injected or damaged initiates an increased production of pro-inflammatory mediators such as interleukin-1β, α, β and tumour necrosis factor-α (TNFα) with consequent increased synthesis of PGE_2 in hypothalamus and elevation of body temperature [26]. Yeast induced pyrexia is called pathogenic fever which is known to be due to production of prostaglandins (PGE_2) which set thermoregulatory centre at a higher temperature [27]. The extract decreased the rectal temperature in a dose-dependent manner following yeast-induced pyrexia. This suggests that the mechanism of the extract's antipyretic effect may in part be due to its inhibition of cyclooxygenase-2 expression, thus suppressing PGE_2 biosynthesis.

Amphetamine is an indirectly-acting sympathomimetic agent. Its inhibition shows antagonistic effect on endogenous catecholamines in central adrenergic neurons [28]. The extract caused a dose-dependent reduction in pyrexia induced by amphetamine and depicts this as a possible mechanism of action.

Dinitrophenol (2,4-dinitrophenol) or DNP induces hyperthermia by uncoupling oxidative phosphorylation causing the release of calcium from mitochondrial stores and prevents calcium reuptake. This leads to free intracellular calcium, muscle contraction and hyperthermia [29]. The extract exhibited a dose- and time-dependent decrease in temperature induced by DNP. Therefore, the extract may have caused the stimulation of sarcoplasmic reticulum Ca^{2+}-ATPase thus promoting calcium reuptake into the sarcoplasmic reticulum, muscle relaxation and hypothermia [30].

It has been reported that the leaves of *E. indica* contain phenolic compounds which possess antibacterial and antioxidant activities [16]. This agrees with our findings as the extract and its fractions produced high levels of antioxidant enzymes Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) *in vivo*. This might be associated with the stimulatory effect of the extract and its fractions due to the phytochemical constituents. *In vitro*, the aqueous fraction of *E.indica* expressed the highest free radical scavenging activity with 1,1-diphenyl-2picrylhydrazyl (DPPH) while ethyl acetate had the lowest free radical scavenging activity. The ethyl acetate fraction of *Eleucine indica* has been reported to have the highest chemosuppressive antiplasmodial activity when compared with other fractions,, suggesting that the active ingredients responsible for the antiplasmodial effect may be localized here [31]. However, *in vivo*, the extract and its fractions showed various high degrees of antioxidant activities, including the ethyl acetate fraction.

Lipid peroxidation, a degradative process of membrane lipids, measured indirectly by the degree of inhibition of malondialdehyde (MDA) by the plant extract and its fractions, was also slightly raised, that is, not reduced when compared to the control. MDA and methaemoglobin (MetHb) have oxidant potentials while is oxidant SOD, CAT and GSH are antioxidant enzymes. The increase in levels of MDA and MetHb is evidence of the pro-oxidant effect of the extract and its fractions. Hence the extract and its fractions display a paradoxical role as pro-oxidant and antioxidant. This is not new. Antioxidants have been known to act as proxidants under certain conditions. For example, ascorbate, in the presence of high concentration of ferric iron, is a potent potentiator of lipid peroxidation. Studies suggest that ascorbate sometimes increases DNA damage in humans. Mechanistic studies in the early stage of LDL oxidation show that the role of Vitamin E is not simply that of classical antioxidant. Unless additional compounds are present, Vitamin E can have antioxidant, neutral or pro-oxidant activity. Beta-carotene also can behave as a pro-oxidant in the lungs of smokers. Every antioxidant is in fact a redox agent and might become a pro-oxidant to accelerate lipid peroxidation and induce DNA damage under special conditions and concentrations [32]. Studies have revealed pro-oxidant effects of antioxidant vitamins and several classes of plant-derived polyphenols such as flavonoids [33], tannins [34] and curcumin [35].

The pro-oxidant and antioxidant effect of plant extracts are due to the balance of two activities: free radical-scavenging activity and reducing power on iron ions, which may drive the Fenton reaction via reduction of iron ions. A predominant reducing power (on iron ions) over the free radical scavenging activity in a mixture of compounds results in the pro-oxidant effect [36]. The predominance of free radical scavenging activity over the reducing power of iron ions will result in net antioxidant effect of the whole extract as the activity of *E. indica* suggests, especially from the minimal rise in MDA levels when compared to the rise in antioxidant enzyme levels. Therefore, it is important to take into account the pro-oxidant activity together with the free radical scavenging activity of the extract to determine the net free radical scavenging capability [37].

CONCLUSION

The antipyretic activities of ethanolic extract (200 – 600 mg/kg) of the whole plant in rats showed significant, dose-dependent responses. There were also significantly high levels of superoxide dismutase and increased levels of reduced glutathione, catalase, free radical scavenging activity with DPPH, lipid peroxidation and methaemoglobin in both serum samples and liver homogenates of rats, which depict both antioxidant and pro-oxidant potentials.These properties may in part provide the rationale for use of *E. indica* in the treatment of malaria fever by practitioners of traditional medicine. Further research is required to fully investigate the mechanisms responsible for these observed responses.

Conflict of interest statement

We declare that we have no conflict of interest.

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