Impact of rHu-Epo supplementation on CRF induced Neurobehavioral changes in rats: Studies on correlation with APP ratio, β amyloid and pTau expression

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Regulators for many of the body’s functions and control complex processes and maintain homeostasis.

Receive 20 to 25% of cardiac output/ min; Blood is filtered through the nephrons.
Broscious and Castagnola, 2006

Body water regulation
  - Urine output
  - Blood pressure

Electrolyte balance
  - Sodium
  - Potassium
  - Phosphorus
  - Calcium
  - Magnesium

Normal kidney function in homeostasis

Excretory regulation
  - Nitrogenous waste products
  - Drug metabolites and other wastes
  - Uric acid

Metabolic (endocrine) regulation
  - Erythropoietin
  - Renin-angiotensin-aldosterone
  - Vitamin D

Acid-base balance
  - Metabolic compensation
CHRONIC RENAL FAILURE (CRF)

CKD - major clinical health problem as it is a systemic disorder that causes widespread organ damage and is related to significant morbidity and mortality (Ryuji ikeda et al., 2010)

With a prevalence of 15% in developed nations (Krishnan & Kiernan, 2009).

CKD - can occur as a result of a primary renal disorder or as a complication of multisystem disease (Krishnan & Kiernan, 2009).
5 stages of CRF is classified based on the estimated GFR (eGFR)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Glomerular filtration rate (GFR), mL/min per 1.73 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or increased GFR</td>
<td>≥90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mild decrease in GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderate decrease in GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severe decrease in GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt;15 (or dialysis)</td>
</tr>
</tbody>
</table>

*Chronic kidney disease is defined as either kidney damage or a glomerular filtration rate less than 60 mL/min per 1.73 m² for 3 months or longer. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.*
Manifestations of kidney failure

Chronic kidney disease

- Sodium and water balance
  - Hypertension
    - Increased vascular volume
      - Heart failure
        - Pericarditis
    - Edema
  - Hyperkalemia

- Potassium balance
  - Hyperkalemia

- Elimination of nitrogenous wastes
  - Uremia
    - Coagulopathies
      - Bleeding
        - Acidosis
          - Skeletal buffering
            - Hypocalcemia
              - Hyperparathyroidism
        - Osteodystrophies
    - Acidosis
      - Activation of vitamin D
        - Phosphate elimination
          - Hypocalcemia

- Erythropoietin production
  - Anemia

- Acid-base balance
  - Skeletal buffering
    - Hypocalcemia

- Impaired immune function
- Skin disorders
- Gastrointestinal manifestations
- Neurologic manifestations
- Sexual dysfunction
- Osteodystrophies
Clinical manifestations of chronic kidney disease

**Integumentary**
- Bruises, pruritus, dry skin, skin color changes ashen gray to yellowish, dry brittle hair and nails

**Cardiovascular**
- High blood pressure, increased heart rate, dysrhythmias, electrocardiographic changes, abnormal heart sounds, retinopathy, fluid retention with peripheral edema and/or pulmonary edema

**Respiratory**
- Increased respiratory rate, Kussmaul respirations, crackles, decreased $P_{O_2}$

**Renal**
- Decreased urine output, azotemia, proteinuria, hematuria, hyperuricemia

**Gastrointestinal**
- Anorexia, nausea, vomiting, halitosis, metallic taste in mouth, bleeding in gastrointestinal tract

**Neurological**
- Peripheral neuropathy, restless legs, change in level of consciousness, lethargy, confusion, encephalopathy, altered motor function

**Immune**
- Increased risk of infection

**Musculoskeletal**
- Renal osteodystrophy, decreased calcium, vitamin D impairment, hyperparathyroidism, pathological fractures

**Hematological**
- Anemia, weakness, fatigue, pallor, lethargy, bleeding due to impaired platelet aggregation
Neuropathological changes in the brain that parallel changes in the kidney have been posited as mechanisms explaining relationship between CRF and cognition (Elias et al., 2004).

These include Atherosclerosis, Microvascular disease, Clinical stroke, Silent stroke, Oxidative stress and White matter lesions.
In a study of homebound elderly individuals, Albuminuria was associated with lower levels of executive function ability and with white matter hyper intensities and white matter volume (Weiner et al., 2008)

Uremic toxins and Neurological diseases

Accumulation of Urea, Creatinine, PTH, Myoinositol, β2 microglobulin

Middle molecules (MW 300 -12,000 Kda)

Hyperkalemia

Electrolytes disturbances (hypercalcemia, hypophosphatemia, hyponatremia)

Hyperparathyroidism, Affected Intermediary Metabolism
Cognitive impairment has increasingly been recognized in CRF (potentially affecting up to 80% of patients) (Murray, 2008).

Studies have shown impairment in executive function ranging from 23% - 38% and memory impairment ranging from 28% - 33%.

Compared to general population, increased risk of dementia (Seliger et al., 2004) and poorer performance on tests of global cognitive function, executive function, defects in language and memory (Murray, 2008) have been reported.
Neurological complications of CKD:

- Cognitive dysfunction
- Stroke
- Restless leg syndrome
- Peripheral and autonomic neuropathy
- Carpal tunnel syndrome
- Uremic Myopathy (Krishnan & Kiernan, 2009)
Effects of Renal Transplantation

Girva et al. (2006) gave clear evidence that cognitive function improves following renal transplantation.

Kramer et al. (2006), demonstrated improvement in both neuropsychological tests, (MMSE) and neurophysiological markers of cognitive function, as measured using evoked potential latencies and EEG rhythms.
Amyloid precursor protein (APP) and β amyloid (Aβ)

Dementia is a primary neurodegenerative disorder and it leads to a complete psychological and physical dependency and finally to death within one to two decades.

It involves aberrant protein processing and is characterized by the presence of both intraneuronal protein clusters composed of

(i) Extracellular Aβ protein aggregates (senile plaques)

(ii) Bundles of Paired Helical Filaments (PHF) of abnormal pTau (Intra cellular neuro fibrillary tangles) (Butterfield et al., 2007).
Aβ is a 39-43 residue protein

MW (≈ 4 Kda).

It is derived by proteolytic cleavage of an integral membrane protein known as amyloid precursor protein (APP) by the action of β- and γ- secretases (Butterfield et al., 2007).
Barron et al., 2006. The role of Gonadotrophins in Alzheimer’s disease. Endocrine, Vol 9, 257-269

**Fig. 2.** Amyloidogenic and non-amyloidogenic APP processing pathways. (A) Amyloidogenic APP processing. Membrane-bound full-length APP is cleaved by BACE releasing a soluble N-terminal fragment (β-sAPP). The C-terminal fragment (C99 stub) is then cleaved by γ-secretase releasing the Aβ peptide. (B) Non-amyloidogenic APP processing. α-Secretase cleaves the full-length APP fragment in the Aβ domain releasing a soluble N-terminal APP fragment (α-APP). The remaining C-terminal fragment (C83 stub) is then cleaved by γ-secretase yielding the non-amyloidogenic fragments. (Adapted from ref. 180.)
Oxidative stress - play a crucial role in the pathogenesis of neurodegenerative disorders, cancer, and ischemia.

Oxidative stress and Aβ production are proportionally linked to each other because Aβ induces oxidative stress *invivo* and *invitro* (Tabner et al., 2005), and oxidative stress increases the production of Aβ (Tamagno et al., 2008).

Aβ induces iNOS expression in vivo that produces neurotoxic levels of NO and results in the cholinergic signaling dysfunction and memory impairment (Tan et al., 2001).
Tau Protein and its Hyper phosphorylation

Major microtubule-associated phosphoprotein (~55 Kda), located in axons where it binds to microtubules, thus promoting microtubule assembly and stability

Encoded by single gene located on chromosome 17

Among its post translational modifications, phosphorylation has been most widely studied (Zhang et al., 2009). Many neurodegenerative diseases are characterized by Tau phosphorylation
Tau (mis) localization in neurons, and consequently the development of neurofibrillary tangles (NFT)

In healthy persons –

(i) Involvement in the outgrowth of neural processes,

(ii) Axonal transport,

(iii) Development of neuronal polarity

(iv) Maintenance of normal neuron morphology
PHFs – Main constituent of large pathological structures (NFTs).

Tau hyperphosphorylation affects the morphology and biological functions of the neurons.

As a result of NFTs formation, MAP –Tau is no longer available for cytoskeletal stabilization. The disorganization of the neuronal skeletal contributes to neuronal malfunction, neuronal cell death and eventually dementia.
ERYTHROPOIETIN (EPO)

EPO - Glycoprotein (30.4 kDa) and it is widely known as the major growth factor for the RBC production (Lin zhu et al., 2009)

165 AA residues chain to which four glycans are attached

The first purification of human EPO from urine of aplastic anemia patient in 1977 led to the cloning of human EPO gene and permitted mass production of recombinant human EPO (Katavetin et al., 2007)
EPO was first characterized as a hematopoietic growth factor and has been in clinical use by millions of patients over the decade for the treatment of anemia (Anna-leena Siren et al., 2000).

The clinical relevance for the use of EPO as a neuroprotective agent was enhanced when it was found to cross the blood-brain barrier after peripheral administration (Brines et al., 2000).

Epo has been show to act as antioxidant by decreasing NO and increasing GSH (Moran et al., 2009).
Neuroprotection by EPO has been shown to associate with anti-apoptosis, neuroregeneration and anti-inflammation (Sola et al., 2005).

Sun et al. (2008) reported that Epo prevented tau hyperphosphorylation in SH-SY5Y cells exposed to the β amyloid peptide.

In mice, Epo treatment improves hippocampus dependent memory by modulating plasticity, synaptic connectivity and activity of memory related neuronal networks (Adamcio et al., 2008).
Anaemia is an important risk factor for cognitive impairment in CRF and the major factor is a relative deficiency of erythropoietin synthesis by the failing kidneys (Eschbach, 1989).

Epo is used routinely to treat anemia in CRF. It has been shown that along with the level of Hb, cognitive function also improves (Pickett et al., 1999).

Lacunae

There is no study on the expression of APP, β Amyloid and phospho Tau in CRF induced experimental animals

Impact of antianemic drug EPO on those protein abnormalities in CRF induced animals (?)
Hypothesis

1. CRF could induce changes in the expression of APP, Aβ and hyperphosphorylated tau protein (pTau) levels in cerebellum, cerebral cortex and hippocampus of wistar strain albino rats.

2. Epo could protect the CRF induced protein abnormalities along with correcting hematological parameters

Aim

To study the impact of Epo supplementation on CRF induced changes in expression of APP, Aβ and hyperphosphorylated tau protein levels in cerebral cortex, cerebellum and hippocampus
Materials and methods

Reviewed and approved by the Institutional Animal Ethics Committee, SRM University, INDIA.

This study was done on 48 Adult Male Wistar rats 120 – 150 gm in weight

After 10 days of acclimatization the animals were randomly assigned to either the experimental groups or control group.
Were housed in Central Animal house of SRM Medical College Hospital

Each 3 animal has given individual labeled cages

They were maintained in controlled laboratory conditions of 12 hour dark/light cycle, 20-22° C temp.

All the animals were weighed alternative days through out the study and water intake was also measured daily.
6 rats per group (n = 6)

PHASE 1 (Total period 28 days – Simultaneous treatment of EPO)

Group I: Control

Group II: Adenine 0.75% in feed for 4 weeks

Group III: Adenine 0.75% in feed for 4 weeks and simultaneous administration of Erythropoietin 100 IU per kg body weight thrice weekly

Group V: EPO (100 IU/ Kg bwt) thrice per week

After end of the treatment (28 days) animals were sacrificed simultaneously for experimental procedures
PHASE II (Total period 40 days – Post treatment of EPO)

Group I: Control

Group II: Adenine 0.75% in feed for 4 weeks

Group III: Adenine 0.75% in feed for 4 weeks + after 4 weeks post treatment of Erythropoietin (100 IU/ Kg bwt.) 12 days, daily once.

Group IV: EPO (100 IU/ Kg bwt) thrice per week

After end of the treatment (40 days) all animals were sacrificed simultaneously.
The most commonly used rat model for progressive renal failure is the “remnant kidney” model (also called 5/6 nephrectomy) (Kujal & Vernerová, 2008).

The other valid, widely used model, is the adenine-induced CRF in rats (Ormrod & Miller, 1980)

Adenine is given mixed with the feed at a concentration of 0.75%, w/w, for 4 weeks. Orally-administered adenine is metabolized to 2,8-dihydroxyadenine, which precipitates and forms tubular crystals that injure the renal tissue (Shuvy et al., 2011)
Chemicals

Adenine was purchased from Sisco Research Lab (SRL), India.

EPO was purchased from Serum Institute of India, Chennai.

Primary and secondary antibodies for western blotting were purchased from Cell Signaling Technology
Primary Antibodies

GAPDH (D16H11) – Rabbit mAb # 5174BC

β Amyloid (D54D2) – Rabbit mAb # 8243

Phospho Tau (Ser 202) – Rabbit Polyclonal Ab # 11834

Total Tau (Tau 46) – Mouse mAb # 4019

APP – Rabbit polyclonal Ab # 2452

Quantification of the bands was done by using image J software
Methods

Rectangular maze procedures has been done weekly once from day 0, till the last day of treatment to find out the changes in memory and learning as the treatment progress.

After ending the treatment period, the animals were taken to record the behavioral changes with the help of plus maze.

At last the animals were sacrificed and then blood samples and organs were collected for analysis.
Rectangular maze
Plus maze

Introduced by Handley and Mithani in 1984,

widely used model to assess anxiety-related behavior in rodents.

- Two open arms and two arms that are enclosed by high walls. The open arms are perpendicular to the closed arms, with the four arms intersecting to form the shape of a plus sign.
Usually elevated approximately 50 cms. above the floor. Security is provided by the closed arms whereas the open arms offer exploratory value.

Therefore, one would expect anxious rats to spend less time in the open arms than those that are less fearful (Salum et al., 2000).

Environmental temperature was maintained equal to the temperature measured in the housing room.
A weak cider vinegar solution (10%) was used to clean the apparatus prior to the introduction of each animal.

Each rat was placed at the center of the elevated plus-maze with its head facing the open arms and allowed to freely explore for 5 min.

After each observation, the EPM was cleaned with ethyl alcohol (10%) to remove scent cues left from the preceding subject.
During this 5 min experiment, the behavior of the rat was recorded as

Percent time spent in open arm (Time spent in open arm *100/ 300)

Number of entries in open arm

Percent time spent in closed arm (Time spent in closed arm *100/ 300)

Number of entries in Closed arm

Percent time spent in centre
**SAP (Stretched Attend Posture)** - rat stretches its head and shoulders forward and then returns to the original position.

**Rearing** - rat maintains an erect posture, sitting on the hind paws only.
Grooming - rat licks/rubs its face and/or body

Defecation – number of fecal boli produced

Head Dip - scanning over the sides of the maze towards the floor
Hematological analysis

After recording the behavioral changes, blood has been collected from the posterior cava vein in each animal, under ether anesthesia, to determine hematological parameters.

Two blood samples has been taken with and without EDTA.

Samples without EDTA was centrifuged at 5000 rpm for 20 minutes and the samples were stored in -70 ºC until analysis.
Blood samples with EDTA were analyzed for blood parameters namely RBC(millions/cumm), Hemoglobin(g%), and Hematocrit(%).

Based on these values MCV(fl), MCH(pg) and MCHC (g/dl) was calculated

\[
\text{MCV} = \frac{\text{Ht}}{\text{RBC}} \times 10
\]
\[
\text{MCH} = \frac{\text{Hb}}{\text{RBC}} \times 10
\]
\[
\text{MCHC} = \frac{\text{Hb}}{\text{Ht}} \times 100
\]
Serum samples stored in -70°C was used for the analysis of biochemical parameters like urea, creatinine and creatine kinase level.

Serum creatinine and urea were measured by spectrophotometric method because they are the important indicator of renal health.

Creatine Kinase were also measured. It catalyzes the conversion of creatine and consumes ATP to create phosphocreatine ADP.
24 hours after last treatment, the animals were sacrificed and brain was immediately removed and washed in ice-cold physiological saline repeatedly and brain was dissected over ice-cold glass slides to the following regions:

Cerebral cortex and Hippocampus and cerebellum (Glowinski and Iverson, 1966).

Regions from each of the brain tissue were blotted, weighed accurately
All the organs weighed separately and brain and kidney relative weight was calculated based on the formula (organ weight / final body weight X 100)

Body weight was calculated based on the formula (body weight change = final weight - initial weight  X 100)

The brain regions were placed in chilled 0.1 mol/L Tris–HCl buffer, pH 7.4.

The samples were homogenized using a Potter-Elvehjem homogenizer to produce 10% homogenates.

The samples were centrifuged at 12,000 x g for 30 min. The supernatant was collected and used for the experiments
Phase I – Haematological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (millions/cumm)</td>
<td>7.05 ±0.52</td>
<td>4.42 ±0.31 **</td>
<td>7.3 ± 0.43NS</td>
<td>11.3 ±0.88***</td>
</tr>
<tr>
<td>HB (g%)</td>
<td>13.13±0.62</td>
<td>6.65±0.51 ***</td>
<td>10.2±0.25 *</td>
<td>17.6±1.31 ***</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>43.08±2.73</td>
<td>27.33 ± 1.80 ***</td>
<td>37.83 ±2.15 NS</td>
<td>58.50 ±2.36 **</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>60.92±1.29</td>
<td>61.78 ± 1.27 NS</td>
<td>51.80 ±2.56 *</td>
<td>54.76 ± 1.02 NS</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.95± 0.69</td>
<td>13.81 ± 0.19 **</td>
<td>14.15 ± 0.76 **</td>
<td>16.61 ± 0.32 NS</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>28.62±0.74</td>
<td>21.27 ± 0.81 ***</td>
<td>27.37 ± 0.88 NS</td>
<td>35.82 ± 0.70 *</td>
</tr>
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</table>
## Phase II – Haematological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3a</th>
<th>Group 3b</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBC (millions/cumm)</strong></td>
<td>7.58 ±0.46</td>
<td>3.87±0.18 ***</td>
<td>4.48± 0.32 **</td>
<td>6.53±0.41 NS</td>
<td>12.3±0.71 ***</td>
</tr>
<tr>
<td><strong>HB (g%)</strong></td>
<td>12.63±0.52</td>
<td>6.08±0.38 ***</td>
<td>5.96±0.30 ***</td>
<td>10.85±0.32 NS</td>
<td>18.6±1.11 ***</td>
</tr>
<tr>
<td><strong>PCV (%)</strong></td>
<td>46.00±2.29</td>
<td>24.33 ± 1.49 ***</td>
<td>27.50±1.17 ***</td>
<td>35.17±1.35 **</td>
<td>56.27±2.15 **</td>
</tr>
<tr>
<td><strong>MCV (fl)</strong></td>
<td>57.92±1.34</td>
<td>62.09 ± 1.79 NS</td>
<td>62.16 ± 2.37 NS</td>
<td>54.47± 2.43 NS</td>
<td>52.39± 1.92 NS</td>
</tr>
<tr>
<td><strong>MCH (pg)</strong></td>
<td>17.45 ±0.69</td>
<td>12.97 ± 0.24 **</td>
<td>13.52 ± 0.72 ***</td>
<td>15.18± 0.45 *</td>
<td>24.31± 0.52 **</td>
</tr>
<tr>
<td><strong>MCHC (g/dl)</strong></td>
<td>29.85±0.24</td>
<td>22.33 ± 0.67 ***</td>
<td>21.73 ± 0.74 ***</td>
<td>34.25± 0.88 *</td>
<td>38.12± 0.65 **</td>
</tr>
</tbody>
</table>
(A) Latency period in rectangular maze

Latency period in secs

Day 0  Day 7  Day 14  Day 21  Day 28

Group 1  Group 2  Group 3  Group 4

Phase I - Simultaneous treatment phase
Latency period of rectangular maze in CRF induced and EPO treated animals in both (A) – Simultaneous treatment phase and (B) – Post treatment phase. Results are expressed as mean ± SEM of 6 rats in each group; (a) -P < 0.001, (b) - P < 0.01, (c) P < 0.05, NS – Not Significant
## Phase I – Plus Maze

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>14.39 ± 2.14</td>
<td>6.50 ± 1.05</td>
<td>8.56 ± 1.34</td>
<td>12.07 ± 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>**</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CA</td>
<td>55.33 ± 3.73</td>
<td>64.33 ± 5.28</td>
<td>56.33 ± 4.01</td>
<td>49.24 ± 4.15</td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Centre</td>
<td>30.28 ± 1.77</td>
<td>29.28 ± 4.27</td>
<td>35.06 ± 4.73</td>
<td>37.97 ± 3.16</td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>EOA</td>
<td>6.33 ±1.11</td>
<td>2.86 ± 0.16</td>
<td>3.50 ± 0.42</td>
<td>5.97 ± 0.8</td>
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<tr>
<td></td>
<td></td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ECA</td>
<td>4.67 ±0.55</td>
<td>5.67 ±0.33</td>
<td>6.33 ± 0.66</td>
<td>5.50 ± 0.56</td>
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<tr>
<td></td>
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<td>NS</td>
<td>NS</td>
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<tr>
<td>SAP</td>
<td>4.17 ±0.66</td>
<td>8.13 ± 1.15</td>
<td>5.00 ± 0.57</td>
<td>7.83 ± 0.61</td>
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<tr>
<td></td>
<td></td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Rearing</td>
<td>16.73 ± 1.04</td>
<td>29.83 ± 1.24</td>
<td>23.83 ± 1.4</td>
<td>24.17 ± 1.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Grooming</td>
<td>3.50 ± 0.76</td>
<td>6.15 ±0.51</td>
<td>3.83 ±0.6</td>
<td>4.45 ± 0.48</td>
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<tr>
<td></td>
<td></td>
<td>*</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Defecation</td>
<td>1.43 ±0.7</td>
<td>3.83 ±0.47</td>
<td>2.83 ±0.47</td>
<td>2.50 ±0.42</td>
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<tr>
<td></td>
<td></td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Head dips</td>
<td>3.67 ±0.55</td>
<td>9.09 ±0.81</td>
<td>6.17 ±0.47</td>
<td>4.54 ±0.33</td>
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<tr>
<td></td>
<td></td>
<td>***</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>
## Phase II – Plus Maze

<table>
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<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3a</th>
<th>Group 3b</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td>17.19 ± 1.14</td>
<td>5.58 ± 0.95</td>
<td>5.61 ± 1.07</td>
<td>7.22 ± 1.11</td>
<td>11.67 ± 0.79</td>
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<tr>
<td>CA</td>
<td>53.27 ± 3.28</td>
<td>67.69 ± 6.65</td>
<td>65.17 ± 5.21</td>
<td>57.50 ±4.71</td>
<td>45.67 ± 3.47</td>
</tr>
<tr>
<td>Centre</td>
<td>29.51 ± 2.03</td>
<td>28.28 ± 3.95</td>
<td>29.39 ±4.24</td>
<td>35.33 ± 4.9</td>
<td>42.67 ± 2.68</td>
</tr>
<tr>
<td>EOA</td>
<td>5.33 ± 1.27</td>
<td>3.00 ± 0.36</td>
<td>2.67 ± 0.42</td>
<td>3.17 ± 0.87</td>
<td>6.17 ± 0.7</td>
</tr>
<tr>
<td>ECA</td>
<td>3.27 ± 0.71</td>
<td>6.17 ± 0.41</td>
<td>5.17 ± 0.3</td>
<td>5.33 ± 0.49</td>
<td>4.13 ± 0.26</td>
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<tr>
<td>SAP</td>
<td>3.67 ± 0.45</td>
<td>7.00 ± 1.15</td>
<td>9.17 ± 0.6</td>
<td>7.83 ± 0.7</td>
<td>7.33 ± 0.55</td>
</tr>
<tr>
<td>Rearing</td>
<td>18.23 ± 1.84</td>
<td>31.83 ± 1.08</td>
<td>32.17 ± 2.38</td>
<td>27.83 ± 2.57</td>
<td>22.77 ± 1.31</td>
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<tr>
<td>Grooming</td>
<td>2.87 ± 0.17</td>
<td>5.45 ± 0.44</td>
<td>5.00 ± 0.57</td>
<td>5.33 ± 0.49</td>
<td>3.50 ± 0.61</td>
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<tr>
<td>Defecation</td>
<td>1.83 ± 0.3</td>
<td>4.26 ± 0.67</td>
<td>3.50 ± 0.42</td>
<td>3.00 ± 0.36</td>
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<td>Head dips</td>
<td>3.01 ± 0.22</td>
<td>9.17 ± 0.94</td>
<td>9.33 ± 0.76</td>
<td>7.00 ± 0.93</td>
<td>4.00 ± 0.73</td>
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Blood Urea Nitrogen (BUN) levels in CRF induced and EPO treated animals in both (A) – Simultaneous treatment phase and (B) – Post treatment phase. Results are expressed as mean ± SEM of 6 rats in each group; * P < 0.05, ** P < 0.01, *** P < 0.001, NS – Not Significant
Serum Creatinine levels in CRF induced and EPO treated animals in both (A) – Simultaneous treatment phase and (B) – Post treatment phase. Results are expressed as mean ± SEM of 6 rats in each group;  * P < 0.05, ** P < 0.01, *** P < 0.001, NS – Not Significant
Serum Creatine kinase levels in CRF induced and EPO treated animals in both (A) – Simultaneous treatment phase and (B) – Post treatment phase. Results are expressed as mean ± SEM of 6 rats in each group;  * P < 0.05, ** P < 0.01, *** P < 0.001, NS – Not Significant
Brain creatine kinase activity in CRF induced and EPO treated animals in both (A) – Simultaneous treatment phase and (B) – Post treatment phase. Results are expressed as mean ± SEM of 6 rats in each group; (a) - P < 0.001, (b) - P < 0.01, (c) P < 0.05, NS – Not Significant.
Western blot – Phase I Simultaneous Treatment

Cerebral Cortex

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<thead>
<tr>
<th>Groups</th>
<th>GAPDH</th>
<th>Total Tau</th>
<th>P Tau</th>
<th>Beta amyloid</th>
<th>APP</th>
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Cerebellum

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<th>Intensity (% of control)</th>
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Hippocampus

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<th>Groups</th>
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Phase II – Post Treatment

Cerebral cortex

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<tr>
<th></th>
<th>APP (100 – 140 Kda)</th>
<th>Beta Amyloid (5 Kda)</th>
<th>pTau (50-80 Kda)</th>
<th>Total Tau (50-80 Kda)</th>
<th>GAPDH (37 Kda)</th>
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<td>Intensity (% of control)</td>
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Cerebellum

APP (100 – 140 Kda)

Beta Amyloid (5 Kda)

pTau (50-80 Kda)

Total Tau (50-80 Kda)

GAPDH (37 Kda)

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Hippocampus

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APP (100 – 140 Kda)
Beta Amyloid (5 Kda)
pTau (50-80 Kda)
Total Tau (50-80 Kda)
GAPDH (37 Kda)
Conclusion

Increased Aβ and Hyperphosphorylated tau proteins (ser 202) were observed in selected brain regions of CRF induced experimental animals. Creatine kinase (CK) activity also decreased in CRF induced animals.

Supplementation of EPO protects the CRF induced protein abnormalities and CK alterations significantly in both simultaneous and post treatment.

This study proves the clinical usefulness of Epo as supplemental therapeutic agent in cognitive dysfunction in CKD.
THANK YOU