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Histological alteration and oxidative variables in Wistar rats with induced-sepsis: the protective effect of tomato pomace powder

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Abstract

Background: Sepsis is a systemic inflammatory response to infection causing morbidity and mortality and has oxidative damage as one of the mechanisms. We induced sepsis in rats by caecal ligation and perforation (CLP) and then investigated the possible protective effect of tomato pomace powder (TPP) on the sepsis-induced neuropathy using vitamin E (VIT E) as a standard antioxidant.

Methods: Thirty-nine male Wistar rats were randomized into six groups: Control (Cont) (N=5) received food and water; TPP (N=5) received TPP (50 mg/kg); VIT E (N=5) received VIT E (500 mg/kg); CLP (N=8) had CLP; TPP+CLP (N=8) received TPP (50 mg/kg) plus CLP; VIT E+CLP (N=8) received VIT E (500 mg/kg) plus CLP. The CLP was done on first day while all other administration lasted 21days after which, neurobehavioural tests were done, animals sacrificed and tissues processed for haematological, biochemical and histological tests.

Results: The CLP group had significant ($p<0.05$) increase in lipid peroxidation (LPO) level, a reduction of the glutathione (GSH) level and an increase in the activity of catalase enzyme activity when compared with the control, all of which were reversed to near control in the co-treated groups ($p < 0.05$). Total white cell and neutrophil counts were significantly ($p < 0.05$) higher in the CLP group compared with the control group. Histological alterations induced by sepsis included degeneration of Purkinje, granule and pyramidal neurons which were ameliorated by TPP and VIT E.

Conclusion: Our results indicated that TPP and VIT E demonstrated protective effects from brain damage caused by CLP-induced sepsis via maintenance of the anti-oxidant status.

Keywords: *Caecal Ligation and Perforation, Lycopersicon esculentum, Cerebellum, Hippocampal formation and Sepsis.*

Résumé

Contexte :L'état septique est une réponse inflammatoire systémique à une infection entraînant une morbidité et une mortalité et a undommage oxydatifcomme l'un des mécanismes. Nous avons induitl'état septique chez le rat par la ligature et perforationcaecale (CLP) et ensuite étudié l'effet de protection possible de la purée en poudre de tomates (TPP) sur la neuropathie induite par l'état septique à l'aide de la vitamine E (VIT E) comme un antioxydant standard.

Méthodes : Trente-neuf rats Wistar mâles ont été randomisés en six groupes :contrôle (cont) (N = 5) reçu la nourriture et l'eau ; TPP (N = 5) reçu TPP (50 mg / kg); VIT E (N = 5) reçu VIT E (500 mg / kg); CLP (N = 8) avait CLP; TPP + CLP (N = 8) reçu TPP (50 mg / kg) en plus de CLP; VIT E + CLP (N = 8) a reçu VIT E (500 mg / kg) plus de CLP.CLP a été réalisée le premier jour alors que toutes les autres administrations ont duré 21 jours, au terme desquels des tests neurocomportementaux ont été effectués, les animaux sacrifiés et les tissus traités pour des tests hématologiques, biochimiques et histologiques.

Résultats: Le groupe CLP présentait une augmentation significative ($p <0,05$) du taux de peroxydation lipidique (LPO), une réduction du taux de glutathion (GSH) et une augmentation dans l'activité de l'activité de l'enzyme catalase par rapport au contrôle, qui étaient tous inversé à proche du contrôle dans les groupes co-traités ($p <0,05$). Le nombre total de globules blancs et de neutrophiles était significativement plus élevé ($p <0,05$) dans le groupe CLP par rapport au groupe témoin. Les altérations histologiques induites par l'état septique comprenaient la dégénérescence de neurones de Purkinje, de granules et pyramidaux qui étaient améliorés par TPP et VIT E.

Conclusion: Nos résultats indiquent que TPP et VIT E ont démontré des effets protecteurs contre les dommages au cerveau causés par un état septique induit par CLP à travers le maintien du statut antioxydant.

Mots clés : *Ligature et perforation caecale, Lycopersicon esculentum, cervelet, formation hippocampique et état septique.*

Introduction

Sepsis is a systemic inflammatory response to infection and a major cause of morbidity and mortality worldwide [1]. The aetiology of sepsis among others is faecal peritonitis and might experimentally result in neuronal degeneration, peri-

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microvessel oedema, and disruption of astrocyte processes [2]. However, secondary bacterial peritonitis in human as in acute intestinal perforation is an important cause of sepsis and death in surgical practice due to an intra-abdominal infectious focus. Sepsis leads to the production of reactive oxygen species which have been reported to play a role in the induction of many pro-inflammatory cytokines and mediators important in producing the acute inflammatory responses associated with sepsis [1].

Oxidative stress occurs when the body's antioxidant system is overwhelmed by excess reactive oxygen species [3]. Hence the need to protect the body from the deleterious effects of oxidative stress. The protective effect of phytochemicals in mitigating damage from oxidative stress appears promising. *Lycopersicon esculentum* (tomato), family Solanaceae, are rich sources of numerous beneficial nutrients and anti-oxidants which include lycopene, choline, alpha-lipolic acid, beta-carotene, lutein, vitamins A and C, folic acid, small amounts of magnesium and potassium [4]. Lycopene an open chain highly unsaturated carotenoid responsible for 80% anti-oxidant property of tomato reportedly inhibited iron catalysed lipid peroxidation and nitric oxide production in rat brain homogenates exposed to ischaemic brain injury [5]. Similarly, *Lycopersicon esculentum* demonstrated neuroprotection against cisplatin-induced alteration of microanatomy of rat cerebellum, dentate gyrus and Cornu Ammonis3 (CA3) of rat brain [6]. Vitamin E (α-tocopherol) is a fat-soluble vitamin found in many foods, fats, and oils. The main function of Vitamin E in humans appears to be that of an antioxidant to neutralize free radicals formed primarily in the body during normal metabolism and also upon exposure to environmental factors, such as cigarette smoke or pollutants [7]. Aside from maintaining the integrity of cell membranes throughout the body, α-tocopherol also protects the fats in low-density lipoproteins (LDLs) from oxidation.

Although the cerebellum accounts only for approximately 10% of the brain's volume, it contains over 50% of the total number of neurons in the brain [8]. The cerebellar cortex is divisible into three functional areas [9] namely: spinocerebellum; vestibulocerebellum, cerebrocerebellum whereas its medulla contains the four masses of cerebellar nuclei namely: dentate, emboliformis, globose, and fastigial all of which are composed of large, multipolar neurons with simple branching dendrites and from which the cerebellar efferents arise. The major functions of the cerebellum include: maintenance of balance, posture and muscle tone; motor coordination of voluntary movements; maintenance of learning and cognitive functions; regulation of saccadic and smooth eye movements [10, 11] and making movements more adaptive and accurate by modifying motor commands of the descending pathways [8]. The hippocampal formation is among others involved in long term spatial and episodic memory storage [12]. Alteration of the structural integrity of either the cerebellum or the hippocampal formation by sepsis may have untoward effect on their functions.

Previous studies using CLP have demonstrated oxidative parameter changes [1, 13, 14] and brain structural alterations [2]; however, no study has so far reported on the effect of *Lycopersicon esculentum* on this technique. In the present work, we aimed to test the beneficial effects of *Lycopersicon esculentum* as Tomato Pomace Powder (TPP) and vitamin E on the brain of adult male Wister rats. To that end, we induced sepsis by Caecal Ligation and Perforation (CLP) technique to study oxidative parameters, haematology and behavioural alterations and brain structural responses by light microscopy. We hypothesized that TPP could prevent CLP-induced brain tissue injury by inhibiting the ROS generation triggered by sepsis and thus answer the question of whether TPP can protect rat brain from induced sepsis. The findings (if protective) could stimulate further research in harnessing tomato's potential as an adjunct in sepsis management.

Materials and methods

Experimental animals

Male Wistar rats weighing 90–180 g were obtained from the College of Medicine animal house, University of Ibadan, Nigeria. They were housed in plastic cages with dimensions 29 cm x 27 cm x 30 cm, with wood shavings in a fly-proof, freely ventilated and naturally illuminated animal rooms at room temperature with a 12 hr light/dark cycle. The animals were acclimatized for two weeks and then divided into experimental and control groups by random sampling techniques, fed with commercial mouse cubes (Ladokun Feeds Nig. Ltd, Ibadan, Nigeria) and drinking water *ad libitum*. All procedures on animal handling were in accordance with ethical use of animals in research [15].

Processing and administration of tomato pomace powder (TPP)

Fresh tomatoes were purchased from Bodija market, Ibadan, Nigeria. The tomatoes were washed, sliced and squeezed to reduce the water content, the squeezed remains were dried in an oven at 50° Celsius for two hours after which it

was grounded into powder which was stored as tomato pomace powder (TPP) in an air tight container as described by Owoeye and Onwuka [6]. The TPP was administered at 50 mg/kg using propylene glycol as the vehicle.

Preparation and administration of α-tocopherol (Vitamin E)
Each soft gelatine capsule containing 100 mg of DL-α-tocopheryl acetate as 100 mg vitamin E acetate was punctured and withdrawn with a new size 21 G needle attached to a new 1 mL insulin syringe. The syringe was attached to an intra-gastric tube for gavage to administer 500 mg/kg body weight daily for 21days.

Research Design
The thirty-nine rats were randomized into six groups and treated as follows:
Group 1 (Cont) (N=5) animals received water and food, served as control.
Group 2 (TPP) (N=5) animals treated with 50 mg/kg of tomato pomace powder (TPP).
Group 3 (VIT E) (N=5) animals treated with 500 mg/kg of vitamin E (VIT E).
Group 4 (CLP) (N=8) animals subjected to caecal ligation and perforation (CLP).
Group 5 (TPP+CLP) (N=8) animals treated with 50 mg/kg of TPP and subjected to CLP.
Group 6 (VIT E+CLP) (N=8) animals treated with 50 mg/kg of VIT E and subjected to CLP.

The doses used in the present study were selected based on previously published data: VIT E [16, 17]. The CLP was performed on first day of experiment while other treatments were given orally by gavage and lasted 21 days.

Induction of sepsis by Caecal Ligation and Perforation (CLP) technique
Rats in groups 4, 5 and 6 were subjected to sepsis by caecal ligation and perforation (CLP) method as previously described [13] on first day of the experiment. Briefly, they were anaesthetized with ketamine (80 mg/kg body weight, i.m.). Under aseptic conditions, a 3 cm midline laparotomy was performed to expose the caecum and adjoining intestine. The caecum was tightly ligated with a 3.0 silk suture at its base, below the ileocaecal valve, and was perforated once with 14-gauge needle. The caecum was then squeezed gently to extrude about (0.5 cm^3) of faecal matter through the perforation site and was then returned to the peritoneal cavity, and the laparotomy was closed with 4.0 silk sutures.

Post operation management
After CLP each animal was allowed to recover from anaesthesia. Operated animals were given a single dose of antibiotics i.m. (ceftriaxone at 50 mg/kg and gentamycin 25 mg/kg) and then returned to their cages to recover from anaesthesia. The rats were observed after CLP for the presence of signs of infection (piloerection, lethargy, tachypnea, and weight loss). On regaining consciousness, the animals were allowed access to feeds and water *ad libitium*.

Behavioural tests
Behavioural tests were performed on all the groups of animals both experimental and control on day 21 to evaluate motor function and equilibrium. Parameters assessed included: exploratory tests, motor function tests and equilibrium tests.

Line crossing and rearing
The apparatus used was a slight modification of published method [18]. It consisted of a square arena (56 cm × 56 cm × 20 cm) made of white wood and its floor divided by lines into 16 squares that allowed the definition of central and peripheral parts. At the beginning of the session, each rat was individually placed in the centre of the arena and its activity was recorded for 5 min. The number of squares crossed with all paws (crossing) and standing on hind legs (rearing) were evaluated during 5 minute sessions. The crossing numbers were indicators of locomotor while the rearing numbers indicated vertical and exploratory activities. At the end of each session, rats were removed from the open field and the experimental chamber was thoroughly cleaned with 70% ethanol and dried before introducing a fresh rat so as to eliminate olfactory bias [19].

Negative Geotaxis
This was used to assess motor coordination of animals when challenged on a sloped surface. The testing apparatus consists of sloped platform of 45 degrees from horizontal to the desktop; the rat was placed on the highest point facing downwards. The latency to turn and orient themselves to face up the slope was recorded. The duration of attempt for each animal was 2 minutes and the mean of two trials was recorded [20].

Haematological analysis
Blood for haematological parameters was obtained from the retro-ocular plexus of the animals using heparinized capillary tubes on day 21 after the behavioural tests into Ethylene Di-amine Tetra Acetic

(EDTA) acid treated sample bottles for the determination of full blood count which included: Red Blood Cell count, Haemoglobin count, Packed Cell Volume Count (PCV), White Blood Cell count and differential cell count (Lymphocytes, Monocytes, Neutrophils, Eosinophils). Analysis was done in the haematology laboratory of the Department of Veterinary Medicine, University of Ibadan

Sacrifice and sample collection

After the last administration on day 21 of the experiment, animals were weighed, blood samples collected, and were then euthanized with ketamine (80 mg/kg body weight) after which cervical dislocation was gently performed. The rat's brains were quickly dissected out, rinsed, mopped and weighed and then divided into two with the right half used for histology and the left half preserved for biochemical assays based on the method of Owoeye and Ojora [20]. The tissues meant for histological analysis were fixed in 10% formalin and later processed for histology by paraffin wax embedment method while those for biochemical analysis were kept in freshly prepared cold phosphate buffer solution at pH 7.4. The biochemical samples were later homogenized in phosphate buffer (pH 7.4) and the resulting homogenate was centrifuged at 10,000 x g for 15 min at minus 4°C, the supernatant obtained was thereafter used for the biochemical estimations.

(SOD) was determined according to the method of Del-Maestro *et al* [23], Catalase (CAT) activity was also determined according to method of Sinha [24]. All the biochemical tests were conducted in the Drug Metabolism & Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Statistical analysis

Descriptive data were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using GraphPad software version 5.04, San Diego, CA, USA. and post-hoc test using Dunnet's test. Groups were compared by Student's t-test and one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p<0.05$.

Results

General observations

Animals in the Control (Cont), tomato pomace powder (TPP) and vitamin E (VIT E) were generally active and fed well. There was no mortality in the groups without surgical interventions but there was 12.5% mortality in CLP exposed rats (2 animals in CLP group and 1 animal in TPP+CLP group). The first week post-op, rats that underwent CLP showed clinical symptoms of sepsis by exhibiting the following features: lethargy, reduced activity, reduced feeding and drinking, piloerection and alopecia.

Table 1: Effect of treatments on body weight differences and mean brain weight.

Groups	Cont	TPP	VIT E	CLP	TPP+CLP	VIT E+CLP
Body wt. diff. (g)	36.00±16.70	36.00±16.70	32.00±11.51	29.71±2.00*	13.04±3.00**	8.54±1.25**
Mean brain wt. (g)	1.64±0.23	1.50±0.14	1.46±0.09	1.60±0.10	1.60±0.19	1.66±0.15

Data are presented as Mean ± S.D. Control, Cont; Tomato pomace powder, TPP; Vitamin E, VIT E; Caecal ligation and perforation, CLP; Weight, wt.; Difference, diff. *P<0.05 versus Control. **P<0.05 versus CLP.

Assessment of Oxidative Stress and Antioxidant indices in the rat's whole brain

The left hemisphere of the brain preserved for biochemical assays as described above was used for biochemical assays. The level of reduced Glutathione (GSH) was estimated based on the method of Beutler *et al* [21] and expressed in µg/ml/mg. Malondialdehyde (MDA) level was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale [22]. The activity of Superoxide dismutase

Body and brain weight changes

As shown in the table 1, the weight difference was lower in all the CLP groups when compared with the control group while there was a significant reduction of body weight in the TPP+CLP and VIT E+CLP groups relative to the CLP group ($p<0.05$). However, the body weight differences did not appear to affect the brain weight as shown in table 1.

Biochemical analysis

As shown in figure 1, sepsis (CLP) elicited oxidative stress as indicated by a significant ($p<0.05$) increase

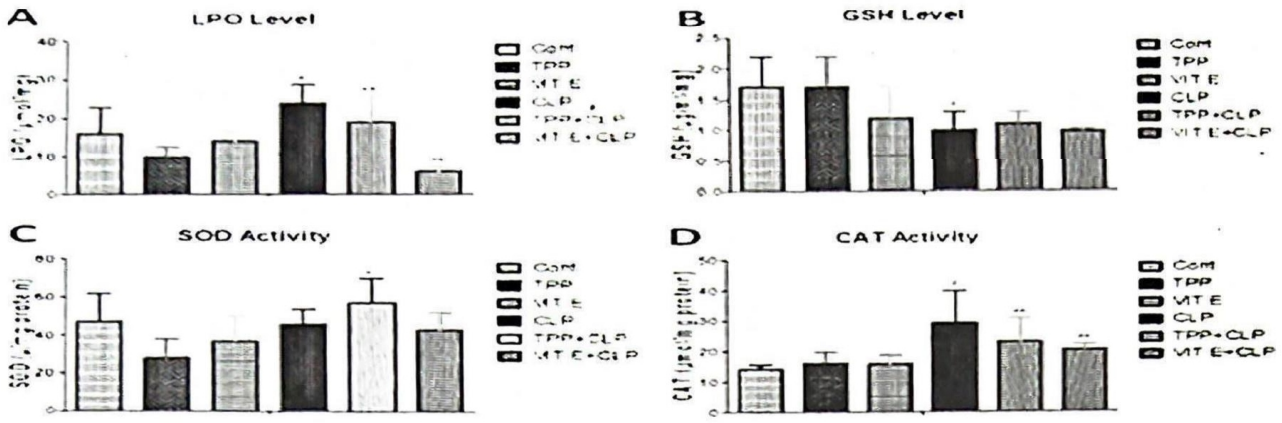

*Fig. 1: Effect of treatment on Superoxide Dismutase (SOD), reduced glutathione (GSH), Catalase (CAT), and Lipid peroxidation (LPO) in rat whole brain. Data are presented as Mean ± S.D. Control, Cont; Tomato pomace powder, TPP; Vitamin E, VIT E; Caecal ligation and perforation, CLP. *P<0.05 versus Control. **P<0.05 versus CLP.*

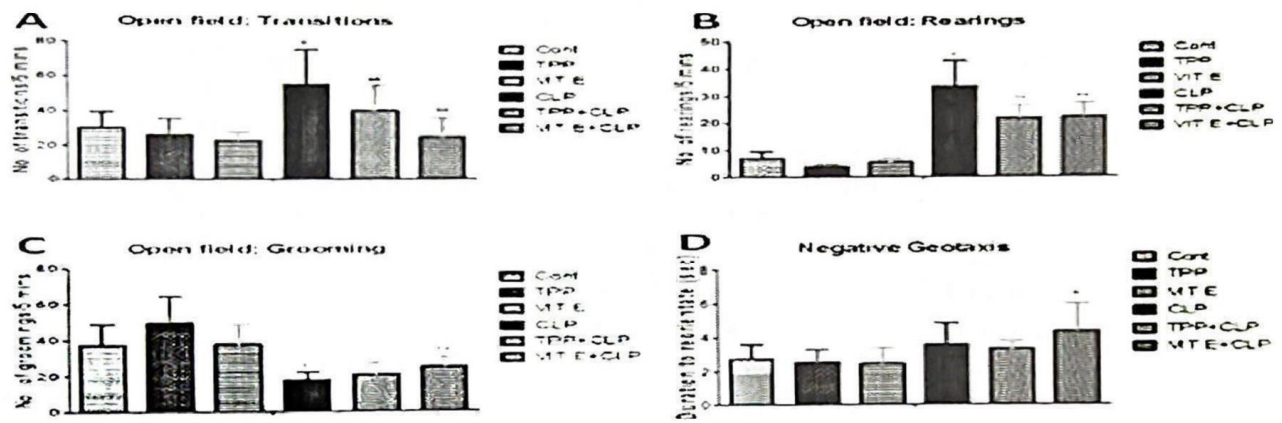

*Fig. 2: Histogram of behavioural tests in the control and treated groups. A: Transitions or horizontal movements measured as number of open field transitions, B: Vertical movements measured as number of open field rearings, C: Grooming measured as the number of grooms, D: Negative geotaxis, measured as time taken for rats to re-orient in a head-up direction. Values are expressed as mean ± S.D. Control, Cont; Tomato pomace powder, TPP; Vitamin E, VIT E; Caecal ligation and perforation, CLP. *P<0.05 versus Control. **P<0.05 versus CLP.*

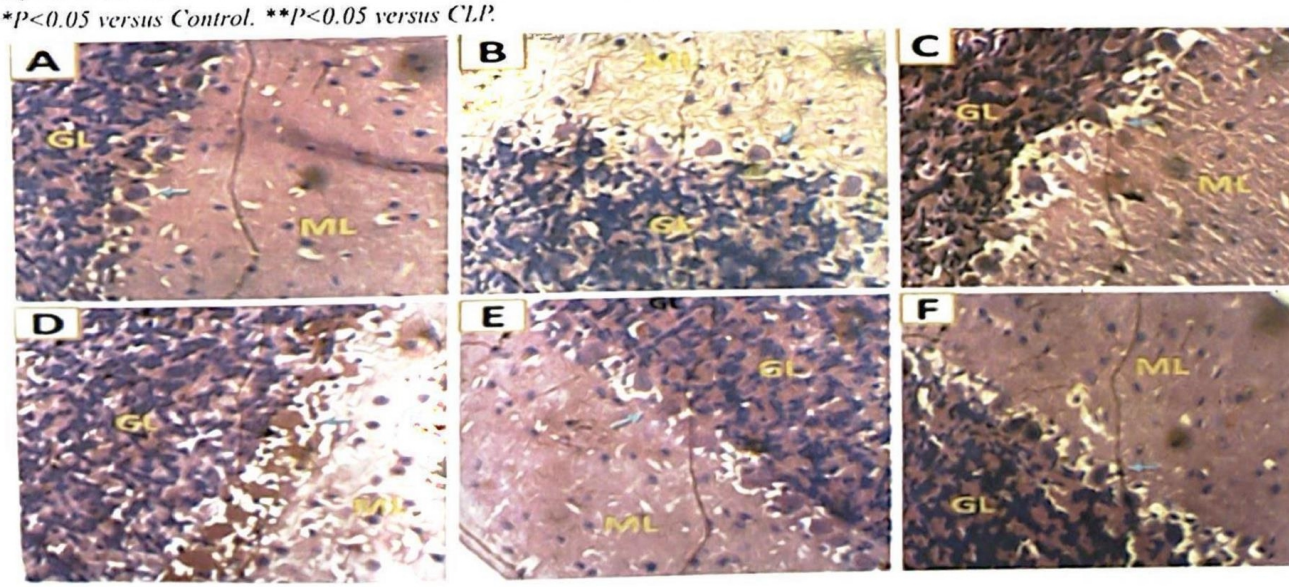

Fig. 3: Representative Photomicrographs of Cerebellar Cortex of Rats. A- Cont group; B- TPP; C- VIT E; D, CLP; E, TPP+CLP; F, VIT E+CLP. Control, Cont; Tomato pomace powder; Vitamin E, VIT E; Tomato pomace powder, TPP; Caecal ligation and perforation, CLP. ML, Molecular layer; PL, Purkinje cell layer; GL-Granular cell layer; blue arrows show Purkinje neurons. Group D Purkinje neurons show eosinophilic nuclei. H&E-stained sections. Final magnifications: 400x

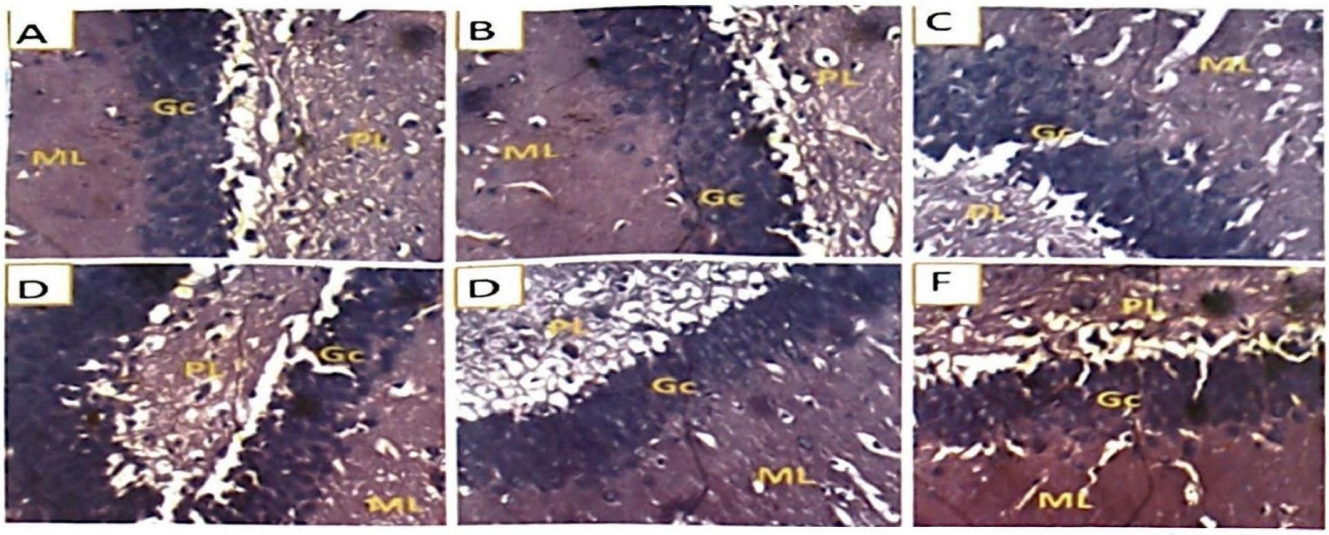

Fig. 4: Representative Photomicrographs of Dentate Gyrus of Rats. A- Cont group; B- TPP; C- VIT E; D, CLP; E, TPP+CLP, F, VIT E+CLP. Control, Cont: Tomato pomace powder; Vitamin E, VIT E; Tomato pomace powder, TPP; Caecal ligation and perforation, CLP; Molecular layer, ML; Polymorphic layer, PL; Granular cell layer, GCL. H&E-stained sections. Final magnifications: 400x

in the level of lipid peroxidation and a reduction in the level of GSH when compared with control group. Similarly, sepsis elicited a significant ($p<0.05$) increase in the activity of CAT relative to control. However, co-treatment of TPP and VIT E with CLP significantly reduced the level of LPO and the activity of CAT when compared with the sepsis (CLA) group as demonstrated in figure 1.

increases in the number of transitions and rearings when compared with the control. The number of grooming was significantly reduced in the CLP when compared with the control. However, co-treatment of CLP with TPP and VIT E both ameliorated these parameters. Negative geotaxis measured by the duration rats needed to re-orientate from the sloping position was prolonged in the CLP-treated groups when compared with the control as shown in figure 2.

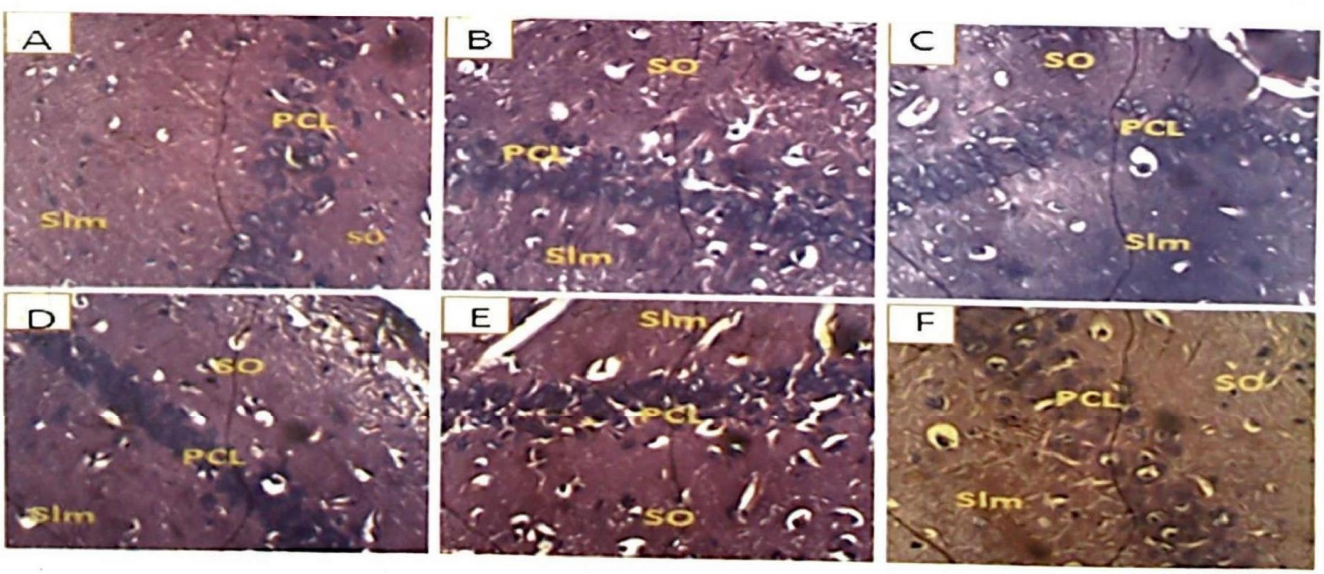

Fig. 5: Representative Photomicrographs of Cornu Ammonis3 (Ca3) Field of Hippocampus of Rats. A- Cont group; B- TPP; C- VIT E; D, CLP; E, TPP+CLP; F, VIT E+CLP. Control, Cont; Tomato pomace powder; Vitamin E, VIT E; Tomato pomace powder, TPP; Caecal ligation and perforation, CLP; stratum oriens, SO; pyramidalis cell layer, PCL; stratum lacunosum moleculare, Slm. H&E-stained sections. Final magnifications: 400x.

Behavioural tests

The data presented in figure 2A and 2B show that rats subjected to CLP were hyperactive at the end of the experiment as shown by the significant ($p<0.05$)

Haematology parameters

There was no significant differences between the means of red blood cell parameters and were therefore not displayed. However, CLP induced the

white blood parameters alterations shown in table 2, evidenced by the significant reduction of the lymphocytes but increased the total white cell count (TWCC) and neutrophils when compared with the control. The significant elevation ($p<0.05$) of the TWCC by 86% and neutrophils by 58% by CLP relative to control was reduced by the TPP+CLP treatment to 11% (TWCC) and 5% (neutrophils). Similarly, VIT E+CLP treatment reduced the effect of CLP to 31% and 16% for the TWCC and neutrophils respectively.

and pyramidal neurons of cornu ammonis3 of hippocampus. However, concurrent treatment with TPP and VIT E significantly reversed some of these alterations.

The mortality rate of 12.5% CLP groups recorded in our experiment fared better than the 33% mortality recorded in the experiments of [26]. This might be due to the prophylactic parenteral intramuscular injection of antibiotics (ceftriaxone and gentamycin) we administered to the rats

Table 2: Effect of treatments on the total white cell count and differential white cell count values.

Groups	TWCC ($x10^3$/iL)	Neutrophils ($x10^3$/iL)	Lymphocytes ($x10^3$/iL)	Eosinophils ($x10^3$/iL)
Cont	5.93±0.30	2.67±0.31	7.03±0.32	1.67±0.58
TPP	6.33±0.41	2.63±0.59	6.97±0.45	1.67 ± 1.16
VIT E	5.80±0.36	2.33±0.15	6.90±0.36	2.00 ±1.00
CLP	11.00±0.45*	4.23 ±0.25*	5.33 ±0.36*	3.00±0.00
TPP+CLP	9.77± 0.15**	2.90±0.30**	6.63±0.25	2.33±0.58
VIT E+CLP	10.50± 0.71	3.55±0.21	6.00±0.14	3.00±0.00

Data are presented as Mean ± S.D. Total white cell count, TWCC; Control, Cont; Tomato pomace powder, TPP; Vitamin E, VIT E; Caecal ligation and perforation, CLP. *P<0.05 versus Control. **P<0.05 versus CLP.

Histological evaluation of the cerebellar cortex, dentate gyrus and cornu ammonis3 (CA3) of hippocampal formation

The histology of the cerebellar cortex showed the normal three cellular layers in the Control, TPP and VIT E groups in addition to the normal large Purkinje neurons with basophilic-staining nuclei. The Purkinje cells of the CLP group (Figure 3D) showed eosinophilic nuclei indicating nuclear karyolysis when compared with the control group as shown in figure 3A. The Purkinje neurons of the TPP+CLP and VIT E+CLP groups are comparable with the Control cerebellum.

Granule neurons of dentate gyrus of CLP group (Figure 4D) and pyramidal neurons of the CA3 of CLP group (Figure 5D) are pyknotic or dark, whereas the neurons of control and other experimental groups exhibit open chromatin.

Discussion

This study investigated the effects of TPP and VIT E on CLP-induced sepsis. The results showed that CLP treatment caused mortality and reduction in the body weight of rats, induced oxidative stress, elicited leukocytosis, altered behavioural parameters and caused degenerative changes in the Purkinje neurons of the cerebellum, granule neurons of dentate gyrus

immediately after CLP induction to control possible surgery-associated infection, whereas they did not administer antibiotics to the operated animals which might explain our report of lower mortality. Our results also compares favourably with the reports of similarly low mortality rate of 15% reported by [13] who administered both antibiotics and fluid resuscitation after CLP. This suggests the importance of antibiotics as prophylaxis in potential septic cases. The observation of clinical features of sepsis in the CLP rats was supported by similar reports of [26]. Although changes in organ weight and weight coefficients induced by chemical substances have been shown to be a reliable and cheap marker of toxicity [27], the body weight reductions in all the CLP groups in this experiment had no effect on the brain weight of the animals suggesting that the toxicity was not quantifiable by brain weight changes.

The sepsis-induced increase in LPO and reduction of glutathione (GSH) levels in our study indicated a state of oxidative stress which was in consonance with earlier reports [1, 14]. Subsequent to activation, infiltrating neutrophils are reported to produce abundant oxygen radicals resulting in lipid and protein oxidation which might contribute to the increased LPO [28]. The observation that SOD enzyme activity was unchanged in this experiment

contradicted published reports [1, 14] who reported significant elevation of SOD enzyme. However, the sepsis significantly increased the activity of the enzyme CAT which breaks down the generated (H_2O_2), in agreement with previous findings [14]. The GSH reduction imposes an oxidative stress that can impair other cellular functions, in particular those regulated by the redox mechanism [29] hence the suggestion that decrease in GSH concentration is one of the most significant alteration in the antioxidant defense [30]. It was however observed that animals concurrently treated with TPP, VIT E and CLP had reductions in LPO level, increased level of GSH and increased SOD and CAT activities. Of note is the elevation of GSH which is important as a constituent of intracellular protective mechanisms against oxidative stress [1], as this might help in improving the oxidant status of the animals.

The host response towards invading pathogens from the introduced sepsis is usually characterized by systemic pro-inflammatory response that is primarily mediated by cytokines, plasma coagulation and complement cascades, and acute phase proteins release, and a cellular component involving leukocytes, especially neutrophils and vascular endothelium [28]. In this experiment, the significant elevation of the total white cell count by eighty-six percent and neutrophils by fifty-eight percent by CLP treatment accentuated the neutrophil response as the main effector cells in acute inflammation in the induced sepsis. This is similar to responses obtained in faecal perforation of peritonitis. Following activation, margination and trans-endothelial emigration from microvessels, infiltrating neutrophils are reported to produce abundant oxygen radicals resulting in lipid and protein oxidation and mitochondrial impairment which might cause further damage to tissues and can induce cell death [28]. However, the observation that co-treatment of TPP and VIT E lowered the total white cell count by eleven and five percent respectively relative to CLP suggested that both items demonstrated the capacity to reduce the toxicity induced by the sepsis.

The histological effect of sepsis demonstrated in the CLP group on the cerebella of the rats include the eosinophilic degeneration of Purkinje cell while the granule cells of the dentate gyrus (DG) and pyramidal neurons of the stratum pyramidalis of the cornu ammonis3 of the hippocampus exhibited pyknotic or dark neurons, evidences of the toxicity of sepsis. Activated neutrophils, which were elevated in this study, are known to generate increased oxygen radicals which might have resulted in oxidative damage. This is because toxicity associated with excessive free radical generation resulting in lipid peroxidation and oxidative damage could induce damages in the membranes of the cell and mitochondria, which might eventually lead to cell apoptosis and necrosis [1, 28, 31, 32]. The prolonged time taken for all CLP rats to re-orientate in the head-up direction suggested a possible vestibular or cerebellar injury which was demonstrated histologically in this experiment. However, the amelioration of the neuronal alterations observed in the TPP+CLP and VIT E+CLP brains demonstrated the capacity of both TPP and VIT E to mitigate the damaging oxidative effects of sepsis. Tomato pomace had earlier been reported to have ameliorated rat cerebellar and hippocampal neuronal damage by other toxicants like cisplatin, mercuric chloride, lead acetate and gamma radiation [6, 17, 20, 33] by reduction of the oxidative damage induced by these substances. In like manner, TPP and VIT E have demonstrated protective effects against CLP in this experiment by attenuating the brain tissue damage and also by decreasing oxidative stress, as confirmed by the histological study and biochemical results.

The consequence of the injury to the Purkinje cells, granule neurons and pyramidal neurons are possible in coordination of skeletal movements secondary to cerebellar injury, disruption of the memory recording (episodic, semantic and spatial), resulting from damage to the trisynaptic pathway of the perforant path which the hippocampal formation mediates [34]. The amelioration by TPP and VIT E of these possible effects of sepsis would prevent these important neurons from injury hence enabling them to perform their functions optimally. The limitation of this study included the lack of immunohistochemical studies to explain the neuro-inflammation and the pattern of neutrophilic reaction that accompanied the sepsis.

Conclusion

The results of this study showed that tomato pomace powder (TPP) and vitamin E demonstrated protective effects in reducing the brain damage caused by CLP-induced sepsis via maintenance of the anti-oxidant status. The gap in knowledge concerning the effects of TPP on sepsis-exposed subjects which our study aimed for has thus been filled and has answered the research question of whether TPP can protect rat brain from induced sepsis. The findings may stimulate further research to harness tomato's potential as a possible adjunct in sepsis management.

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