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Neuroprotective effect of herbal extracts inhibiting soluble Epoxide Hydrolase (sEH) and Cyclooxygenase (COX) against chemotherapy-induced cognitive impairment in mice

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Abstract

Chemotherapy-induced cognitive impairment (CICI) is a novel clinical condition characterized by memory, learning, and motor function deficits. Oxidative stress and inflammation are potential factors contributing to chemotherapy's adverse effects on the brain. Inhibition of soluble epoxide hydrolase (sEH) has been proven effective and beneficial in neuroinflammation and reversal of memory impairment. The research aims to evaluate the memory protective effect of sEH inhibitor and dual inhibitor of sEH and COX and compare its impact with herbal extracts with known nootropic activity in an animal model of CICI. *In vitro* sEH, the inhibitory activity of hydroalcoholic extracts of *Sizygium aromaticum*, *Nigella sativa*, and *Mesua ferrea* was tested on murine and human sEH enzyme as per the protocol, and IC_{50} was determined. Cyclophosphamide (50 mg/kg), methotrexate (5 mg/kg), and fluorouracil (5 mg/kg) combination (CMF) were administered intraperitoneally to induce CICI. The known herbal sEH inhibitor, *Lepidium meyenii* and the dual inhibitor of COX and sEH (PTUPB) were tested for their protective effect in the CICI model. The herbal formulation with known nootropic activity viz *Bacopa monnieri* and commercial formulation (Mentat) were also used to compare the efficacy in the CICI model. Behavioral parameter such cognitive function was assessed by Morris Water Maze besides investigating oxidative stress (GSH and LPO) and inflammatory (TNFα, IL-6, BDNF and COX-2)

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Author contributions

Rachana Kulkarni: execution of study, Richa Mehta: original draft preparation; Sumanta Kumar Goswami: concept, study design, and manuscript review; Bruce Hammock: concept, study design, manuscript review; Christophe Morisseau: in vitro screening of herbal extracts; Sung Hee Hwang: synthesis dual sEH inhibitor, reviewing and editing; Onkarmurthy Mallappa: Execution of study, Data acquisition and Data analysis; Mohammed Azeemuddin Mukhram: Execution of study, Data acquisition and Data analysis, Mohamed Rafiq; Supervision and design of the study and S. N Manjula; conceptualization, study design, manuscript review and supervision.

Declaration of competing interest

Bruce D. Hammock, SungHee Hwang and Christophe Morisseau have patents regarding use of sEH inhibitors.

markers in the brain. CMF induced CICI, which was associated with increased oxidative stress and inflammation in the brain. However, treatment with PTUPB or herbal extracts inhibiting sEH preserved spatial memory via ameliorating oxidative stress and inflammation. *S. aromaticum* and *N. sativa* inhibited COX2, but *M. Ferrea* did not affect COX2 activity. *Lepidium meyenii* was the least effective, and mentat showed superior activity over *Bacopa monnieri* in preserving memory. Compared to untreated animals, the mice treated with PTUPB or hydroalcoholic extracts showed a discernible improvement in cognitive function in CICI.

Keywords

Chemotherapy-induced cognitive impairments; sEH; *Sizygium aromaticum* ; *Nigella sativa* ; *Mesua ferrea* ; *Lepidium meyenii*

1. Introduction

Cancer is a critical global health issue; chemotherapy extends cancer patients' lives. As more people survive cancer, there is a growing need for more research on the long-term effects of chemotherapy and other medical procedures and how they affect patients' quality of life. Chemotherapy-induced cognitive impairment (CICI), also referred to as "chemo-brain" or "brain fog", can be a significant obstacle that survivors must overcome after treatment. Cognitive impairments caused by chemotherapy include temporary or permanent difficulties with learning, memory, attention, processing speed, and executive function [1,2]. In addition to surgery and radiation therapy, chemotherapy is still considered a first-line treatment for both primary and secondary malignancies. Nonetheless, neurotoxicity resulting to memory impairment is a common adverse effect of cancer chemotherapy [3]. Memory, learning, attention, processing speed, and visuospatial skills negatively impact patients with CICI [4]. Evidence from animal studies suggests that changes in oxidative stress, long-term potentiation, plasma membrane pump activity in the CNS, a lack of blood flow, weakened immune system, and apolipoprotein E gene expression may have a role in causing chemobrain [5]. An estimated 10 million cancer survivors in the US experience cognitive impairment due to chemotherapy. According to data from all cancer cases, about 30% of survivors experience cognitive impairment before therapy, more than 70% during treatment, and more than 30% may have CICI for up to 20 years after treatment [6]. Memory loss, trouble retaining new information, difficulty switching gears, an inability to think critically and an inability to establish meaningful associations are all symptoms of chemobrain [7]. Epoxide hydrolases are enzymes that catalyse the conversion of epoxides 1,2 or dihydrodiols [8]. Epoxyeicosatrienoic acid (EETs) are produced when soluble epoxide hydrolase (sEH) is inhibited, leading to a class of EpFA with anti-inflammatory properties [9]. These EpFA alleviate inflammation and cellular ageing by rebalancing cellular components via the endoplasmic reticulum stress pathway [10]. EpFA have neuroprotective effects contributing to the reversal of cognitive impairment [11]. Inhibition of the COX, including COX-2 enzyme reduces inflammation [12] and angiogenesis associated with cancer [13], but may impair memory [14]. Earlier studies have reported that soluble sEHI preserve memory in diabetes-induced memory impairment [11,15] and a dual inhibitor of sEH and the cyclooxygenase enzyme, COX-2, potentiates the anti-tumour activity of anti-cancer

medicines by inhibiting angiogenesis and tumour growth [16] and anti-tumour activity of anti-cancer medicines [17]. Numerous studies have shown that inhibiting sEH reduces the side effects of NSAIDs and COXIBs. The combination is dramatically synergistic in reducing inflammation and pain as well as the side effects of chemotherapeutic agents [18]. We hypothesize that inhibiting sEH would protect memory in CICI or be considered a potential pathway in treating CICI [19].

2. Materials and Methods

2.1. Chemicals

Cyclophosphamide, methotrexate, and 5-fluorouracil combination is used to treat cancers [19]. CMF-induced cognitive impairment in mice was used as a model to study effect of treatments, including 4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl) benzene sulfonamide (PTUPB) on CICI, chronic neuro-inflammation and oxidative [20,21]. CMF treatment regimen includes cyclophosphamide- 50 mg/kg, methotrexate- 5mg/kg, and 5-fluorouracil-50 mg/kg and was administered intraperitoneally [22] to induce chemo brain. PTUPB was prepared at the laboratory of Professor Bruce Hammock, University of California, Davis [23].

2.2. Herbal extracts and formulation.

The hydroalcoholic extracts of *Syzygium aromaticum* buds, *Nigella sativa* seeds and *Mesua ferrea* seeds were chosen for the study based on their reported neuroprotective and memory enhancement activities [24,25,26]. *Syzygium aromaticum*, *Nigella sativa* and *Mesua ferrea* are called Lavanga, Kalonji and Nagakesar, respectively, were procured from the JSS Ayurveda College, Mysuru, Karnataka, India. *Lepidium meyenii*, known to inhibit she [27] was prepared at Hammock laboratory and administered to compare its effect with the other herbal extracts in CICI. Similarly, *Bacopa monnieri* [28] and herbal formulation, Mentat [29] which are known to improve memory were collected from Himalaya Wellness Company, Makali, Bengaluru, and were used to compare the effectiveness of test herbal extracts in CICI

2.3. Animals

Eight week male Swiss albino mice weighing 30-40g were procured from the animal house of The Himalaya Wellness Company. The animals were kept in an environment with a constant light/dark cycle (12h each), temperature (24 ± 2 °C), and humidity (about 60%). Before experimentation, they were given seven days for acclimatization to the laboratory conditions. The Himalaya Wellness Company's study (Protocol no. 206/20) was approved by the institution's animal ethics committee, and experiment was conducted in accordance with the Committee for The Purpose of Control and Supervision of Experiments on Animals guidelines for laboratory animals and ethics, Department of animal welfare, Government of India.

2.4. Induction of chemobrain by CMF [30]

The intraperitoneal dose of CMF was 10 ml/kg and was formulated in saline [31,32]. 5-fluorouracil and methotrexate were administered with a gap of 4h between both the

treatments to all groups except standard control. Cyclophosphamide was administered to all treatment groups after two days except for normal control. Animals were subjected to the Morris Water Maze experiment after the acclimatization period. The randomization was based on a five-day acquisition trial followed by a one-day retention trial. After randomization, the animals were divided into nine groups of seven each (see Table 1).

2.5. Preparation Of Extract

The crude drugs viz. *Syzygium aromaticum*, *Nigella sativa* and *Mesua ferrea* were procured from the JSS Ayurveda College, Mysuru, Karnataka, India. The drugs were coarsely powdered and around 100g of seeds of Nagakesar and kalonji and buds of clove were macerated with 500 mL of absolute ethanol for 4h. The product obtained was refluxed for 90min at 90°C then filtered using Whatman filter paper. The filtered solution was reduced in volume with a rotary evaporator, and the volatile gas was discarded. The resulting viscous oil end product was transferred to a Petri plate and dried on a Ringer water bath at 100°C. Subsequently, the semi-dried products were dried in a vacuum chamber at 450mmHg of pressure at room temperature. The dried extracts were weighed and transferred to an air-tight container. The % yield of extract calculated was approximately 25% for both Lavanga and Kalonji and 15% for Nagakesar.

2.6. Procedure For sEH inhibition assay

The *in vitro* sEH assay to evaluate the inhibitory potential of herbal extracts was performed as per Jones *et al.* protocol [32]. Activities of human and murine sEH were studied using [^{3}H]*t*-DPPO as a substrate. The efficacy of the hydroalcoholic extracts of *the S. aromaticum, N. sativa and M. ferrea* to inhibit hydrolysis of the substrate was evaluated at multiple concentrations and IC_{50} were determined.

2.7. Procedure to evaluate memory function

The mice were trained in the Morris water maze for five days after acclimatization [33]. The retention time, swim latency and escape latency were recorded. Animals were randomized into nine groups based on their performance in the Morris water maze. The animals which could not be trained in the Morris water maze were excluded from the study. The selected trained animals were grouped as given above and treated as per schedule for 21 days. A behavioral assessment was carried out on the 22nd day after the end of the dosing schedule [34]. Under anesthesia, blood samples were collected on the 22nd day post-dosing, and immediately the animals were euthanized using a high dose of anesthesia, and brain samples were collected. Samples of the brains were rapidly isolated, and stored at −80 °C until further estimations were performed [35]. (Fig. 1)

2.8. Body weight measurement

The body weights of the animals in each group were monitored every day from week 1 to week 4 as chemotherapy leads to weight loss and weakness.

2.9. Hematological parameters

An automatic blood cell counter (PCE210 Fully Automatic Blood Cell Counter, ERMA Inc., Tokyo, Japan [36] was used to assess and estimate hematological parameters in order to examine therapy efficacy. White blood cell count (103 /µl), numerous monocytes (103 /µl), lymphocytes (103 /µl), granulocytes (103 /µl), and platelet count (103 /µl) were also measured.

2.10. Effect of treatment on brain biochemistry

Blood samples were collected before sacrificing the animals by cervical dislocation. Scissors were used to separate the head, and the scalpel was used to envision the skull. In the midline of the skull, an anterior post-cut was made, followed by a mediolateral cut above the eyes. Finally, the brain was isolated with the help of a spatula by cutting the meninges and cranial nerve with utmost care [37]. Right after removal, the brain was washed with saline and frozen in phosphate buffer (pH 7) at −20 degrees Celsius for later analysis.

2.11. Effect of treatment on brain oxidative stress

Glutathione(GSH) and lipid peroxidase (LPO) levels in brain tissues were measured to assess the impact of therapy on oxidative stress.

2.11.1. GSH—500µl of tissue homogenate and TCA reagent were spun at 5000 revolutions per minute for 10min, For 10min, 50µl both supernatant and DTNB solution were incubated in an ELISA plate. The absorbance was measured at 412 nm. The blank control was composed of a 50:50 mixture of distilled water and DTNB solution. GSH concentration was calculated using a standard curve and reported in units of µmoles/mg of total protein. Multiple tests were performed.

2.11.2. LPO—100 µl of tissue homogenates were prepared, and then 500 µl of TBA-TCA-HCl reagent was added. For 10min, the solution was heated to 90 degrees Celsius, or until it turned pink. In order to separate the supernatant, we centrifuged the sample at 2000 rpm for 5 min. The absorbance at 525nm was measured after collecting 300 µl of supernatant. 300 µl of TBA-TCA-HCl reagent were used as a blank. LPO concentration was calculated by comparing the measured value to a standard curve and is reported in terms of nanomoles per milligram of total protein. Multiple tests were performed.

2.12. Effect of treatment on brain inflammation—Following the manufacturer's instructions, we measured the concentrations of TNF-, IL-6, and BDNF in the brain tissue samples using the mouse TNF-α GENLISATM ELISA kit, the mouse IL-6 Development GenBulkTM ELISA kit, and the mouse BDNF GENLISATM ELISA kit, respectively.

2.13. Statistical analysis—The data was analyzed by one-way ANOVA followed by Dunnett’s post hoc test and presented as Mean ± SEM of 3-7 observations.

3. Results

3.1 Evaluated herbal extracts inhibit sEH

The hydroalcoholic extracts showed promising inhibitory effects on human and murine sEH enzyme in the *invitro* assay. The hydroalcoholic extracts of *Nigella sativa*, *Mesua ferrea* and *Syzygium aromaticum* inhibited half of the human sEH enzyme activity at 5.23 μg/mL, 11.57 μg/mL and 9.22 μg/mL, respectively. Similarly, the hydroalcoholic extracts of *Nigella sativa*, *Mesua ferrea* and *Syzygium aromaticum* inhibited half of the murine sEH enzyme activity at 19.14 μg/mL, 18.58 μg/mL, and 29.32 g/mL, respectively.

3.2. Inhibition of sEH protects against loss in body weight (Figure 2)

3.3. Inhibition of sEH protects cognition in CICI (Figure 3)

3.4. Inhibition of sEH protects against CICD-induced hematological alteration (Figure 4)

3.11. Inhibition of sEH maintains brain homeostasis (Figure 5)

4. Discussion

Mice in this study were subjected to a CMF chemotherapeutic challenge. The ‘chemo-brain’ typically manifests as cognitive dysfunction, increased proinflammatory cytokines, increased neuronal cell death, and a substantial decrease in neurogenesis. These pathological conditions were achieved by CMF treatment three times in 21-day cycle, proving the efficacy of the model. PTUPB, Mentat, and extracts of Mesua ferrea, and Syzygium aromaticum protected against CMF-induced weight loss, cognitive impairment, hematological abnormalities, oxidative stress, and inflammation.

In behaviour study by Morris water maze CMF- treated animals took longer time to reach the platform compared to the normal control suggesting the impairment of recalling power. The mice that were administered PTUPB, herbal extracts, or Mentat showed better spatial memory compared to CMF alone treated disease control group. The extracts' potential protective effect on memory function may be attributable, in part, to their ability to block sEH, as these extracts (Nigella sativa, Mesua ferrea, and Syzygium aromaticum) showed in-vitro sEH inhibitory activity in the present study. Nigella sativa and Syzygium aromaticum hydroalcoholic extracts reduced inflammatory cytokine (COX-2) production in the brains of CMF-treated mice. Inhibition of cyclooxygenase-2 (COX-2) activity has been linked to impaired memory performance following exercise, although the impact of COX-2 inhibition on memory function in CICI is unknown. Inhibition of COX-2 by Mentat, S. aromaticum, and N. sativa did not diminish their protective impact on memory in CICI, as shown in our trial. To establish a link between the behavioral data and biochemical and hematologic markers, a battery of assays and estimations was carried out.

Because chemotherapy kills rapidly dividing cells like blood cells, anemia is indicated by a drop in RBC and hemoglobin levels in CMF-treated mice. In comparison to the CMF group, the standard and test groups had significantly higher levels of red blood cells and hemoglobin. The immune response appeared to normalize in the therapy groups despite the lack of myelosuppression. The increased RBC and hemoglobin levels observed in the therapy groups may have been caused, in part, by a decrease in oxidative stress. After CMF

therapy, platelet counts rose significantly, a sign of acute immunological responses. A rise in platelet levels by CMF may be to blame for the death of cells, including neurons, which leads to memory impairment because of apoptosis [39]. Our results on platelet counts in the CMF group also confirm those of a prior study by Mills et al., which found an uptick in inflammatory markers such vascular endothelial and platelet activation after chemotherapy [40]. PTUPB, herbal extracts, and Mentat may have a protective effect because they reduce the platelet increase caused by CMF. Treatments for CMF may be protective since they reduce inflammation, just as platelets can cause it [41]. CMF also increases brain oxidative stress, whereas PTUPB and herbal medicines reduce this stress, suggesting they have a protective effect.

Antioxidant activity in the brain was evaluated by measuring levels of GSH and LPO. Treatment with CMF significantly reduced GSH levels but increased LPO levels in the brain in comparison to a conventional control, indicating oxidative stress generated by chemotherapy. Treatments with PTUPB and herbal items (with the exception of Mesua ferrea) reduced the level of LPO in CMF-treated rats, indicating that they had an antioxidant effect. However, these treatments had no effect on CMF-induced alterations in GSH.

High amounts of inflammatory markers including TNF- α and IL-6 were found in the brains of those who were treated with CMF. The CMF group showed a statistically significant elevation in BDNF over the normative control. This rise was consistent with a previous study that found prolonged microglial cell stimulation leading to BDNF secretion [22]. Microglia activation was observed to be persistent in the CMF model [42]. Inflammation was reduced after treatment with PTUPB, herbal extracts, or mentat due to a decrease in TNF-, IL-6, or BDNF.

Since sEHI have been shown to have antioxidant and anti-inflammatory effects [11,15] they were investigated in this work. sEH is the enzyme responsible for converting the EETs to the equivalent vicinal diols. Blocking sEH in animal models of brain with deletion or pharmacological inhibitors increases in vivo levels of epoxyeicosanoids, which provide substantial protection. Pharmacological inhibition of sEH results in decreased neuroinflammation and higher EET. Improvements in spatial learning and memory have been linked to sEHI. There are a number of sEHI known, but none of them have been developed with CNS application in mind. Maca increased the expression of proteins involved in autophagy in the cerebral cortex of middle-aged mice [43]. It also enhanced the animals' cognitive abilities, motor skills, and stamina. B. monnieri extract has many pharmacological effects, such as neuroprotection, improvement of cognitive impairment, increased cerebral blood flow, enhancement of antioxidant enzyme activity and intracellular signaling pathways [44]. PTUPB, a synthetic dual inhibitor of sEH and COX employed in the study, was found to improve memory in the treated mice. In this scenario, where COX-2 activity was elevated by CMF, the plant-derived sEH inhibitor maca extract had no effect on COX in the brain. Overall, the antioxidant and anti-inflammatory impact of PTUPB, herbal extracts, and Mentat were linked to their protective effect.

5. Conclusion

According to the findings of the current study, the cognitive dysfunction measured by the Morris water maze test was significantly reduced after treatment with hydroalcoholic extracts of Syzygium aromaticum, Nigella sativa and Mesua ferrea. The reference medications Mentat and Bacopa both performed better than expected. When compared to the CMF group, the therapy groups lost less weight overall. Understanding the complex mechanisms of pathogenesis and the pathways responsible for the treatment medications' ability to restore cognition requires extensive research. Isolating the active chemicals in Lavanga, Kalonji, and Nagakesar that are important for memory enhancement is essential for understanding and determining the mechanistic pathways. In conclusion, much more study and research are needed to find a cure for this illness.

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Data Availability Statement

The datasets generated during or analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations:

BDNF	brain-derived neurotrophic factor
CICI	chemotherapy-induced cognitive impairment
CMF	a combination of Cyclophosphamide, Methotrexate and 5-Fluorouracil
CNS	central nervous system
COX-2	cyclooxygenase-2
EETs	epoxyeicosatrienoic acids
EpFA	epoxy fatty acids
GSH	glutathione
IL-6	interleukin-6
LPO	lipid peroxidase
PTUPB	4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl) benzenesulfonamide
RBC	red blood cells
sEH	soluble epoxide hydrolase

sEHI	soluble Epoxide Hydrolase: inhibitors
TNF	tumour necrosis factor
WBC	white blood cells

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Highlights

- Soluble epoxide hydrolase converts neuroprotective Epoxyeicosatrienoic acid (EET) to less active trans-dihydro diols.
- EETs alleviate inflammation, and cellular ageing by rebalancing cellular components via the ER stress pathway.
- Inhibition of EH results in increased levels of EET, which has a neuroprotective effect conferring the reversal of cognitive impairment.

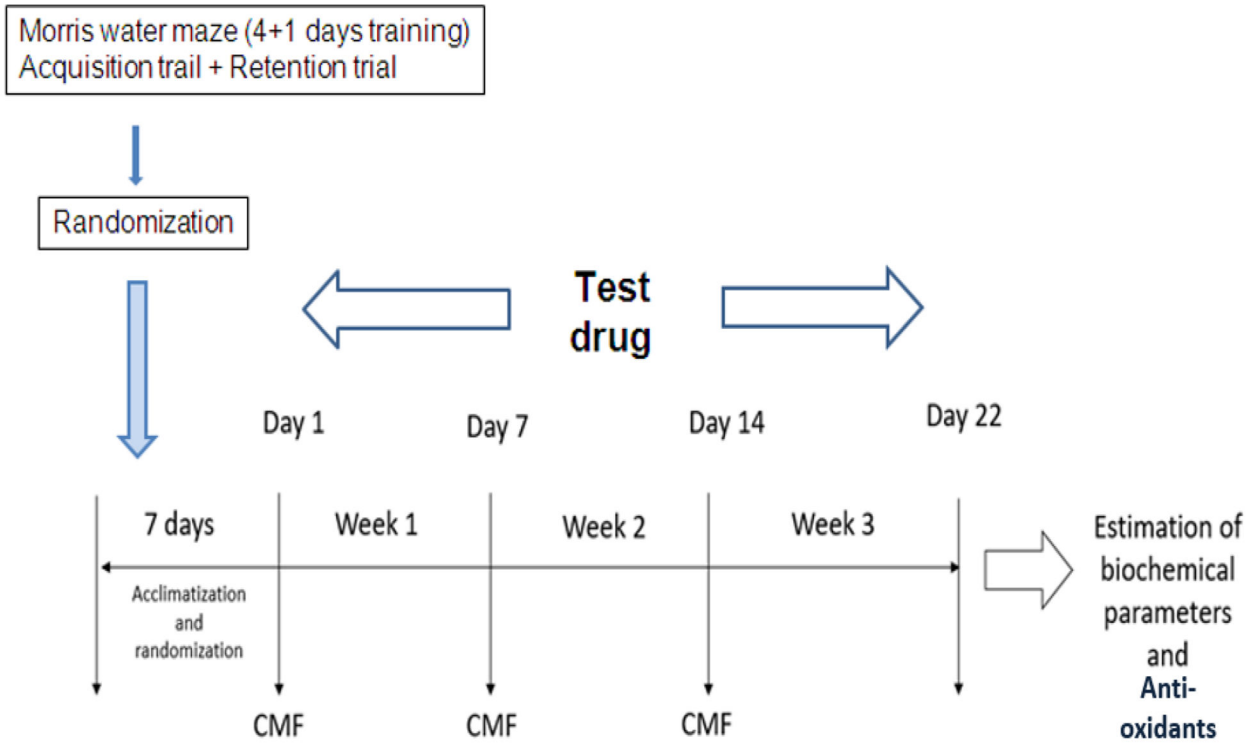


Fig. 1. Experimental Study Design-

The animals were trained on the Morris water maze and successful mice were randomized into different groups. CMF treatment was started on day 1. CMF was treated 3 times during the 14 days of the study period. Herbal extracts and chemicals inhibiting sEH were treated for 21 days and at the end of the study memory function was evaluated using a Morris water maze. Blood and brain samples were collected for biochemical, hematological and brain endogenous antioxidant enzymes respectively.

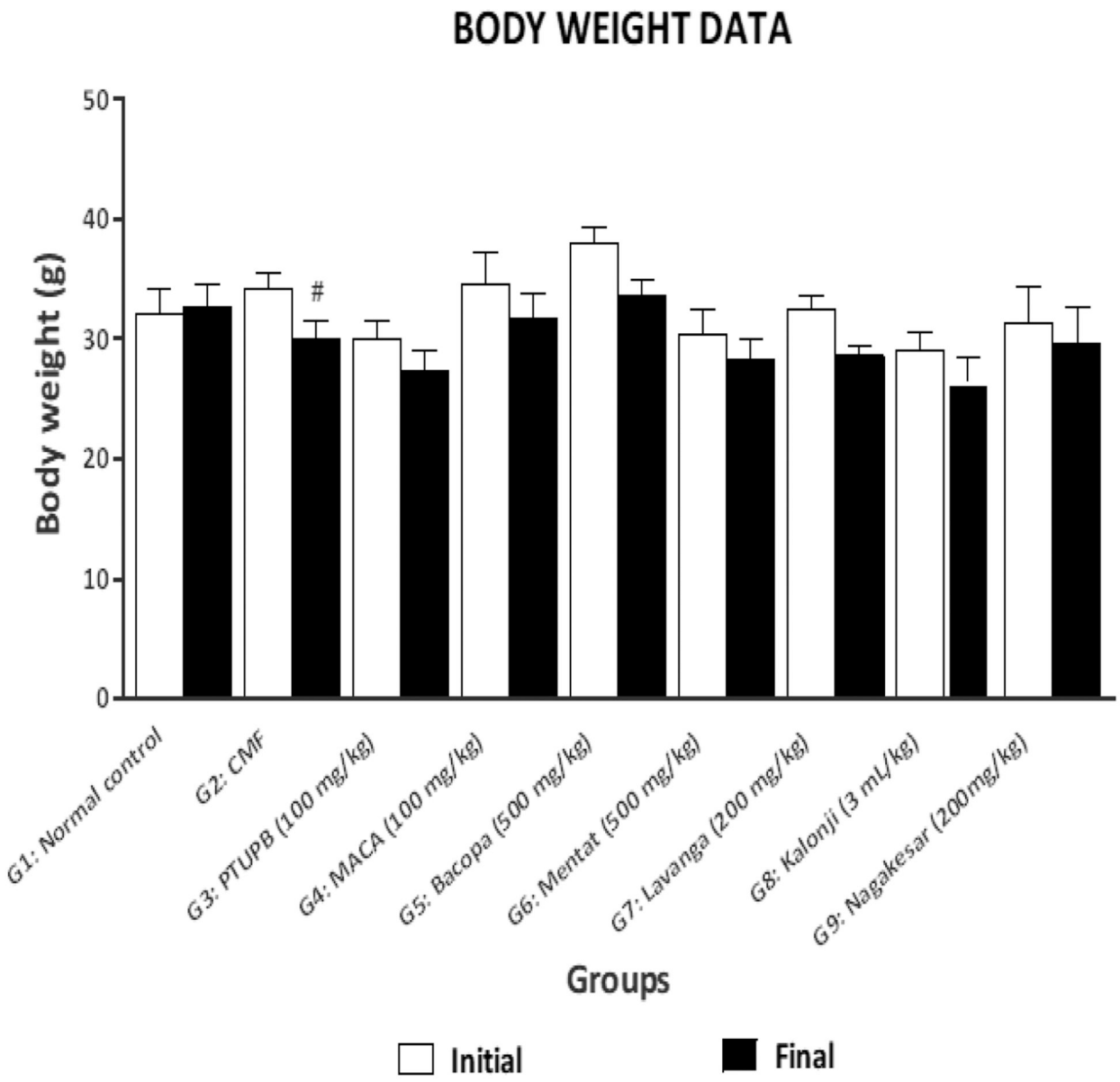


Figure 2. Effect of treatment on body weight-

A significant decrease in body weight was observed in CMF treated group after 21 days of treatment compared to the normal control group. # $p < 0.05$. Treatment with PTUPB, herbal extract or herbal formulation prevented loss of body weight at the end of the treatment period. The data was analyzed by one-way ANOVA followed by Dunnett's post hoc test and presented as Mean ± SEM of 7 observations.

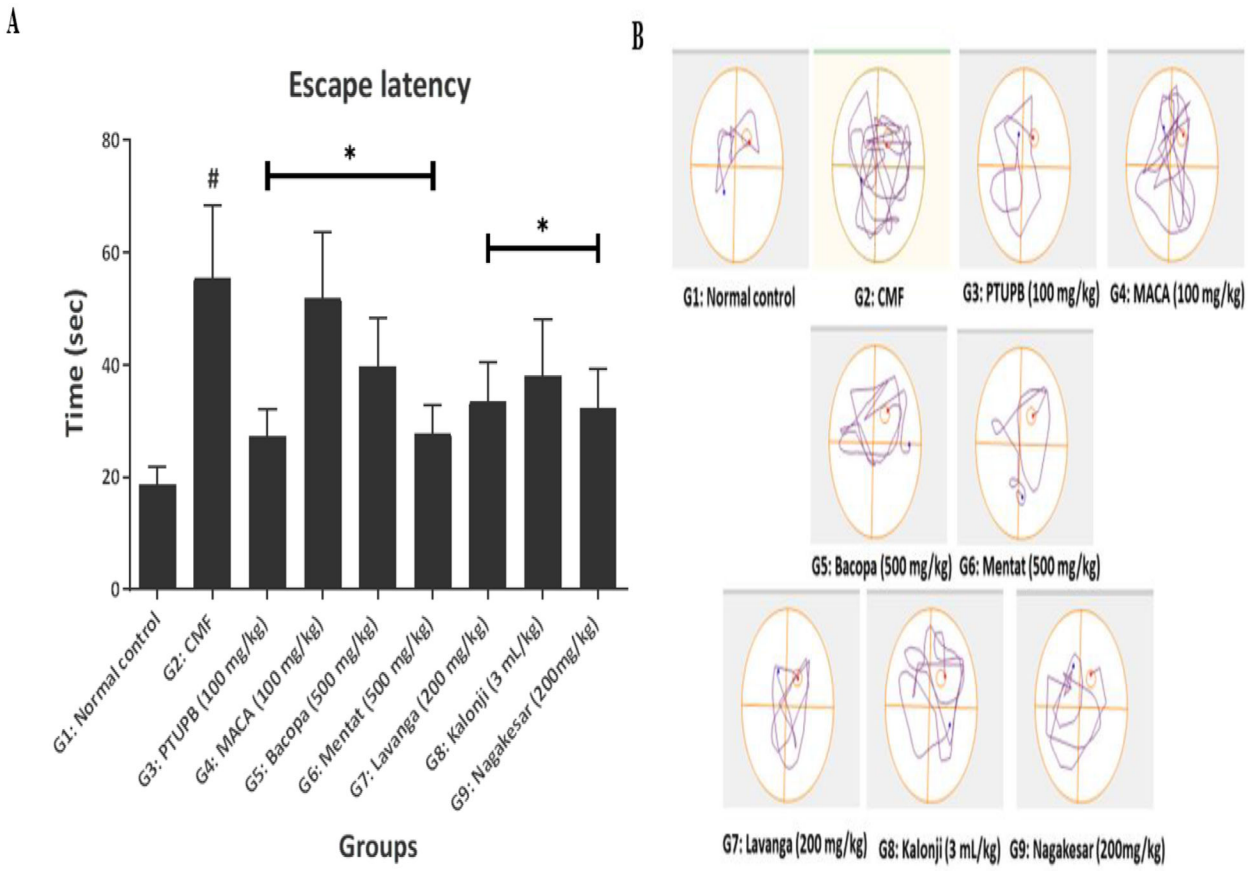


Figure 3. Effect of sEH inhibition on cognition-

(A) Escape latency of the CMF-treated mice were significantly increased compared to normal control mice (###p<0.001) suggesting loss in cognition, whereas the escape latency of the animals belonging to *g*roup 3- 9 was significantly decreased compared to CMF-treated mice (* p< 0.05 & **p<0.01). (B) The distance travelled by CMF-treated mice in water before finding an escape platform was increased compared to normal control mice, suggesting memory impairment. However, the distance travelled by animals belonging to *g*roups 3- 9 was less compared to CMF-treated mice, suggesting protection of memory. The data was analyzed by one-way ANOVA followed by Dunnett's post hoc test and presented as Mean ± SEM of 7 observations.

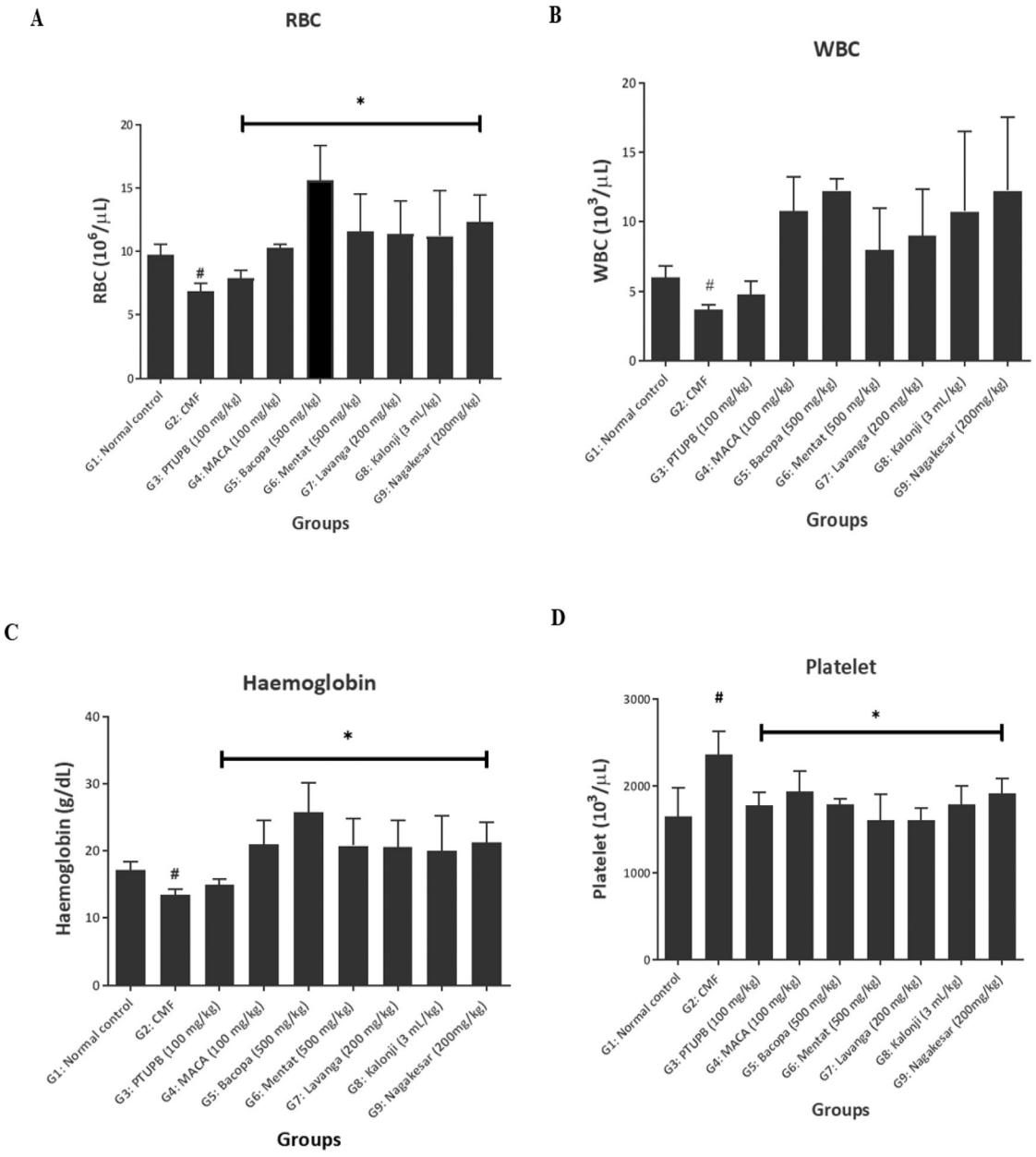
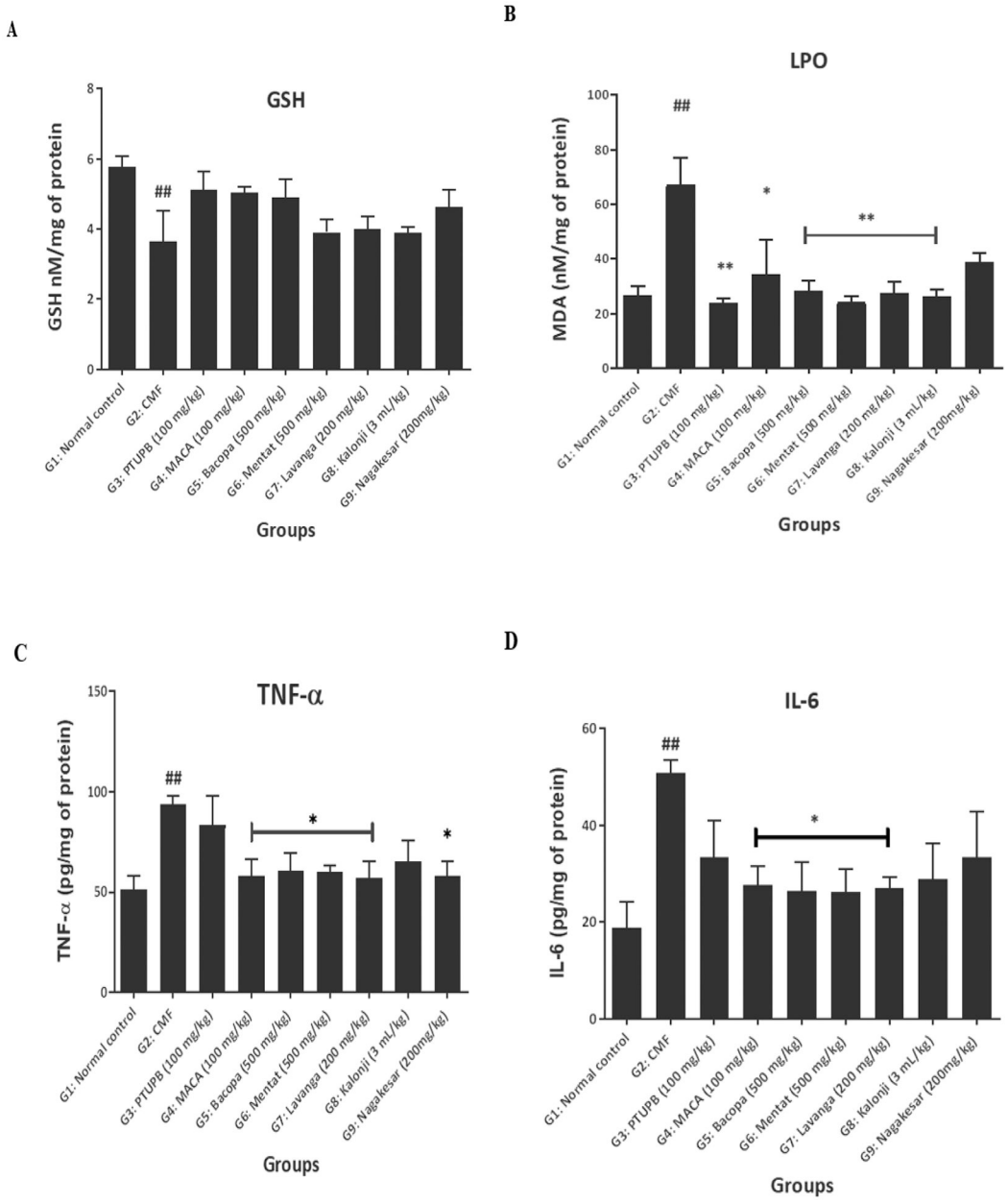


Figure 4. Effect of treatment on hematological parameters-

(A) A significant reduction in the level of the RBC (normal range of RBC 9.17-12.1 x106/μl [38]) was observed in the CMF-treated mice compared to normal control mice. #$p < 0.05$. Treatment with PTUPB, herbal extracts or formulation significantly increased the total RBC count when compared to the CMF group. *$p < 0.05$. This data confers that the standard and test drugs were able to protect the RBC count of animals against the administration of chemotherapeutic drugs. (B) A significant reduction in the level of WBC (normal range of WBC- 3.63–16.7 x103/μl [38]) was observed in the CMF-treated mice compared to normal control mice. #$p < 0.05$. Treatment with PTUPB, herbal extracts or formulation did not alter total WBC count when compared to the CMF group. (C) A significant reduction in the level of hemoglobin (normal range of hemoglobin 13.1–17.9

g/dL [38]) was observed in the CMF-treated mice compared to normal control mice. #p < 0.05. Treatment with PTUPB, herbal extracts or formulation significantly minimized a reduction in the total Hb count when compared to the CMF group. *p < 0.05. One can infer from these data that the CMF administered animals were prone to anemia, whereas, the animals treated with PTUPB, herbal extracts or formulations were able to effectively combat this condition, thereby, maintaining the levels of hemoglobin significantly higher than those in the pathological control group. (D) A significant increase in the level of platelets (normal range of platelets 445–1815 x103/μl [38]) was observed in the CMF-treated mice compared to normal control mice. #p < 0.05. Treatment with PTUPB, herbal extracts or formulations significantly decreased the platelet count when compared to the CMF group. *p < 0.05. This infers that the treatment with CMF may increase the chances of clotting and immunological reaction, whereas treatment with PTUPB, herbal extracts or formulations would minimize the increase in platelets by CMF. The data was analyzed by one-way ANOVA followed by Dunnett's post hoc test and presented as Mean ± SEM of 3–7 observations.



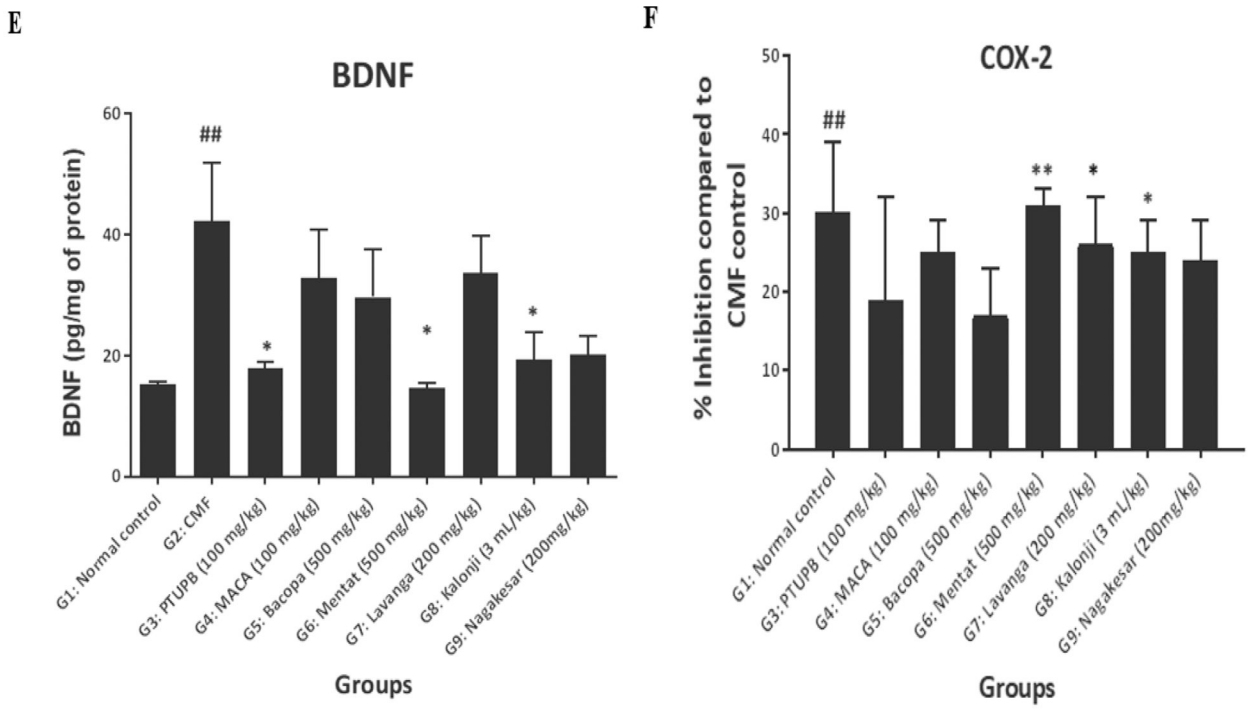


Figure 5. Effect of treatment on brain biochemistry-

A significant decrease in the level of (A) GSH, but an increase in the level of (B) LPO was observed in the brain of CMF-treated mice compared to normal control mice. ##$p < 0.01$. Treatment with PTUPB, herbal extracts (except M. ferrea) or Mentat significantly decreased oxidative stress compared to the CMF group. *$p < 0.05$. **$p < 0.01$. A significant increase in the levels of (C) TNFα, (D) IL-6, (E) BDNF was observed in the brain of CMF-treated mice compared to normal mice suggesting an increase in the level of inflammation. ##$p < 0.01$. Treatment with PTUPB, herbal extract or mentat decreased the level of inflammation either by decreasing the level of TNFα, IL-6 or BDNF. *$p < 0.05$. **$p < 0.01$. (F) It seems the COX-activity was increased in the CMF-treated group compared to normal control group. Inhibition of the activity of COX-2 with herbal extract (Lavanga and Kalonji) or mentat alleviated the inflammatory effects of the combined chemotherapy. ##$p < 0.01$* $p < 0.05$. **$p < 0.01$ The data was analyzed by one-way ANOVA followed by Dunnett's post hoc test and presented as Mean ± SEM of 3–7 observations.

Table 1

Grouping of animals

Group number	Treatment and dose
1.	Healthy control mice receiving Saline (10 ml/kg)
2.	Pathological control receiving CMF (50 mg/kg, 5 mg/kg, 50 mg/kg, intraperitoneally)
3.	Mice receiving both CMF and PTUPB (100 mg/kg, oral)
4.	Mice receiving both CMF and *Lepidium meyenii* or Maca extract (100 mg/kg, oral)
5.	Mice receiving both CMF and *Bacopa monnieri* or Bacopa extract (500 mg/kg, oral)
6.	Mice receiving both CMF and solution of crushed Mentat tablet (500 mg/kg, oral)
7.	Mice receiving both CMF and *Sizygium aromaticum* or Lavanga extract (200 mg/kg, oral)
8.	Mice receiving both CMF and *Nigella sativa* or Kalonji extract (3 ml/kg)
9.	Mice receiving both CMF and *Mesua ferrea* or Nagakesar extract (200 mg/kg, oral)

Abbreviations: CMF: Cyclophosphamide, Methotrexate and 5-Fluorouracil combination.