Human-dose equivalent 5-fluorouracil triggers a pathophysiological cascade of neuroinflammation, cortical remodeling, and behavioural disruption in Wistar rats

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Abstract

5-Fluorouracil (5-FU) is an antimetabolite widely used in cancer chemotherapy. Despite its central role in many anticancer regimens information on its potential effects on the central nervous system (CNS) remains limited. This study investigated the effects of human dose equivalent 5-FU on the cerebral cortex of rats. Fifty male rats were randomly assigned into five groups (n=10). The control group received intraperitoneal (i.p.) saline, while four experimental groups received i.p. 5-FU at 12.5 25 50 or 100 mg/kg body weight. Saline or 5-FU was administered for four consecutive days, followed by alternate-day dosing until day 12. Behavioral assessments were conducted and animals were sacrificed 24 hours after the final test. Cortical tissues were analyzed using biochemical assays histology and immunohistochemistry. 5-FU administration caused dose-dependent decreases in body weight food intake and locomotor activity. Treated rats also showed impaired spatial working memory and reduced time spent in

the open arms of the elevated plus maze. Biochemically 5-FU significantly increased cortical malondialdehyde and tumor necrosis factor-alpha levels while total antioxidant capacity and interleukin-10 levels decreased. Histological analysis revealed progressive disruption of cortical cytoarchitecture with increasing doses. 5-FU induces dose-dependent neurotoxicity in the rat cerebral cortex characterized by behavioral deficits, oxidative stress neuroinflammation and histomorphological alterations. These findings highlight the need to better understand and mitigate CNS toxicity associated with 5-FU–based chemotherapy.

Keywords: Antimetabolite, Anxiety, Memory loss, Neurotoxicity, Neurodegeneration, Neuroinflammation

1. Introduction

Fluoropyrimidines, including floxuridine, capecitabine, and fluorouracil (5-FU), are antimetabolite chemotherapeutic agents that have remained central to the management of solid organ cancers for several decades [1,2]. Both monotherapy and combination regimens incorporating fluoropyrimidines continue to be standard in the treatment of metastatic disease and in adjuvant protocols [2]. Among them, 5-FU, first developed in the 1950s, is one of the most widely prescribed agents for gastrointestinal, breast, and other solid tumors [1].

The cytotoxic effects of 5-FU are mediated primarily through inhibition of thymidylate synthase, which disrupts DNA synthesis and replication, as well as through incorporation of its metabolites into RNA and DNA [1,4–7]. Clinically, intravenous 5-FU is approved for gastric, breast, pancreatic, and colorectal adenocarcinomas, while topical formulations are employed for premalignant and malignant skin conditions such as actinic keratoses and superficial basal cell carcinomas [3]. Despite these therapeutic benefits, 5-FU is associated with systemic toxicities that limit its clinical use.

Beyond peripheral toxicity, chemotherapeutic agents, including 5-FU have been increasingly recognized to exert adverse effects on the central nervous system (CNS). These effects manifest as deficits in attention, memory, and executive function, collectively termed “chemo-brain” or “chemo-fog” [8–11]. While often considered nonspecific consequences of treatment, persistent cognitive impairment suggests that certain agents may induce specific neurotoxic changes [10,11]. Indeed, although less frequently reported, 5-FU has been implicated in neurological syndromes such as multifocal leukoencephalopathy [12] and Wernicke’s encephalopathy [13]. Experimental studies have further demonstrated that 5-FU can induce anxiety-like behavior and impair spatial memory in rodents [14, 15].

Despite its longstanding use and widespread clinical relevance, the neurotoxic profile of 5-FU particularly its impact on cortical integrity and function remains poorly characterized. The cerebral cortex, being integral to higher-order cognitive processes, represents a critical but understudied target of 5-FU toxicity. This study therefore seeks to examine the effects of 5-FU

on the cerebral cortex of rats, with the aim of elucidating neurobehavioral, neurochemical, and structural alterations. Findings from this work may advance understanding of chemotherapy-associated cognitive dysfunction and provide insights into mechanisms underlying 5-FU–induced CNS toxicity.

2. Methods

2.1 Chemicals and drugs

5-fluorouracil (5 Flucel®, 250 mg/5 ml injection), Normal Saline, Assay kits

2.2 Animals

Animals used in this study were sourced from the animal house of the Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria. Rats were housed in wooden cages that measured 20 x 10 x 12 inches in temperature-controlled (22.5°C ±2.5°C) quarters with lights on at 7.00 am. Rats were allowed free access to food and water. All procedures were conducted in accordance with the approved protocols of the Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology and within the provisions for animal care and use according to the European Council Directive, (EU2010/63) on scientific procedures in living animals.

2.3 Diet

All animals were fed commercially-available chow (29% protein, 58% carbohydrate. 11% fat) throughout the study.

2.4 Experimental methodology

Fifty adult male rats weighing 150-170g each were assigned into five groups of ten (n-10) rats each. The groups included vehicle control that were administered intraperitoneal (i.p.) injection of saline at 2 ml/kg, and four other groups administered i.p. 5-Fluoruracil at 12.5, 25, 50 and 100 mg/kg body weight respectively. Saline and 5-Fluorouracil were administered for four consecutive days initially, then on alternate days 6, 8, 10 and 12 [16]. The doses of 5-Fu translate to human equivalent doses that are within the clinical dose range [14]. At the end of the experimental period, animals were exposed to the open-field arena (for the assessment of locomotor, rearing and grooming) behaviours, the elevated plus maze (EPM) for anxiety related behaviours, and the Y-maze for the assessment of spatial working-memory. Twenty-four hours after the last behavioural test, animals were sacrificed. The brains of the rats were excised, sections of the cerebral cortex were homogenised (for the estimation of oxidative stress, antioxidant status, and inflammatory markers); or processed for paraffin-embedding, and stained

with haematoxylin/eosin and cresyl violet for general histological study. Immunohistochemical staining was performed for neuron-specific enolase.

2.5 Estimation of body weight and feed intake

Estimation of body weight of animals in all groups was done weekly, while the estimation of daily feed consumption was made an electronic weighing scale (Mettler Toledo Type BD6000, Switzerland) as previously described [17, 18]. The percentage change in body weight or food intake for each animal was calculated using the following equation, and the results for all animals were computed to find the statistical mean:

2.6 Behavioural tests

2.6.1 Open-field behaviours

The open-field arena in rodents is used to assess central effects of a drug or chemical compound as measured by exploratory activity, rearing and grooming behaviours. Locomotion, rearing and self-grooming behaviours were scored during ten minutes of exposure to the arena. The open-field arena is a rectangular-shaped box with a hard floor that measures 36 x 36 x 26 cm; and is made of white-painted wood. Its floor is divided into 16 equal squares with a permanent red marker. Placement of animals and scoring of the various parameters are as previously described [19, 20].

2.6.2 Y-Maze Test

This Y-maze spatial working-memory test is based on the natural propensity of rodents to explore novel environments. The Y-maze paradigm is made of wood and has three identical arms arranged in the shape of a “Y.” Each arm is usually 15 inches long and 3.5 inches wide, with 3-inch-high walls. Each rat was placed in one of the arms of the Y maze and allowed to move freely within the compartments until its tail completely enters another arm. The sequence of arm entries was recorded as described previously [21, 22].

2.6.3 Elevated plus Maze

The elevated plus maze (EPM) test was developed to examine the possible anxiolytic effects of drugs in rodents. The paradigm is based on the propensity of rodents for closed spaces. It is a cross-shaped platform with two open (50 x 10 x 0 cm) and two closed arms (50 x 10 x 30 cm) placed 50–70 cm above the ground. Placement of animals and scoring of the various parameters are as previously described [23, 24].

2.7 Brain Homogenisation

The cerebral cortex was homogenised (1:10, w/v) in ice-cold phosphate-buffered saline using a Teflon-glass homogeniser as previously described [25]. The homogenate was then centrifuged

for 15 minutes at 5,000 revolutions/minute at 4 °C. The supernatant was immediately used to assay respective markers.

2.8 Biochemical Test

2.8.1 Estimation of Lipid peroxidation

Lipid peroxidation levels were measured as malondialdehyde content as previously described [26, 27] Change in colour was measured using a spectrophotometer at 532 nm.

2.8.2 Antioxidant activity

Total antioxidant capacity was determined using commercially available assay kit. Colour changes were measured as described previously described [28, 29].

2.8.3 Tumour necrosis factor-α, Interleukin (IL) -10 and interleukin-6

Tumour necrosis factor-α and interleukin (IL)-10 were measured using enzyme-linked immunosorbent assay (ELISA) techniques with commercially available kits (Enzo Life Sciences Inc. NY, USA) designed to measure the 'total' (bound and unbound) amount of the respective cytokines as previously described [30. 31]. Interleukin 6 level was assayed using enzyme-linked immunosorbent assay (ELISA) techniques with commercially available kits (Enzo Life Sciences Inc. NY, USA) as previously described [32, 33].

2.9 Protocol for neuron specific enolase (NSE) Immunohistochemistry

Immunohistochemical test for neuron specific enolase (NSE) was performed using the NovocastraTM and NovolinkDM polymer detection system (Leica Biosystems, UK) and appropriate primary monoclonal antibodies as previously described [34, 35].

2.10 Photomicrography

Sections of the cerebral cortex were examined using a Sellon-Olympus trinocular microscope (XSZ-107E, China) with a digital camera (Canon Powershot 2500) attached, and photomicrographs taken.

2.11 Statistical analysis

Data was analysed using Chris Rorden's ezANOVA for windows. One-way ANOVA was used to test the hypothesis. Tukey (HSD) test was used for Posthoc analysis. Results were expressed as mean ± S.E.M, and $p < 0.001$ considered significant.

3.0 Results

3.1 Effect of 5-fluorouracil on body weight and feed intake

Figure 1 shows the effect of 5-fluorouracil on mean relative change in body weight (upper panel) and mean relative change in feed intake (lower panel). Mean relative body weight decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to

control. Mean relative feed intake also decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control.

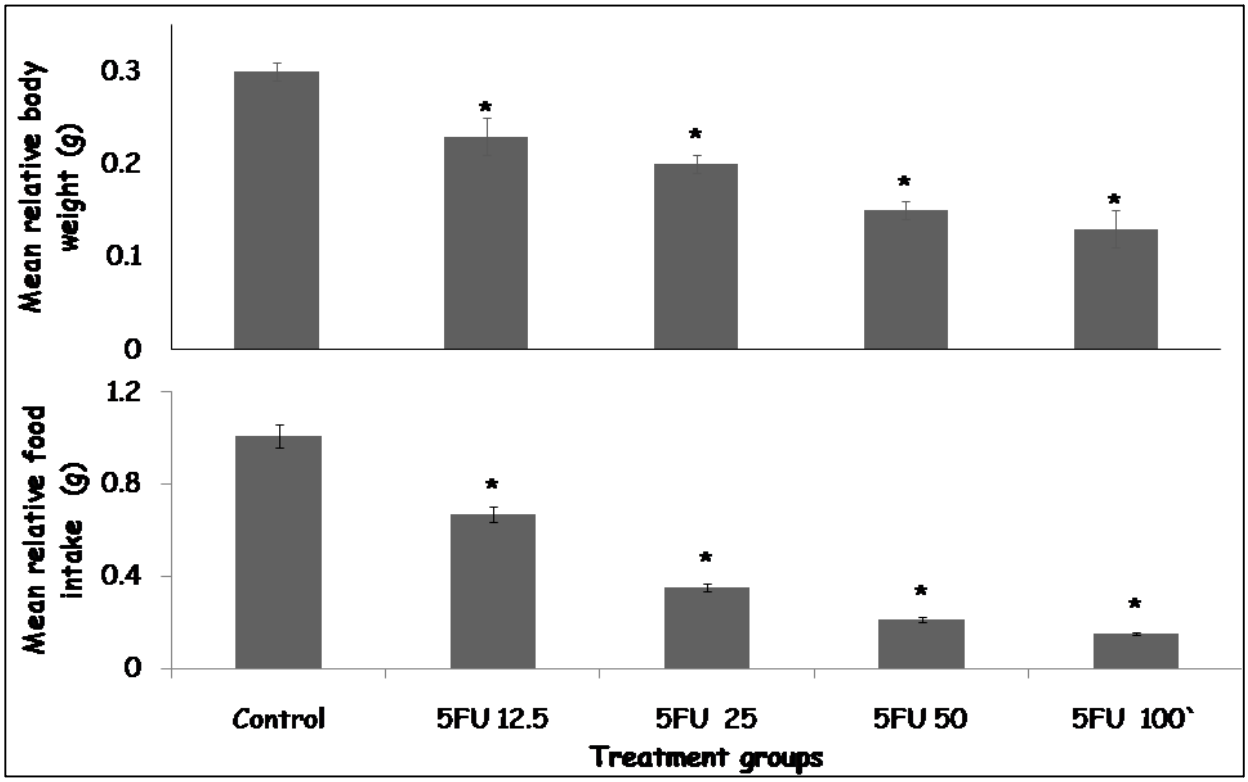

Figure 1: Effect 5-Fluorouracil on mean relative change in body weight (upper panel) and mean relative change in food intake (lower panel) in rats. Each bar represents Mean ± S.E.M, *$p < 0.05$ significant difference from control, number of rats per treatment group =10. 5FU: 5-Fluorouracil

3.2 Effect of 5-fluorouracil on exploratory behaviours in the open-field Arena

Figure 2 shows the effect of 5-fluorouracil on exploratory behaviours in the open field box scored as line crossing (upper panel) and rearing activity (lower panel). Line crossing decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control. Rearing activity decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control.

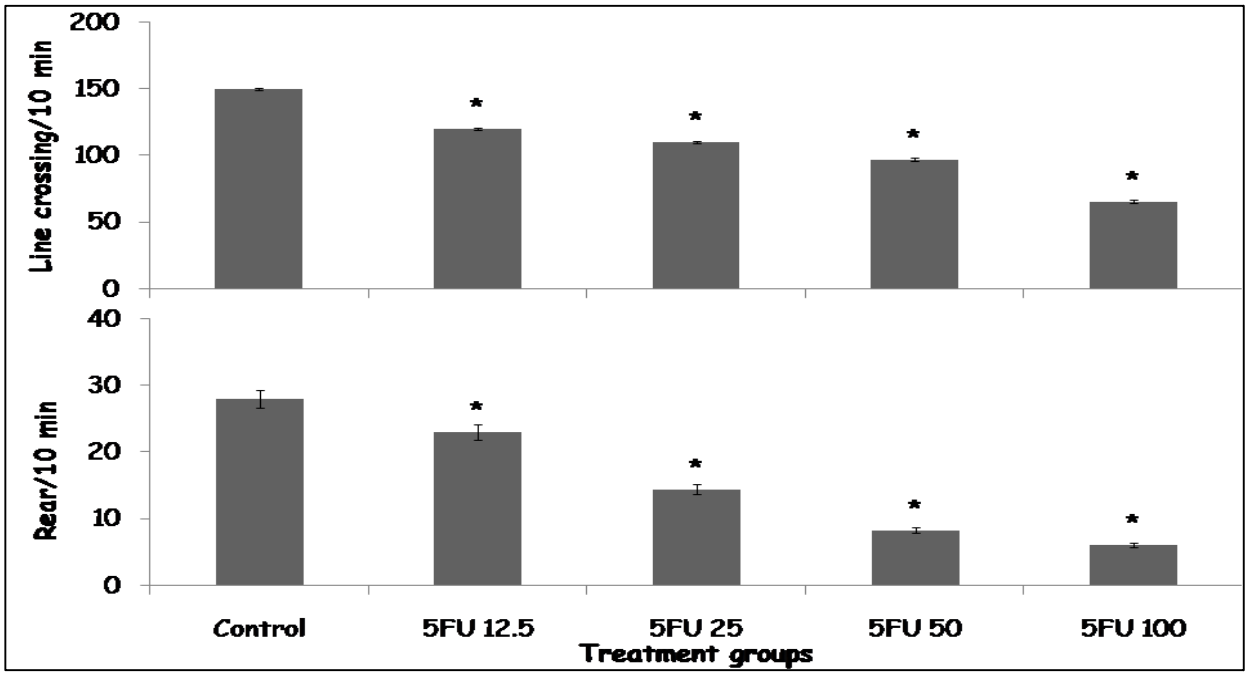

Figure 2: Effect 5-Fluorouracil on line crossing (upper panel) and rearing activity (lower panel) in rats. Each bar represents Mean ± S.E.M, *p < 0.05 significant difference from control, number of rats per treatment group =10. 5FU: 5-Fluorouracil.

3.3 Effect of 5-fluorouracil on Self-grooming in the open-field box

Figure 3 shows the effect of 5-fluorouracil on self-grooming behaviours in the open field box. Self-grooming decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control.

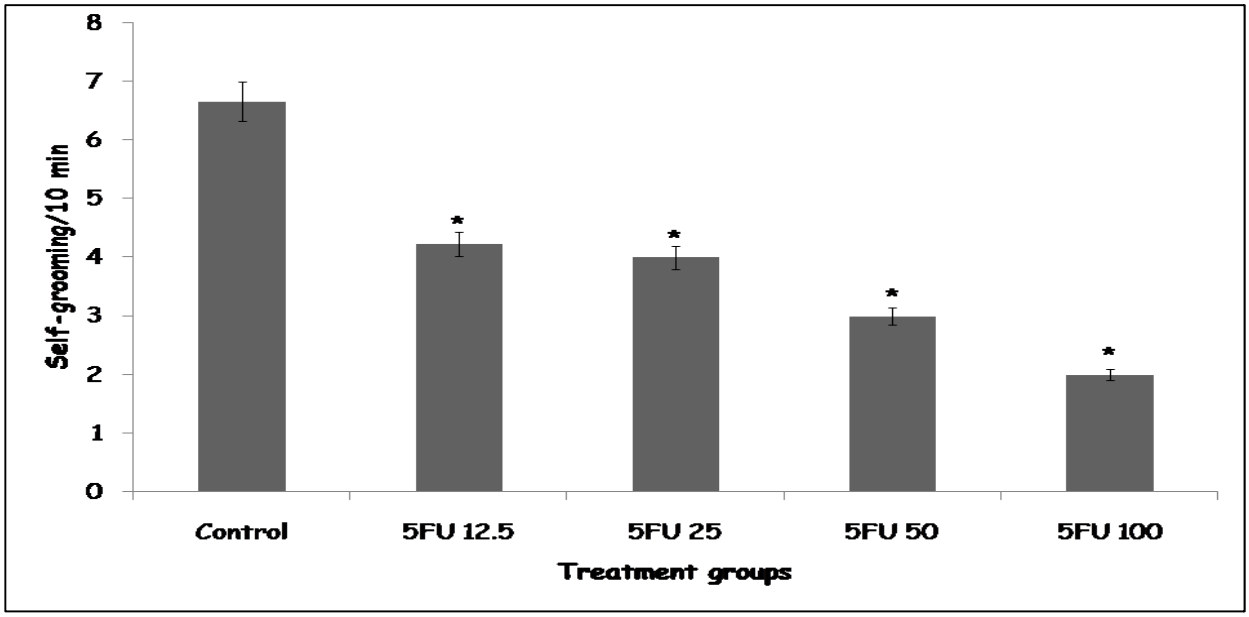

Figure 3: Effect 5-Fluorouracil on self-grooming behaviours in rats. Each bar represents Mean ± S.E.M, *p < 0.05 significant difference from control, number of rats per treatment group =10. 5FU: 5-Fluorouracil.

3.4 Effect of 5-fluorouracil on anxiety-related behaviours in the Elevated plus maze

Figure 4 shows the effect of 5-fluorouracil on anxiety-related behaviours in the elevated plus maze scored as time spent in the open arm (upper panel) and closed arm (lower panel). Time spent in the open arm decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control. Time spent in the closed arm increased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control.

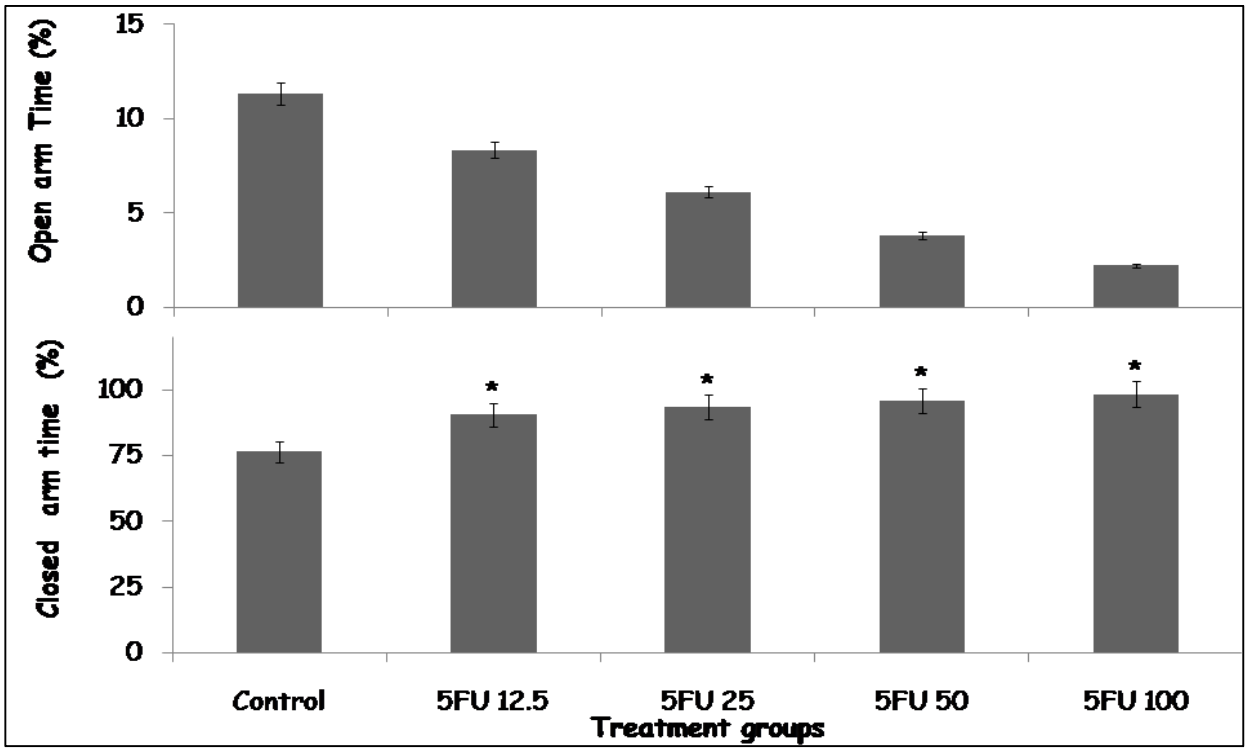

Figure 4: Effect 5-Fluorouracil on time spent in the open arm (upper panel) and closed arm (lower panel) of the elevated plus maze in rats. Each bar represents Mean ± S.E.M, *$p < 0.05$ significant difference from control, number of rats per treatment group =10. 5FU: 5-Fluorouracil.

3.5 Effect of 5-fluorouracil on spatial working memory tasks in the Y and Radial arm maze

Figure 5 shows the effect of 5-fluorouracil on spatial working memory tasks in the Radial arm (upper panel) maze scored as alternation index and Y maze (lower panel) scored as percentage alternation in 5 minutes. Alternation index in the radial arm maze and percentage alternation in the Y maze decreased significantly ($p < 0.001$) with 5-FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control.

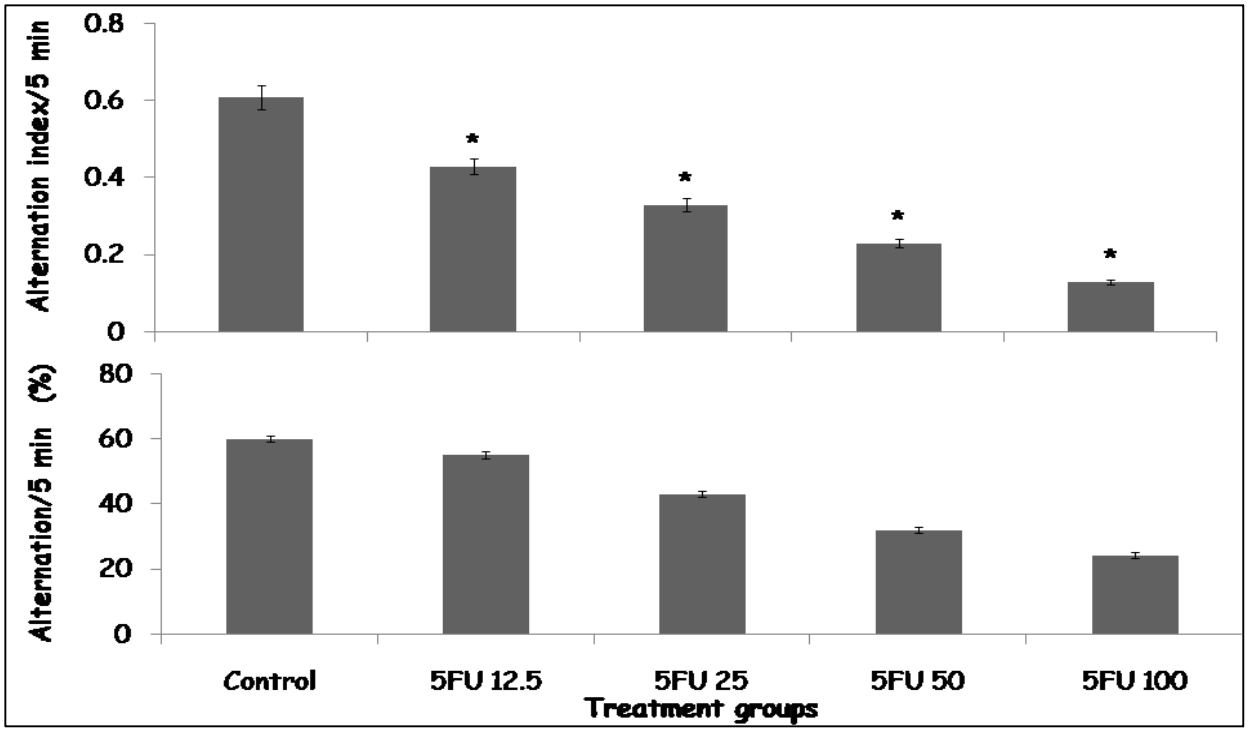

Figure 5: Effect 5-Fluorouracil on alternation index in the radial arm maze (upper panel) and percentage alternation in the Y maze (lower panel). Each bar represents Mean ± S.E.M, *p < 0.05 significant difference from control, number of rats per treatment group =10. 5FU: 5-Fluorouracil.

3.6 Effect of 5-fluorouracil on levels of lipid peroxidation, antioxidant capacity and inflammatory markers in the cerebral cortex

Table 1 shows the effect of 5-fluorouracil on lipid peroxidation, total antioxidant capacity, and inflammatory marker levels in the cerebral cortex. Lipid peroxidation measured as Malondialdehyde (MDA), Tumour necrosis factor (TNF)-α and Interleukin-6 levels increased significantly ($p < 0.001$) with 5FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control. Total antioxidant capacity (TAC) and interleukin 10 levels decreased significantly ($p < 0.001$) with 5FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control

Table 1: Effect of 5-fluorouracil on lipid peroxidation, antioxidant capacity and inflammatory markers

Groups	MDA (µm)	TAC (mM TE)	TNF-α ng/L	IL-10 pg/ml	IL-6 pg/ml
Control	2.23±0.12	12.34 ±0.11	25.23±1.11	30.22±0.10	5.57 ± 1.23
5FU 12.5	7.80 ±0.34*	6.32±0.10*	40.20±1.10*	19.20±0.10*	14.20 ± 1.56*
5FU 25	10.24±0.81*	4.22±0.10*	50.20±0.30*	17.22±0.23*	21.33 ± 2.25*
5FU 50	15.50±0.92*	3.78±0.10*	62.11±1.10*	13.12±0.11*	22.45 ± 2.56*
5FU 100	21.12±0.91*	2.52±0.11*	82.20±1.5*	11.14±0.04*	24.21 ± 2.63*

Mean ± S.E.M, *p < 0.05 significant difference from control, number of rats per treatment group =10. 5FU: 5-Fluorouracil.

3.7 Effect of 5-fluorouracil on neurotransmitter levels in the cerebral cortex

Table 2 shows the effect of 5-fluorouracil on neurotransmitter levels in the cerebral cortex. There was a significant decrease in the levels of brain derived neurotropic factor (BDNF) with 5FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control. Levels of acetylcholine in the cerebral cortex also decreased significantly with 5FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control Dopamine levels decreased significantly with 5FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control. Serotonin levels also decreased significantly 5FU at 12.5, 25, 50 and 100 mg/kg body weight compared to control.

Table 3: Effect of 5-fluorouracil on cerebral cortex neurotransmitter levels

Groups	**BDNF (pmol/mg tissue)**	**Acetylcholine (pmol/mg tissue)**	**Dopamine (pmol/mg tissue)**	**Serotonin (pmol/mg tissue)**
Control	25.11±0.10	3.50±0.01	48.15±2.22	9.20±0.10
5FU 12.5	23.22±0.12	3.42±0.19	40.22±1.11*	7.12±0.10*
5FU 25	15.91±0.10*	2.22±0.11*	35.42±1.21*	6.52±0.14*
5FU 50	13.23±0.20*	1.82±0.10*	33.55±0.22**	6.40±0.31*
5FU 100	9.21±0.20*	1.10±0.10*	20.13±0.23*	4.12±0.11#

Data presented as Mean ± S.E.M, *$p < 0.05$ vs. control, #$p<0.05$ significant difference from CYP, number of rats/group =6, BDNF: Brain derived neurotropic factor.

3.8 Effect of 5-fluorouracil on histomorphology of the cerebral cortex

Figures 6 (A, B, C, D and E) and 7 (A, B, C, D and E) are representative haematoxylin and eosin (H&E) and cresyl fast violet (CFV) stained sections of the rat cerebral cortex respectively. Examination of the H&E-stained slides of the group of rats administered vehicle revealed characteristic well delineated layers of the cerebral cortex. Scattered within the neuropil are multipolar shaped pyramidal cell with large vesicular nucleus, granule neurons with large open-faced nuclei and scanty cytoplasm and small sized neuroglial cells in rats administered vehicle (Figure 6A). The neuropil, which is pink staining in the H & E slides is well preserved in the CFV stained slides (Figure 7A). In the groups administered 5FU at 12.5 and 25 mg/kg body weight, normal shaped pyramidal cell can be observed interspersed between granule cells and neuroglial cells in the H&E Figure 6B and 6C) and CFV (Figure 7B and 7C) stained slides respectively. In the groups administered 5FU at 50 (Figure 6D and 7D) and 100 mg/kg (Figure 6E and 7E), observed were numerous neuroglial cells, degenerating pyramidal and granule cells interspersed between normal pyramidal and granule cells. Evidence of neuronal degeneration and neuronal injury included presence of pale-staining neurons with shrunken nuclei.

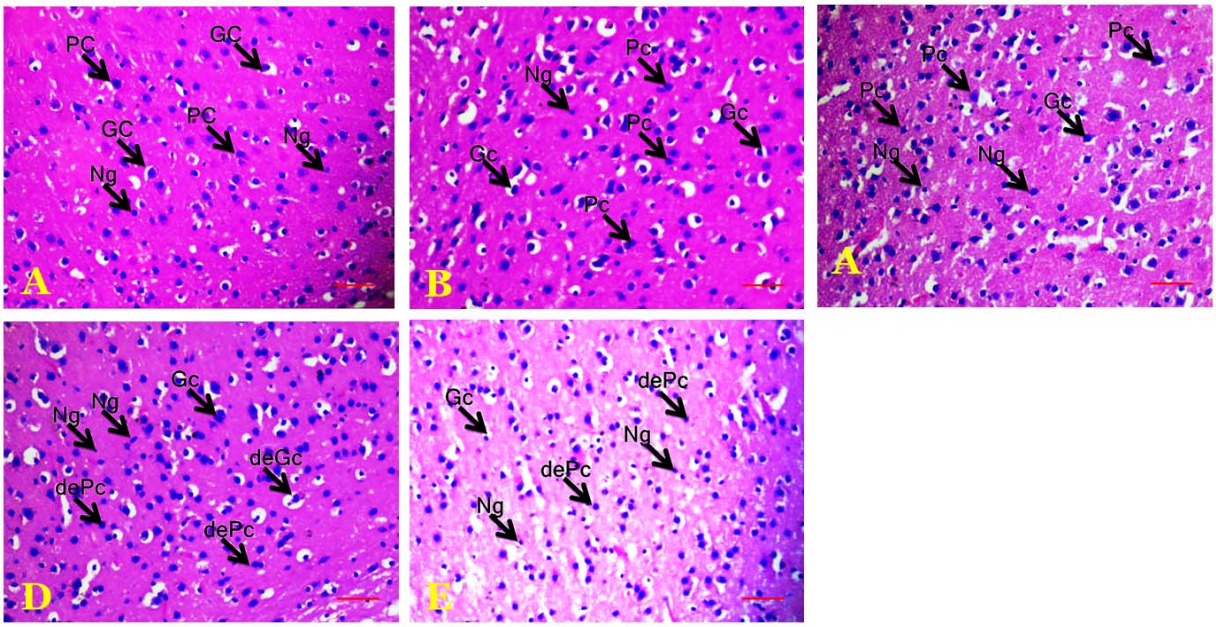

Figure 6 (A, B, C, D and E) are representative haematoxylin and eosin (H&E) stained sections of the rat cerebral cortex. PC: Pyramidal cells, GC: Granule Cells, Ng: Neuroglia, DePc: Degenerating Pyramidal cells. De Gc: Degenerating granule cells. Magnification: X100

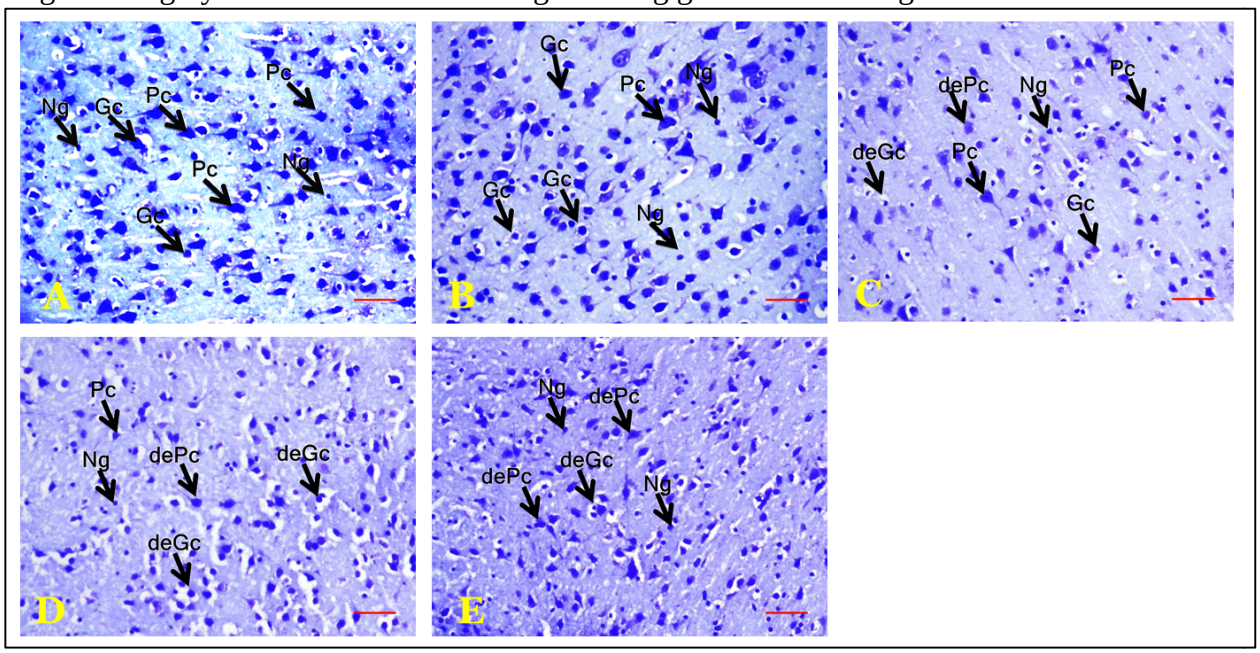

Figure 7 (A, B, C, D and E) are representative cresyl fast violet (CFV) stained sections of the rat cerebral cortex. PC: Pyramidal cells, GC: Granule Cells, Ng: Neuroglia, DePc: Degenerating Pyramidal cells. De Gc: Degenerating granule cells. Magnification: X100

3.9 Effect of 5-fluorouracil on Neuron Specific Enolase (NSE) immunoreactivity in the cerebral cortex

Figure (8 A-E) are representative NSE immunohistochemistry-stained sections of the rat cerebral cortex. Examination revealed the presence of numerous NSE-positive intact neurons with bluish staining nucleus and a brown rim in the groups administered vehicle (Figure 8A) and 5FU at 12.5 (Figure 8B) and 25 mg/kg (Figure 8C). The NSE-positive cells have pyramidal and granule

cell morphologies, whilst the glial cells have no stain, appearing white. In the groups administered 5FU at 50 (Figure 8D) and 100 (Figure 8E) there is a decrease in the number of and intensity of NSE-positive cells. Also, the cell morphologies are altered with numerous degenerating pyramidal and granule cells.

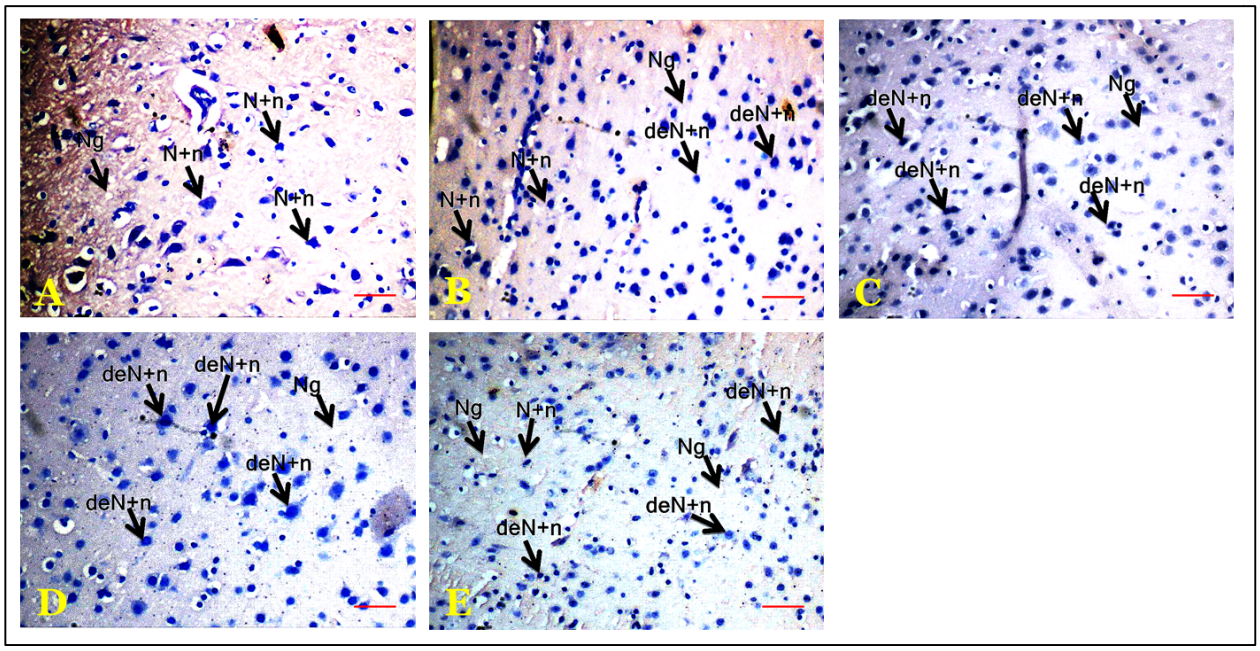

Figure 8 (A-E) are representative NSE immunohistochemistry-stained sections of the rat cerebral cortex. A: control, B5FU at 12.5 mg/kg body weight. C: 5FU at 25mg/kg body weight D: 5FU at 50 mg/kg body weight and E: 5FU at 100 mg/kg body weight. N+N neuron specific enolase reactive neurons, DeN+ n Degenerating Neuron specific enolase reactive neurons. Magx100

Discussion

Advances in our understanding of the adverse nervous system effects of cancer chemotherapy agents would assist in determining regimens that considerably reduce the risk of neurotoxicity. Therefore, the current study evaluated the effects of four doses of 5FU on neurobehavioural, neurochemical, and neuromorphological changes in the cerebral cortex of rats. Results showed that 5-FU administration was associated with decrease in weight gain, feed intake, locomotor activity, rearing activity, self-grooming, spatial working memory, total antioxidant capacity, and interleukin 10 levels. Also observed were increased anxiety, lipid peroxidation and tumor necrosis factor– α and IL-6 levels. A decrease in the cerebral cortex levels of BDNF, dopamine, serotonin and acetylcholine levels were also observed.

In this study, the administration of 5-fluorouracil produced a marked reduction in both body weight and feed intake across all treatment groups when compared with controls. The decline in body weight is in line with a number of previous studies that had reported similar effects [36-38];

with feed intake and weight gain reduction. It also supports the well-documented systemic toxicity of 5-FU, which is known to affect rapidly dividing cells, including those of the gastrointestinal tract resulting commonly in mucositis, nausea, and gastrointestinal irritation reducing appetite and nutrient absorption, thereby contributing to weight loss [36-38]. The significant reduction in feed intake observed in treated animals likely reflects anorexigenic effects and gastrointestinal discomfort induced by 5-FU. Previous studies have reported similar findings, attributing decreased feed consumption to chemotherapy-induced alterations in taste perception, intestinal inflammation, and metabolic stress. Reduced nutrient intake further exacerbates weight loss, creating a vicious cycle of malnutrition and systemic weakening [39]. Also, the decrease in feed intake and resultant weight reduction observed following 5FU has also been linked to reduction in the MRNA encoding peptide YY [38]. Body weight and appetite loss in experimental animals are considered surrogate indicators of a generalized toxicity and declining health status [40]. The consistent decline across all 5-FU doses in this study reinforces the notion that even lower doses of the drug can impair feeding behavior and body mass regulation. These findings are in line with clinical observations in cancer patients receiving 5-FU, many of whom experience cachexia, anorexia, and weight loss during therapy [39].

Exploratory behaviours, measured as line crossing and rearing frequency, were significantly reduced in 5-FU–treated rats when compared with controls, with the decline being consistent across all dose groups. In the open field paradigm, line crossing reflects horizontal locomotor activity, while rearing is an index of vertical exploration and curiosity-driven behaviour [41]. Together, these measures provide insight into the animal's general activity level, motivation, and emotional state. The results of this study are consistent with previous studies which had associated 5-FU administration with a significant reduction in the locomotor/exploration activity of C57Bl/6j mouse [42]. The reduction in line crossing observed across all dose groups in this study would suggests that 5-FU impairs spontaneous locomotor activity, which could be attributable to fatigue, muscle weakness, or central nervous system suppression of motor activity. Similarly, the marked reduction in rearing indicates diminished exploratory drive and environmental engagement, possibly reflecting reduced motivation, increased anxiety or both [41-43]. These behavioural alterations are in keeping with the concept of chemotherapy-induced sickness behaviour, characterized by lethargy, decreased activity, and loss of interest in the environment [43]. Clinically, these findings mirror seemingly patient reports of fatigue, psychomotor slowing, and reduced initiative during and after chemotherapy with fluoropyrimidines. Thus, the decreased line crossing and rearing observed in this study may represent preclinical correlates of chemotherapy-associated behavioural impairments.

Self-grooming is a complex, stereotyped behaviour in rodents that serves both a maintenance function (cleaning and thermoregulation) and an emotional function, as it is modulated by stress, anxiety, and central neurotransmitter activity. In the open field paradigm, changes in grooming frequency are often interpreted as indicators of the animal's emotional reactivity, motivational state, and neurochemical balance [45-47]. In this study, 5-FU administration produced a significant reduction in grooming across all treated groups. This finding suggests that 5-FU exerts a suppressive effect on motivated, self-directed behaviours, which may reflect neurotoxicity, sickness behaviour, and emotional blunting [47]. Reduced grooming is consistent with chemotherapy-induced malaise and anorexia observed in parallel with body weight and feed intake reductions, pointing to a general decline in the animals' well-being. Neurobiologically, grooming behaviour is strongly regulated by dopaminergic and serotonergic systems, particularly in the striatum, prefrontal cortex, and limbic structures [44, 48]. The central inhibitory response observe across all three behaviours could be associated with chemotherapy-induced oxidative stress and an increase in proinflammatory cytokines also observed in this study These changes could impair neural circuits underlying the initiation and sequencing of grooming patterns and behaviours generally. Additionally, inflammatory cytokines such as TNF-α and Il-6 are known to modulate motivational behaviour, and their upregulation may contribute to the observed suppression. A few studies have associated the increase in this cytokine with decreased appetite, fatigue, and other motivational suppression [49, 50].

In the elevated plus maze, 5-FU was associated with a significant reduction in the time spent in the open arms and a corresponding increase in the time spent in the closed arms across all doses. Since the open arms of the maze are perceived as aversive due to their height and exposure, while the closed arms provide relative safety, these results suggest that 5-FU enhances anxiety-like behaviour in rats; with higher doses evoking a higher anxiogenic response. This finding aligns with previous experimental reports demonstrating that systemic 5-FU administration increases indices of anxiety in rodents [42]. In the current study, the increased anxiety response may be related to the changes in the central neurotransmitters that were seen with 5-FU administration.

In this study, behavioural manifestations of the cognitive deficits associated with 5-FU administration were seen as reduced scores in the Y-maze and Radial arm mazes. The alternation index in the radial arm maze and the percentage alternation in the Y maze are established measures of spatial working memory, as they rely on the animal's capacity to remember previously visited arms and avoid revisits within a short period [51, 52]. The observed impairments therefore indicate that 5-FU compromises short-term memory and spatial learning

abilities. A previous report had shown spatial cognitive impairments following 5-FU administration [42]. 5-Fluorouracil administration was observed to evoke spatial cognitive impairments with the attendant morphological manifestation of a decreased neurogenesis within the hippocampus. Some other studies deduced that the cognitive impact of 5-FU can be linked to alteration of cell proliferation and neurogenesis in the dentate gyrus [53]. Also, a reduction in dendritic spines and increased in the inflammatory cytokines in the brain have been implicated [54]. The findings in our current study generally agree with these.

Biochemical analysis of the cerebral cortex revealed a clear shift toward oxidative stress and neuroinflammation following 5-FU treatment. Lipid peroxidation, indexed by malondialdehyde (MDA), increased significantly across all doses, indicating enhanced free radical generation and membrane lipid damage. This was accompanied by significant elevations in pro-inflammatory cytokines including tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), while protective factors, including total antioxidant capacity (TAC) and the anti-inflammatory cytokine interleukin-10 (IL-10), were markedly reduced. This biochemical profile is consistent with the well-recognized role of 5-FU in generating reactive oxygen species (ROS) and disrupting redox homeostasis and is consistent with the results of other studies that had reported the association between 5-FU and the triggering of oxidation stress/lipid peroxidation [55, 56]. Excess ROS can trigger lipid peroxidation, protein oxidation, and DNA damage, thereby contributing to neuronal dysfunction [55, 56]. The parallel increase in pro-inflammatory cytokines suggests that oxidative imbalance may activate glial cells and perpetuate a cycle of neuroinflammation, which is known to impair synaptic plasticity and cognitive processing [57]. The reduction in IL-10, a critical anti-inflammatory cytokine, further underscores the loss of protective regulatory mechanisms, allowing unchecked inflammation to exacerbate neural injury [58, 59].

Administration of 5-FU was associated with a broad suppression of central cortical neurochemical systems critical for cognition, mood regulation, and behavioural control. Brain-derived neurotrophic factor (BDNF) levels decreased significantly across all treatment doses. BDNF plays a central role in neuronal survival, synaptic plasticity, and memory formation; its reduction suggests impaired neuroplastic capacity, which may underlie the observed deficits in spatial working memory and exploratory behaviours. Similarly, cortical acetylcholine levels were markedly reduced. Acetylcholine is a key neurotransmitter for attention, learning, and memory processes, particularly within prefrontal–hippocampal circuits. Its depletion provides a direct mechanistic link to the impaired alternation performance in both the radial arm and Y maze. In parallel, dopamine and serotonin levels were also significantly decreased by 5-FU. Dopamine regulates motivation, reward-driven behaviours, and motor activity, while serotonin

modulates mood, anxiety, and affective states. The reductions in these monoamines align with the suppressed exploration, decreased grooming, and increased anxiety-like behaviour observed in the open field and elevated plus maze. Together, these changes indicate that 5-FU disrupts multiple neurotransmitter systems in a manner consistent with the emergence of apathy, psychomotor slowing, and heightened anxiety.

Histological evaluation of the rat cerebral cortex provided further evidence of 5-FU-induced neurotoxicity in a dose-dependent manner. In control animals, H&E and CFV-stained sections revealed a well-preserved cortical architecture, with clearly delineated cortical layers and normal populations of pyramidal neurons, granule neurons, and glial cells. Neurons displayed large vesicular nuclei and prominent nucleoli, consistent with healthy morphology, while the neuropil was intact and well stained. At low doses of 5-FU (12.5 and 25 mg/kg), cortical sections retained largely normal architecture, with preserved pyramidal and granule cells interspersed with glial cells. This suggests that at these doses, cortical neurons can partly resist or compensate for the cytotoxic effects of 5-FU. However, at higher doses (50 and 100 mg/kg), profound neuronal degeneration and architectural disruption became evident. Pyramidal and granule cells exhibited morphological hallmarks of neuronal injury, including pale-staining cytoplasm, shrunken or pyknotic nuclei, and loss of normal cell shape. These degenerative changes were accompanied by a marked increase in glial cell density, which may reflect gliosis, a reactive process secondary to neuronal injury. The CFV-stained sections further confirmed disruption of Nissl substance, indicative of impaired protein synthesis and neuronal metabolic stress.

Neuron-specific enolase (NSE) immunostaining further confirmed the dose-dependent neurotoxic effects of 5-FU on the cerebral cortex**.** Neuron-specific enolase is a neuronal subtype and one of the five isomers of the glycolytic enzyme enolase [60, 61]. It was initially identified in brain tissue extracts and later in the amine precursor uptake and decarboxylation (APUD) cells and neurons of the neuroendocrine system. In health it is localized within the central and peripheral neurons however it is not present in glial cells [60, 61]. In this study Immunohistochemical examination of the rat cerebral cortex revealed a decrease in the neuron specific enolase positive neurons with increasing doses of 5FU which is associated with neuronal injury. studies have reported.

Conclusion

In conclusion these findings provide evidence that 5-FU induces dose-dependent neurobehavioural, neurochemical and neuronal degeneration in the cerebral cortex, with higher doses producing marked reductions in neuronal viability and functional integrity

References

1. Ghafouri-Fard S, Abak A, Tondro Anamag F, Shoorei H, Fattahi F, Javadinia SA, Basiri A, Taheri M. 5-Fluorouracil: A Narrative Review on the Role of Regulatory Mechanisms in Driving Resistance to This Chemotherapeutic Agent. Front Oncol. 2021 Apr 19;11:658636. doi: 10.3389/fonc.2021.658636.
2. Anaka M, Abdel-Rahman O. Managing 5FU Cardiotoxicity in Colorectal Cancer Treatment. Cancer Manag Res. 2022 Jan 23;14:273-285. doi: 10.2147/CMAR.S273544.
3. Casale J, Patel P. Fluorouracil. [Updated 2024 Feb 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549808/
4. Sethy C, Kundu CN. 5-Fluorouracil (5-FU) resistance and the new strategy to enhance the sensitivity against cancer: Implication of DNA repair inhibition. Biomed Pharmacother. 2021 May;137:111285. doi: 10.1016/j.biopha.2021.111285.
5. Barathan M, Zulpa AK, Ng SL, Lokanathan Y, Ng MH, Law JX. Innovative Strategies to Combat 5-Fluorouracil Resistance in Colorectal Cancer: The Role of Phytochemicals and Extracellular Vesicles. *International Journal of Molecular Sciences*. 2024; 25(13):7470. https://doi.org/10.3390/ijms25137470
6. Mounira Chalabi-Dchar, Tanguy Fenouil, Christelle Machon, Anne Vincent, Frédéric Catez, Virginie Marcel, Hichem C Mertani, Jean-Christophe Saurin, Philippe Bouvet, Jérôme Guitton, Nicole Dalla Venezia, Jean-Jacques Diaz, A novel view on an old drug, 5-fluorouracil: an unexpected RNA modifier with intriguing impact on cancer cell fate, *NAR Cancer*, 2021;3, (3), zcab032, https://doi.org/10.1093/narcan/zcab032
7. Zhang N, Yin Y, Xu SJ, Chen WS. 5-Fluorouracil: mechanisms of resistance and reversal strategies. Molecules. 2008 Aug 5;13(8):1551-69. doi: 10.3390/molecules13081551.
8. Pendergrass JC, Targum SD, Harrison JE. Cognitive Impairment Associated with Cancer: A Brief Review. Innov Clin Neurosci. 2018 Feb 1;15(1-2):36-44.
9. Wefel, J. S., R. Lenzi, et al. (2004). "The cognitive sequelae of standard-dose adjuvant chemotherapy in women with breast carcinoma: results of a prospective, randomized, longitudinal trial." Cancer 100(11): 2292-9.
10. Bender CM, Sereika SM, Berga SL, Vogel VG, Brufsky AM, Paraska KK, Ryan CM. Cognitive impairment associated with adjuvant therapy in breast cancer. Psychooncology. 2006 May;15(5):422-30. doi: 10.1002/pon.964.
11. Cascella M, Di Napoli R, Carbone D, Cuomo GF, Bimonte S, Muzio MR. Chemotherapy-related cognitive impairment: mechanisms, clinical features and research perspectives. Recenti Prog Med. 2018 Nov;109(11):523-530. doi: 10.1701/3031.30289.
12. Ki SS, Jeong JM, Kim SH, Jeong SH, Lee JH, Han CJ, Kim YC, Lee JO, Hong YJ. A case of neurotoxicity following 5-fluorouracil-based chemotherapy. Korean J Intern Med. 2002 Mar;17(1):73-7. doi: 10.3904/kjim.2002.17.1.73.
13. Cho IJ, Chang HJ, Lee KE, Won HS, Choi MY, Nam EM, Mun YC, Lee SN, Seong CM. A Case of Wernicke's Encephalopathy Following Fluorouracil-based Chemotherapy. J Korean Med Sci. 2009 Aug;24(4):747-750. https://doi.org/10.3346/jkms.2009.24.4.747

14. Groves T, Corley C, Byrum SD, Allen AR. The Effects of 5-Fluorouracil/Leucovorin Chemotherapy on Cognitive Function in Male Mice. Front Mol Biosci. 2021 Oct 28;8:762116. doi: 10.3389/fmolb.2021.762116.
15. Abraham, J., M. W. Haut, et al. (2008). "Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging." Clin Breast Cancer 8(1): 88-91.
16. da Silva MC, Fabiano LC, da Costa Salomão KC, de Freitas PLZ, Neves CQ, Borges SC, de Souza Carvalho MdG, Breithaupt-Faloppa AC, de Thomaz AA, dos Santos AM, et al. A Rodent Model of Human-Dose-Equivalent 5-Fluorouracil: Toxicity in the Liver, Kidneys, and Lungs. *Antioxidants*. 2023; 12(5):1005. https://doi.org/10.3390/antiox12051005
17. Olofinnade AT, Onaolapo TM, Oladimeji S, Fatoki AM, Balogun CI, Onaolapo AY, Onaolapo OJ. An Evaluation of the Effects of Pyridoxal Phosphate in Chlorpromazine induced Parkinsonism using Mice. Cent Nerv Syst Agents Med Chem. 2020;20(1):13-25. doi: 10.2174/1871524920666200120142508.
18. Olofinnade AT, Onaolapo OJ, Onaolapo AY Clomiphene/letrozole administration can modulate neurobehavioural and neurochemical indices in hyperandrogenic female rats. Acta Bioscientia 2025:1(3);111-122. https://doi.org/10.71181/actabioscientia12260
19. Onaolapo AY, Ojo FO, Onaolapo OJ. Biflavonoid quercetin protects against cyclophosphamide-induced organ toxicities via modulation of inflammatory cytokines, brain neurotransmitters, and astrocyte immunoreactivity. Food Chem Toxicol. 2023;178:113879. doi: 10.1016/j.fct.2023.113879.
20. Olofinnade AT, Onaolapo AY, Okunola OB, Onaolapo OJ Zinc Gluconate Supplementation Protects against Methotrexate-induced Neurotoxicity in Rats *via* Downregulation of Oxidative Stress, Inflammation and Neuron-specific Enolase Reactivity in Rats Current Biotechnology, Volume 13, Issue 3, Sep 2024, p. 159 - 173
21. Onaolapo OJ, Onaolapo AY, Akanmu MA, Olayiwola G. Caffeine/sleep-deprivation interaction in mice produces complex memory effects. Ann Neurosci. 2015 Jul;22(3):139-49. doi: 10.5214/ans.0972.7531.220304.
22. Onaolapo OJ, Paul TB, Onaolapo AY. Comparative effects of sertraline, haloperidol or olanzapine treatments on ketamine-induced changes in mouse behaviours. Metab Brain Dis. 2017 Oct;32(5):1475-1489. doi: 10.1007/s11011-017-0031-3. Epub 2017 May 15. Erratum in: Metab Brain Dis. 2017 Dec;32(6):2161. doi: 10.1007/s11011-017-0117-y.
23. Onaolapo OJ, Odeniyi AO, Jonathan SO, Samuel MO, Amadiegwu D, Olawale A, Tiamiyu AO, Ojo FO, Yahaya HA, Ayeni OJ, Onaolapo AY. An Investigation of the Anti-Parkinsonism Potential of Co-enzyme Q_{10} and Co-enzyme Q_{10} /Levodopa-carbidopa Combination in Mice. Curr Aging Sci. 2021;14(1):62-75. doi: 10.2174/1874609812666191023153724.
24. Onaolapo OJ, Onaolapo AY, Omololu TA, Oludimu AT, Segun-Busari T, Omoleke T. Exogenous Testosterone, Aging, and Changes in Behavioral Response of Gonadally Intact Male Mice. J Exp Neurosci. 2016 May 2;10:59-70. doi: 10.4137/JEN.S39042
25. Onaolapo AY, Abdusalam SZ, Onaolapo OJ. Silymarin attenuates aspartame-induced variation in mouse behaviour, cerebrocortical morphology and oxidative stress markers. Pathophysiology. 2017 Jun;24(2):51-62. doi: 10.1016/j.pathophys.2017.01.002.
26. Olofinnade AT, Onaolapo AY, Onaolapo OJ, Olowe OA. Hazelnut Modulates Neurobehaviour and Ameliorates Ageing-induced Oxidative Stress, and Caspase-3-Mediated

Apoptosis in Mice. Curr Aging Sci. 2021;14(2):154-162. doi: 10.2174/1874609813666201228112349.

27. Olofinnade AT, Onaolapo TM, Oladimeji S, Fatoki AM, Balogun CI, Onaolapo AY, Onaolapo OJ. An Evaluation of the Effects of Pyridoxal Phosphate in Chlorpromazine induced Parkinsonism using Mice. Cent Nerv Syst Agents Med Chem. 2020;20(1):13-25. doi: 10.2174/1871524920666200120142508
28. Onaderu TA, Onaolapo OJ, Onaolapo AY Post-conceptional melatonin administration mitigates changes in neurobehaviour and cerebral cortex histomorphology in prenatal sodium valproate-exposed rats. Acta Bioscientia **2025**:1(1);38-45 https://doi.org/10.71181/actabioscientia12160
29. Ajao JA, Akinsehinwa AF, Onaolapo OJ, Onaolapo AY. Alcohol Extract of Muira Puama (*Ptychopetalum Olacoides*) ameliorates Aluminium Chloride-induced changes in Behaviour and Cerebral cortex Histomorphology in Wistar Rats. Acta Bioscienctia **2025:1**(1):022-029 https://doi.org/10.71181/actabioscientia12140
30. Onaolapo OJ, Olofinnade AT, Ojo FO, Falade J, Onaolapo AY. Prepubertal Continuous Dietary Folate Fortification Enhances the Brain Function of Adult Mice by Modulating Antioxidant Status, Inflammation, and Brain Neurotransmitter Levels. Antiinflamm Antiallergy Agents Med Chem. 2023;22(3):198-209. doi: 10.2174/0118715230249814230925060325.
31. Olofinnade, Anthony T., Adejoke Yetunde Onaolapo, and Olakunle James Onaolapo. "Graded Effects of Dry-Feed Added Sodium Benzoate/Ascorbic Acid Combination on Neurobehaviour, Oxidative Stress, and Markers of Inflammation in Mice." *Current Bioactive Compounds* 20, no. 1 (2024): 28-37.
32. Lawal EO, Onaolapo OJ, Onaolapo AY Omega-3 fatty acid reverses Ketamine-induced hyperlocomotion, memory deficit and cerebral cortex neuronal injury in rats. Acta Bioscientia 2025:1(1);46-57 https://doi.org/10.71181/actabioscientia12170
33. Famuyide OO, Elijah TK, Onaolapo AY. Assessment of the neurobehavioral and histomorphological effects of quercetin on monosodium glutamate-induced changes in the rat cerebral cortex Acta Bioscienctia 2025:1(1);58-65 https://doi.org/10.71181/actabioscientia12180
34. Onaolapo AY, Onaolapo OJ, Nwoha PU. Methyl aspartylphenylalanine, the pons and cerebellum in mice: An evaluation of motor, morphological, biochemical, immunohistochemical and apoptotic effects. J Chem Neuroanat. 2017 Dec;86:67-77. doi: 10.1016/j.jchemneu.2017.09.001.
35. Onaolapo AY, Onaolapo OJ, Nwoha PU. Aspartame and the hippocampus: Revealing a bi-directional, dose/time-dependent behavioural and morphological shift in mice. Neurobiol Learn Mem. 2017 Mar;139:76-88. doi: 10.1016/j.nlm.2016.12.021.
36. Noguchi C, Matsue K, Nakanishi Y, Asanuma F, Miyata H, Sasaki M. Characterization of 5-Fluorouracil Daily Oral Dosing versus Dietary Restriction on Femoral Growth Plates in Rats. *Toxicologic Pathology*. 2018;47(1):73-81. doi:10.1177/0192623318811836
37. Nakamura K, Tonouchi H, Sasayama A, Yamaji T, Ashida K. Nutritional treatment with an immune-modulating enteral formula alleviates 5-fluorouracil-induced adverse effects in rats. PLoS One. 2019 Nov 26;14(11):e0225389. doi: 10.1371/journal.pone.0225389.

38. Sakai H, Kai Y, Takase K, Sato K, Kimura M, Tabata S, Yaegashi M, Sato F, Yomoto T, Narita M. Role of peptide YY in 5-fluorouracil-induced reduction of dietary intake. Clin Exp Pharmacol Physiol. 2016 Aug;43(8):753-9. doi: 10.1111/1440-1681.12588.
39. Al-Amouri FM, Badrasawi M. Taste alteration and its relationship with nutritional status among cancer patients receiving chemotherapy, cross-sectional study. PLoS One. 2024 May 9;19(5):e0302990. doi: 10.1371/journal.pone.0302990.
40. Dietze S, Lees KR, Fink H, Brosda J, Voigt JP. Food Deprivation, Body Weight Loss and Anxiety-Related Behavior in Rats. Animals (Basel). 2016 Jan 7;6(1):4. doi: 10.3390/ani6010004.
41. Seibenhener ML, Wooten MC. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp. 2015 Feb 6;(96):e52434. doi: 10.3791/52434.
42. Parment R, Dubois M, Desrues L, Mutel A, Dembélé K-P, Belin N, Tron L, Guérin C, Coëffier M, Compère V, et al. A *Panax quinquefolius*-Based Preparation Prevents the Impact of 5-FU on Activity/Exploration Behaviors and Not on Cognitive Functions Mitigating Gut Microbiota and Inflammation in Mice. *Cancers*. 2022; 14(18):4403. https://doi.org/10.3390/cancers14184403
43. VanderVeen BN, Sougiannis AT, Velazquez KT, Carson JA, Fan D and Murphy EA (2020) The Acute Effects of 5 Fluorouracil on Skeletal Muscle Resident and Infiltrating Immune Cells in Mice. Front. Physiol. 11:593468. doi: 10.3389/fphys.2020.593468
44. Kalueff AV, Stewart AM, Song C, Berridge KC, Graybiel AM, Fentress JC. Neurobiology of rodent self-grooming and its value for translational neuroscience. Nat Rev Neurosci. 2016 Jan;17(1):45-59. doi: 10.1038/nrn.2015.8. Epub 2015 Dec 17. Erratum in: Nat Rev Neurosci. 2016 Feb;17(2):118.
45. Onaolapo OJ, Onaolapo AY. Subchronic Oral Bromocriptine Methanesulfonate Enhances Open Field Novelty-Induced Behavior and Spatial Memory in Male Swiss Albino Mice. Neurosci J. 2013;2013:948241. doi: 10.1155/2013/948241.
46. Onaolapo AY, Onaolapo OJ, Ibekwe CB, Hameed SA, Rauf R. "Low-dose L-methionine-associated changes in behavioural indices in young rats." *Int J Neurosci Behav Sci* 2016;4: 11-19
47. Andronikashvili G, Gurashvili T, Dondoladze K, Nikolaishvili M, Makashvili, M. Neurobiology of Supporting Syntactic Chains of Self-Grooming in Rodents and Its Biochecmical Characteristics. *Journal of Biosciences and Medicines*, 2024;**12**,330-352. doi: 10.4236/jbm.2024.124025.
48. Seo D, Patrick CJ, Kennealy PJ. Role of Serotonin and Dopamine System Interactions in the Neurobiology of Impulsive Aggression and its Comorbidity with other Clinical Disorders. Aggress Violent Behav. 2008;13(5):383-395. doi: 10.1016/j.avb.2008.06.003.
49. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. Spring; 2007;45(2):27-37. doi: 10.1097/AIA.0b013e318034194e.
50. Coperchini F, Greco A, Teliti M, Croce L, Chytiris S, Magri F, Gaetano C, Rotondi M. Inflamm-ageing: How cytokines and nutrition shape the trajectory of ageing. Cytokine Growth Factor Rev. 2025 Apr;82:31-42. doi: 10.1016/j.cytogfr.2024.08.004.
51. Onaolapo OJ, Onaolapo AY, Akanmu MA, Olayiwola G. Caffeine/sleep-deprivation interaction in mice produces complex memory effects. Ann Neurosci. 2015;22(3):139-49. doi: 10.5214/ans.0972.7531.220304.

52. Onaolapo AY, Onaolapo OJ. Caffeine's influence on object recognition and working-memory in prepubertal mice and its modulation by gender. Pathophysiology. 2015;22(4):223-30. doi: 10.1016/j.pathophys.2015.09.001.
53. John, J.; Kinra, M.; Mudgal, J.; Viswanatha, G.L.; Nandakumar, K. Animal models of chemotherapy-induced cognitive decline in preclinical drug development. *Psychopharmacology* **2021**;238:3025–3053.
54. Groves TR, Farris R, Anderson JE, Alexander TC, Kiffer F, Carter G, Wang J, Boerma M, Allen AR. 5-Fluorouracil chemotherapy upregulates cytokines and alters hippocampal dendritic complexity in aged mice. *Behav. Brain Res.* **2017**;316:215–224.
55. Al-Asmari AK, Khan AQ, Al-Qasim AM, Al-Yousef Y. Ascorbic acid attenuates antineoplastic drug 5-fluorouracil induced gastrointestinal toxicity in rats by modulating the expression of inflammatory mediators. Toxicol Rep. 2015;2:908-916. doi: 10.1016/j.toxrep.2015.06.006.
56. Lamberti M, Porto S., Zappavigna, S, Stiuso, P., Tirino V, Desiderio, V. Caraglia, M. (2015). Levofolene modulates apoptosis induced by 5-fluorouracil through autophagy inhibition: Clinical and occupational implications. International Journal of Oncology, 46, 1893-1900. https://doi.org/10.3892/ijo.2015.2904)
57. Sălcudean, Andreea, Cristina-Raluca Bodo, Ramona-Amina Popovici, Maria-Melania Cozma, Mariana Păcurar, Ramona-Elena Crăciun, Andrada-Ioana Crisan, Virgil-Radu Enatescu, Ileana Marinescu, Dora-Mihaela Cimpian, and et al. 2025. "Neuroinflammation—A Crucial Factor in the Pathophysiology of Depression—A Comprehensive Review" *Biomolecules* 15, no. 4: 502. https://doi.org/10.339
58. Maziz, Mohammad Nazmul Hasan, Srikumar Chakravarthi, Thidar Aung, Phone Myint Htoo, Wana Hla Shwe, Sergey Gupalo, Manglesh Waran Udayah, Hardev Singh, Mohammed Shahjahan Kabir, Rajesh Thangarajan, and et al. 2025. "Microglia-Mediated Neuroinflammation Through Phosphatidylinositol 3-Kinase Signaling Causes Cognitive Dysfunction" *International Journal of Molecular Sciences* 26, no. 15: 7212. https://doi.org/10.3390/ijms261572120/biom15040502
59. Fakorede S, Lateef OM, Garuba WA, Akosile PO, Okon DA, Aborode AT. Dual impact of neuroinflammation on cognitive and motor impairments in Alzheimer's disease. J Alzheimers Dis Rep. 2025 Jun 2;9:25424823251341870. doi: 10.1177/25424823251341870.
60. Isgrò, M.A., Bottoni, P., Scatena, R. (2015). Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. In: Scatena, R. (eds) Advances in Cancer Biomarkers. Advances in Experimental Medicine and Biology, vol 867. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-7215-0_9
61. Helbok, R., Meyfroidt, G. & Beer, R. Intracranial pressure thresholds in severe traumatic brain injury: Con. *Intensive Care Med* **44**, 1318–1320 (2018). https://doi.org/10.1007/s00134-018-5249-y