Hydroethanolic extract of *Ixora coccinea* leaves inhibits testicular and epididymal toxicity associated with antitumor drug Cisplatin in rats

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GSC Biological and Pharmaceutical Sciences, 2022, 21(01), 256–264

Publication history: Received on 16 September 2022; revised on 20 October 2022; accepted on 23 October 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.1.0401

**Abstract**

Cisplatin (Cp) is one of the most effective chemotherapy antineoplastic drugs. Nevertheless, it causes numerous injurious effects on numerous organs, precisely the testes. *Ixora coccinea* (IC) is a well-known medicinal herb and has a long history of medicinal use as a tonic to promote health. This study revealed the inhibitory effects of hydroethanolic extract of IC leaves (HEICL) on Cp-induced testicular damage in male Wistar rats. Thirty (30) adult male Wistar rats were used and divided into six groups of five rats. These groups were treated as followed: Group 1 (control group) and 2 were administered (0.9% saline) and Cp (10 mg/kg. b.wt) intraperitoneal (IP) route respectively, and groups 3 and 4 were administered HEICL at 150 mg/kg and 300 mg/kg and 5 and 6 were co-treated with Cp + HEICL (150 and 300 mg/kg) respectively. The treatment duration was 28 days. Spermatological profiles and testicular histopathology were assessed. Cp-treated rats showed a significant (p<0.05) reduction in relative organ weight and sperm parameters. On the other hand, Cp treatment increased the percentage of sperm abnormalities relative to the control and the groups administered HEICL only. These results were confirmed by histopathological analysis. Contrarily, there were modulations in cp-induced spermiotoxic and testicular damage following the HEICL pretreatment. Our research showed that Cp treatment exerts damaging consequences on sperm parameters, testicular tissues, and accessory sex organs in rats. Pre-treatment with HEICL for 26 days at doses of 200 and 400 mg/kg bodyweight had a protective role against testicular damage brought on by Cp.

**Keywords:** Cisplatin; *Ixora coccinea*; Spermatozoa; Testes; Albino rats

1. **Introduction**

Male infertility is one of the most common medical conditions that need to be addressed and such problems are linked to a variety of factors, some of which are acquired (such as toxic pollutants) or of natural origin (food and drugs) [1].

The traditional anticancer drug known as cisplatin (CP) is frequently used to treat a variety of human malignant tumors, including ovarian, bladder, lung, liver, and others [2]. CP binds to DNA, and consequently induces apoptosis and inhibits cell growth [3]. Even with its therapeutic and all-encompassing efficacy against various cancers, its clinical usage is restricted due to its unchecked organ toxicities, particularly testicular toxicity, which may be irreversible [4,5]. Recent studies have established that oxidative stress is the precise mechanism for CP-induced testicular toxicity and genotoxicity. [6]. CP treatment can also destroy the semen profile and the testes’ interstitial cell shrinkage and vacuolation [7]. Exposure to this drug may result in azoospermia, oligospermia, a loss of testicular weight, and eventually permanent or temporary male sterility [8].

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Prior experimental studies highlighted several antioxidants’ protective functions against testicular damage caused by CP [9]. Researchers from all over the world have recently switched their attention to compounds derived from plants, and it is known that between 66 and 85% of the world’s population now directly depends on plant products for food, medicine, and other benefits [10]. These plants’ various parts have yielded extracts that have been used in medicine [11, 12, 13], and these plant-derived products have fewer side effects, are easily accessible, and are efficacious [14]. Thus, there is a growing interest in the evaluation of several plant extracts for their medicinal value in the management of organ disorders [15,16].

*Ixora*, a genus of flowering plants in the Rubiaceae family consists of tropical evergreen trees that are native to the tropical regions of Asia [17]. The leaves of IC have been known to contain kaemferol, flavonoids, quercetin, anthocyanidins, ferulic acids, and other phenolic acids with medicinal values [18]. Folkloric medical practices have used IC to treat a variety of illnesses. Root decoction was used to treat anorexia, hiccups, and nausea. The powdered roots are applied topically to sores and persistent ulcers, and fresh leaves and stems are poulticed to treat sprains, eczema, boils, and contusions, and the flowers are used to treat sprains, bronchitis fever, sores, chronic ulcers, scabies, leucorrhoea, dysentery, dysmenorrhea, haemoptysis, hypertension, menstrual irregularities, and skin diseases [19], it has been shown to possess hepatoprotective, chemoprotective, antimicrobial, antioxidant anti-nociceptive, antimitotic, and anti-inflammatory activities [20]. IC has been used to treat a variety of conditions according to several literary works. There has never been any research on IC’s impact on the male reproductive system. Therefore, this study aimed to investigate the protective effects of HEICL against CP-induced testicular damage in albino rats.

2. Material and methods

2.1. Plant material

Fresh leaves of IC were collected from the premises of the Federal University of Agriculture, Makurdi, Benue State, Nigeria. The plant was identified by a taxonomist; a voucher specimen number UAM/FH/237/20 already exists in the College of Forestry herbarium, Federal University of Agriculture, Makurdi, Benue State.

2.2. Preparation of the HEICL

The leaves were washed under running water, air-dried for two (2) weeks at room temperature, and... then pulverized using a mortar and pestle. About 200g of powdered leaves of IC was soaked in a beaker holding 2000ml of aqueous ethanol solvent (80% ethanol). This was stored at room temperature for 48 hours with 2 hourly agitations. After 48 hours, filtration was performed with a clean Muslin cloth and Whatman filter paper no. 1. The filtrate was concentrated in a 45°C water bath. The concentrated HEICL was weighed to determine the yield and kept in a refrigerator at 40°C until needed.

2.3. Animals Treatment

Thirty (30) male Wistar rats were acquired from the Animal House at Benue State University’s College of Medicine in Makurdi and housed under regular environmental conditions (24-25°C, 12h/12h light/dark cycle) and fed a pellet diet. Water was freely accessible. Before the investigation, they were acclimatized for two (2) weeks. The experimental protocol followed the National Institutes of Health guidelines for the care and welfare of research animals [21] and was approved by the Ethics Committee of the Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture, Makurdi, Benue State, Nigeria. The rats were handled according to standard protocols for using laboratory animals in research.

2.4. Drugs and Dosage

Cisplatin (CP) (Unistin 50 mL/50 mg Vial Eimc United Pharmaceutical Badr City. Cairo, Egypt): A single dose (10 mg/kg) of CP was injected intraperitoneally (IP). This dose is widely accepted to induce testicular toxicity in rats.

2.5. Animals and Treatment

The rats were randomly divided into 5 groups of 6 animals each as follows:

- Control group (A) was given saline injection intraperitoneally (IP) for 26-days.
- Group (B) was given normal saline (IP) for 20 days and a single injection of CP (10 mg/kg) on day 21.
- Group C rats received 200mg/kg of HEICL orally for 28 days.
- Group D rats received 400mg/kg body weight of HEICL orally for 28 days.
• Group E rats received 200mg/kg of HEICL orally for 20 days and CP was given (i.p) on the 21st day
• Group F received 400mg/kg of HEICL orally for 20 days and CP was given (i.p) on the 21st day.

2.6. Animal sacrifice and organ collection
On day 27, the rats were fasted overnight, individually weighed, and their ultimate weights were recorded. On day 28, the rats were killed with sodium pentobarbital (100 mg/kg i.p.) and sacrificed by cervical dislocation: The testes, epididymides, and accessory sex organs (seminal vesicles, vas deference, and prostate glands) were removed and dissected, and the index weight (I.W) of the excised organs was computed as follows: 

\[ I.W = \frac{\text{organ weight (g)}}{\text{body weight (g)}} \times 100 \]

and the average value obtained for each paired organ was considered one observation, with values, reported as g/100 g body weight. Each animal’s testis was fixed in Bouin’s fluid for histological examination.

2.7. Semen analysis

2.7.1. Sperm motility
Determination of cauda epididymal sperm motility was done using the method described by [22]. The individual motility was determined by the formula:

\[ \text{Motility (individual) (%) } = \frac{\text{Number of motile sperm}}{\text{Total number of sperm (motile+immobile)}} \times 100 \]

2.7.2. Sperm concentration
Sperm count was determined using an improved Neubauer hemocytometer by the method described by [23, 24]. Epididymal spermatozoa were obtained by the invasive opening of the cauda epididymis and released into a sterile universal specimen bottle, containing 1 ml of normal saline. Briefly, 5 µl of epididymal fluid was delivered onto a glass slide covered with a 22×22 mm coverslip and examined under the light microscope at a magnification of ×400. The microscopic field was scanned systematically and each spermatozoon encountered was assessed.

2.7.3. Sperm viability test
The viability (percentage of live spermatozoa was determined using an eosin nigrosin stain as described by [23, 25].

\[ \text{Viability (%) } = \frac{\text{Number of viable sperm}}{\text{Total number of sperm (viable+non-viable)}} \times 100 \]

2.7.4. Determination of acrosome integrity
The sperm acrosome integrity was determined by the method described by [26]. Acrosome integrity was determined by placing a drop (100 µL) of sperm sample on a clean, grease-free slide and mixed with a single drop of Giemsa stain. The spermatozoa were allowed to interact with the stain for at least 2 min and then a smear was prepared. The prepared smear was air-dried and examined under an oil immersion objective (100× magnification) to determine the percentage of spermatozoa with intact acrosomes.

The spermatozoa that pick the Eosin-Nigrosine stain means Acrosome integrity is compromised or dead. The spermatozoa with intact Acrosome integrity do not pick the stain. The mean results were expressed as per cent intact acrosomes.

2.7.5. Sperm morphologies
Sperm morphology was determined by examining air-dried slides under oil immersion as described by [23]. The sperm cells were scored as follows:

Normal morphology: sperms with normal head and tail. Abnormal morphology: sperm cells with isolated heads – misshapen head or not; head misshapen head with abnormal tail and fused sperm. The percentage of abnormal forms was evaluated; Normal semen has fewer than 30% of abnormal forms [27].

2.8. Histopathological analysis:
The testicular tissues were fixed in 10% neutral buffered formalin for 48 hrs, dehydrated with different concentrations of ethanol, cleared with xylene, and fixed in paraffin. Finally, 4 µm thick sections were prepared and stained with hematoxylin and eosin.
2.9. Statistical analysis

The results were expressed as Mean ± SEM. All the data was subjected to Tuckey's test after a one-way analysis of variance (ANOVA). Graph pad prism 8.01 software was used for comparing various groups. The significant level was adjusted at P<0.05.

3. Results

Table 1: Protective effect of oral administration of HEICL and/or Quercetin for 26 days on body weight of Male Wistar rats treated with CP

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial week (g)</th>
<th>2nd week (g)</th>
<th>3rd week (g)</th>
<th>Final week (g)</th>
<th>Weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dst. Water</td>
<td>156.3±3.18</td>
<td>164.3±0.67</td>
<td>168.7±0.89</td>
<td>170.7±0.88</td>
<td>14.00</td>
</tr>
<tr>
<td>CP</td>
<td>151.0±1.00</td>
<td>162.0±1.16</td>
<td>166.7±0.89</td>
<td>148.3±0.88</td>
<td>3.00</td>
</tr>
<tr>
<td>200mg/kg HEICL</td>
<td>161.0±4.51</td>
<td>165.0±0.58</td>
<td>167.7±0.33</td>
<td>169.3±0.88</td>
<td>8.00</td>
</tr>
<tr>
<td>400mg/kg HEICL</td>
<td>157.7±1.86</td>
<td>161.7±1.67</td>
<td>166.3±0.89</td>
<td>167.7±1.45</td>
<td>10.00</td>
</tr>
<tr>
<td>CP+200mg/kg HEICL</td>
<td>162.7±1.45</td>
<td>164.3±0.67</td>
<td>167.3±0.33</td>
<td>157.3±0.33</td>
<td>5.40</td>
</tr>
<tr>
<td>CP+400mg/kg HEICL</td>
<td>163.0±1.53</td>
<td>163.0±0.58</td>
<td>168.0±0.58</td>
<td>159.0±0.58</td>
<td>7.00</td>
</tr>
</tbody>
</table>

Note: The values are presented as Mean ± SEM (n=6); a Significant differences as compared to normal control (Dst. Water) P<0.05; b Significant differences as compared to positive control (CP) P<0.05; Dst. Water= Distilled water, CP=Cisplatin, HEICL= hydroethanolic extract of *Ixora coccinea* leaf

Accordingly, the loss in body weight of the rats was greater in the groups administered CP and CP+ HEICL) then in the control group. There was a significant (p < 0.05) decrease in body weight in the group treated with CP only when compared with the controls, however, there was an increase in the body weights in the groups co-administered CP + HEICL in a dose-dependent manner.

Table 2: Protective effect of oral administration of HEICL for 26 days on the relative organ weights of male Wistar rats treated with CP

<table>
<thead>
<tr>
<th>Doses (mg/Kg)</th>
<th>Organs Weight (g)</th>
<th>Testes (Lt)</th>
<th>Testes (rt)</th>
<th>Sem Ves</th>
<th>Epid</th>
<th>Vas def</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dst. Water</td>
<td></td>
<td>1.38±0.09</td>
<td>1.64±0.05</td>
<td>0.58±0.04</td>
<td>0.59±0.03</td>
<td>0.06±0.00</td>
<td>0.12±0.00</td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td>0.93±0.05</td>
<td>0.96±0.02</td>
<td>0.33±0.01</td>
<td>0.26±0.01</td>
<td>0.03±0.01</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>200mg/kg HEICL</td>
<td></td>
<td>1.59±0.01</td>
<td>1.45±0.02</td>
<td>0.65±0.27</td>
<td>0.48±0.03</td>
<td>0.04±0.00</td>
<td>0.06±0.00</td>
</tr>
<tr>
<td>400mg/kg HEICL</td>
<td></td>
<td>1.39±0.01</td>
<td>1.38±0.03</td>
<td>0.58±0.40</td>
<td>0.41±0.01</td>
<td>0.04±0.00</td>
<td>0.08±0.00</td>
</tr>
<tr>
<td>CP+200mg/kg HEICL</td>
<td></td>
<td>1.01±0.01</td>
<td>1.11±0.03</td>
<td>0.41±0.12</td>
<td>1.27±0.08</td>
<td>0.70±0.01</td>
<td>0.07±0.00</td>
</tr>
<tr>
<td>CP+400mg/kg HEICL</td>
<td></td>
<td>1.03±0.02</td>
<td>1.27±0.08</td>
<td>0.50±0.26</td>
<td>0.28±0.01</td>
<td>0.04±0.00</td>
<td>0.07±0.00</td>
</tr>
</tbody>
</table>

Note: The values are presented as Mean ± SEM (n=6) a Significant differences as compared to normal control (Dst. Water) P<0.05, b Significant differences as compared to positive control (CP) P<0.05. Dst. Water= Distilled water, CP=Cisplatin, HEICL= Hydroethanolic extract of *Ixora coccinea* leaf, lt= left, rt= right, Vas def= vas deference, epid= epididymis, sem ves= seminal vesicle

Treatment of rats with HEICL alone at graded doses did not affect the parameters studied as compared to the control value. Administration of CP alone caused a significant decrease in sperm motility, and sperm concentration, and a significant increase in the percentage of abnormal sperm and acrosomal integrity (dead) when compared to the control group. Pre-treatment of the rats with HEICL for 21 days before CP treatment significantly showed an increase in the percentage motility (Table 3).
Table 3 Protective effects of oral administration of HEICL and/or Quercetin for 26 days on sperm parameters of CP-treated male Wistar rats

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dst. Water</td>
<td>93.67±1.45</td>
<td>94.00±1.16</td>
<td>124.3±0.88</td>
<td>101.7±1.20</td>
<td>19.67±0.88</td>
<td>110.7±1.45</td>
<td>26.33±1.20</td>
</tr>
<tr>
<td>CP</td>
<td>32.00±1.15</td>
<td>32.00±1.16</td>
<td>72.67±1.45</td>
<td>32.00±1.16</td>
<td>116.3±1.45</td>
<td>16.67±0.33</td>
<td>84.67±1.45</td>
</tr>
<tr>
<td>200mg/kg HEICL</td>
<td>98.67±0.33</td>
<td>98.67±0.33</td>
<td>129.3±1.20</td>
<td>112.7±1.20</td>
<td>21.67±1.20</td>
<td>117.3±2.03</td>
<td>30.67±0.67</td>
</tr>
<tr>
<td>400mg/kg HEICL</td>
<td>96.33±1.20</td>
<td>96.33±1.20</td>
<td>139.7±1.45</td>
<td>108.0±1.16</td>
<td>20.00±1.16</td>
<td>127.3±1.20</td>
<td>28.33±1.33</td>
</tr>
<tr>
<td>CP+200mg/kg HEICL</td>
<td>64.00±2.08</td>
<td>64.00±2.08</td>
<td>81.00±1.16</td>
<td>86.67±1.45</td>
<td>36.33±0.88</td>
<td>113.3±0.88</td>
<td>42.67±1.33</td>
</tr>
<tr>
<td>CP+400mg/kg HEICL</td>
<td>70.33±0.88</td>
<td>70.33±0.88</td>
<td>93.67±2.03</td>
<td>87.33±0.67</td>
<td>24.67±7.36</td>
<td>114.0±2.31</td>
<td>40.33±0.88</td>
</tr>
</tbody>
</table>

TABLE 3. Effect of oral administration of HEICL and CP for 26 days on sperm parameters; Note: The values are presented as Mean ± SEM (n=6). a Significant differences as compared to normal control (Dst. Water) P<0.05; b Significant differences as compared to positive control (CP) P<0.05;

Figure 1 Light micrographs of testicular tissues of rats treated with HEICL and CP

(a) Photomicrograph of the testicular tissue of the control group showing healthy seminiferous tubules at all stages of spermatogenic cells (primary spermatocyte “blue arrow” and spermatozoa “blue arrow”) and the interstitial cells with Leydig cells (black star) filling the space between the seminiferous tubules. (b) Photomicrograph of the testicular tissue of rats treated with CP showing degenerative alterations (blue star) in spermatogenic cells and the detachment of the spermatogenic epithelium. (c) Photomicrograph of the testicular tissue of rats treated with HEICL alone showing a healthy histological structure with seminiferous tubules (dark star), spermatogonia “blue arrow, and spermatozoa (red arrow) (d) Photomicrograph of the testicular tissue of rats treated with HEICL alone showing a healthy histological structure with seminiferous tubules (the dark star) and primary spermatocyte “blue arrow(e) Photomicrograph of the testicular tissue of rats treated with HEICL and CP showing recovery of the testicular tissues including the seminiferous tubules (dark star) and the spermatid (red arrow), and the spermatogonia (blue arrow) (f) Photomicrograph of the testicular tissue of rats treated with HEICL and CP showing recovery of the spermatogenic epithelium (red arrow) in
most seminiferous tubules (dark star) and the spermatid (red arrow). Sections were stained with hematoxylin and eosin (400x).

4. Discussion

Drug-induced reproductive toxicity in the testicular tissues is now one of the areas of concern in toxicology due to the very sensitive cellular composition of the testicular epithelium and the high rate of mitotic activity of the testes. Our interest in Cp’s reproductive toxicity stems from discoveries that the testis is extremely susceptible to Cp, causing severe and permanent testicular damage [28]. The current study findings agree that CP therapy is extremely lethal to male reproductive organs, while HEICL displayed cytoprotective effects in rat testicular tissues. In this investigation, Cp therapy dramatically caused reduced body weight in the treated rats as compared to the control and the groups administered HEICL only. The weight loss might be due to Cp-induced gastrointestinal damage [29]. Cp is extremely emetogenic, causing severe gastrointestinal disturbances such as nausea, anorexia, diarrhoea, and malabsorption, which can lead to weight loss [29, 30]. However, the co-administration of CP and HEICL had a considerable increase in body weight, which may indicate that the HEICL pre-treatment reversed the cachexic effect caused by the Cp. Our study reveals how HEICL decreases Cp-induced cachexia by limiting weight loss, and this has important therapeutic implications. To the best of our knowledge, this is the first investigation into HEICL’s potential to prevent Cp-induced weight loss carried out.

When CP was given to male rats, their testicular and accessory organ weight, two reliable indicators of gonadal toxicity, were significantly decreased, consistent with earlier findings [31, 32]. Previous studies have shown that Cp has a deleterious effect on a variety of reproductive variables, including the weight of the testicles and other accessory sex organs [33]. Most likely, aberrant reactive oxygen species (ROS) production in the testicular tissues is what causes this [34, 35]. The decline in the weight of the reproductive organs following Cp administration was allayed by the pre-treatment with HEICL at 200 mg/kg and 400 mg/kg in groups 5 and 6 respectively. The significant reduction in reproductive organs’ weight in the present study may be a result of inhibited steroidogenesis and spermatogenesis. Since a significant portion of the testicular weight is determined by the amount of differentiated spermatogenic cells, a decrease in testicular weight would seem to imply damage to the germ cells [36].

Spermatogenesis is a key biomarker of chemical toxicity in male reproduction in mammals ([37]. In the present study, it was observed that the exposure to Cp caused a significant (p<0.05) increase in sperm abnormalities (decreased epididymal sperm count, concentration, motility, and dramatically increased aberrant sperm morphology and this aligns …with the findings of [32]. The reduced sperm concentration, motility, and normal sperm morphology in CP-treated rats could be attributed to lipid peroxidation of unsaturated fatty acids in the sperm plasma membrane, which results in a loss of fluidity and function [38]. Consequently, spermatogenic cell death may be the cause of the decrease in epididymal sperm count seen in rats treated with Cp. Sperm motility is a crucial functional indicator of the ability of sperm to fertilize eggs [39]. Any deleterious effect on sperm motility would significantly influence fertility [40]. The toxic impact of Cp on the flagellum, a critical component of the motility mechanism, and/or on sperm cells may be a reason for the marked reduction in sperm motility [41]. Low sperm concentrations and sluggish or immobile sperm are less likely to pass through the cervical mucosa. Therefore, the poor fertilization of the ova causes sterility [42].

Moreover, spermatozoa recovered from the cauda epididymis of rats in groups 5 and 6 pre-treated with HEICL at doses of 200 and 400 mg/kg, respectively, exhibited a significant (p<0.05) increase in epididymal sperm concentration, motility, and a high percentage of normal sperm with intact head, body, and tail. The current study’s findings indicate that HEICL pre-treatment in rats has the potential to increase their reproductive fitness. Excitingly, treatment with HEICL alone at doses of 200 mg/kg and 400 mg/kg boosted sperm count, concentration, and motility and with a high percentage of normal sperm morphology and the sperm cells capable of forwarding movement required for fertilization with no abnormalities in flagella substructures.

The histological section of the testicular tissues confirmed the reproductive toxicity of CP. Testicular sections from groups (A, C, and D), showed an abundance of seminiferous tubules that were evenly spaced with all of the spermatogenic sequence’s cells and interstitial spaces that were essentially normal (slides A, C, and D). On the contrary, rats exposed to CP (group 2) revealed marked disruption of the normal architecture of the testes, including testicular atrophy, sloughing, and degenerative changes in the seminiferous tubules. The groups (E and F) pre-treated with HEICL showed an improvement in the Cp-induced harmful effects in the testicular tissues, which was an interesting finding. The ability of HEICL pre-treatment to preserve structurally and functionally active seminiferous tubules somewhat similar to that of the control revealed their protective effects on the morphology of testicular cells.
Oral administration of HEICL at 200 and 400mg per kg body weight for 26 days has an inhibitory effects against testicular damage induced by CP. This study reveals that pre-treatment with HEICL protected against CP-induced testicular toxicity.

5. Conclusion

This study shows that CP treatment has a deleterious impact on the semen quality, testes, and accessory sex organs (epididymis, prostate, and seminal vesicles). But HEICL pre-treatment played a beneficial role in CP-induced such prior. As a result, HEICL may be thought of as a suitable supplemental substance for individuals receiving CP or other antineoplastic medications. This offers a low-cost defensive tactic for treating chemotherapy-induced organ damage. But more research is required to determine how HEICL affects CP’s anti-cancer activity.

Compliance with ethical standards

Acknowledgements

The authors acknowledge the technical assistance of Mr. Daniel Wisdom Ochanya of the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Federal University of Agriculture, Makurdi.

Disclosure of conflict of interest

Each author has read the document and given their consent to transmit it to this journal. According to all of the authors, the research was conducted without any commercial or financial ties that would have given rise to a conflict of interest.

Statement of ethical approval

All procedures performed in experiments involving experimental animals were approved by the Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

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