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THE HEPATO-RENAL EFFECT OF *AGERATUM CONYZOIDES* AQUEOUS LEAF EXTRACT ON PARACETAMOL-INDUCED TOXICITY IN WISTAR RATS

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Abstract

Herbal preparations from medicinal plants offer an effective remedy to some liver injuries due to their rich phytonutrients. Herbal preparations are made from herbal drugs, such as whole plant, plant parts, algae, fungi, lichen, exudates, in a crude state in dried or fresh form and extracts with the help of different processes such as infusion, decoction, maceration, distillation, expression, fractionation, purification, concentration, fermentation. These herbal preparations include whole plant or parts, comminuted, or powdered herbal drugs, tinctures and extracts, fatty oils, essential oils, expressed juices and processed exudates of herbal materials. This study aimed to investigate the protective potential of aqueous leaf extracts of *Ageratum conyzoides* on activities of specific biomarkers for liver and kidney damage like ALT, AST, Creatinine, and Urea in control and treated animals. An overdose of 1000mg/kg paracetamol significantly ($p<0.05$) increases the activity of ALT, AST, and albumin, levels of total protein, total bilirubin, urea, creatinine, and bicarbonate respectively, whereas the activity of ALP and levels of potassium and sodium were significantly ($p<0.05$) reduced as compared to normal. Of the three experimental drug concentrations, an exposure with 600mg/kg aqueous extract of A. conyzoides yielded the best results as it restored the values of ALT, AST, total bilirubin, urea, creatinine, and bicarbonate compared to control whereas ALP, total protein, albumin, potassium, and sodium levels are not restored by administration of plant extract. It is evident from observations that aqueous extract of A. conyzoides steadily repaired injured hepatic tissues and impaired renal function caused by paracetamol.

Keywords: Ageratum conyzoides, antioxidants, hepato-renal damage, paracetamol, silymarin.

1. Intoduction

Paracetamol is a widely used over-the-counter pain reliever and fever reducer. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In therapeutic doses, paracetamol is usually well tolerated; side effects and interaction with other drugs are usually not observed. Albeit, paracetamol has a narrow therapeutic index which means that its

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therapeutic dose is close to overdose, making it relatively dangerous substance, particularly for people who self-medicate. A single dose of 10 to 15 grams can potentially cause major hepatotoxicity in adult human beings [1]. An overdose of paracetamol can lead to severe liver damage, a well-documented phenomenon with significant public health implications [2]. Although overdoses from prescribed paracetamol are rare, its widespread availability over-the-counter has contributed to cases of toxicity due to self-medication. In animal studies, paracetamol overdose has been associated with acute centrilobular hepatic necrosis, underscoring its hepatotoxic potential [11, 15]. Reactive Oxygen Species (ROS) generation is implicated in various disease conditions, highlighting the importance of antioxidants in mitigating oxidative stress-related damage [13, 14]. These antioxidants are naturally abundant in edible plants, including vegetables, fruits, and medicinal herbs [9]. Ageratum conyzoides, [Fig.1]]a widely utilized medicinal plant in traditional medicine, possesses bioactive compounds of interest to the pharmaceutical industry [5, 10]. Research on white-flowered *Ageratum conyzoides* has demonstrated notable anti-inflammatory effects, particularly attributed to its flavonoid fraction [8]. Additionally, in vitro studies have shown that *Ageratum conyzoides* extract exhibits concentration-dependent scavenging of 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radicals.

Fig. 1. Different organs of *Ageratum conyzoides*: flowers of white flowered (a), flowers of purple flowered (b), flowers of white–purple flowered (c), leaves of white flowered (d), leaves of purple flowered (e), and leaves of white–purple flowered (f) plants.

The accessibility of paracetamol over-the-counter and its potential for self-medication-induced overdose underscore the need for effective therapeutic interventions. This study aims to investigate the protective properties of *Ageratum conyzoides* leaf extract (ACE) against acute paracetamol overdose. By elucidating the bioactive components of ACE, this research seeks to identify potential therapeutic strategies for paracetamol-induced liver damage and associated health conditions. The experimental approach outlined aims to advance scientific understanding of *Ageratum conyzoides* as a promising therapeutic agent against paracetamol-induced liver damage, with implications for future clinical applications and natural remedy development. This investigation contributes to the growing body of research on natural remedies for managing drug-induced toxicity and promoting health through herbal interventions. The findings may inform the development of novel treatments that harness the medicinal properties of Ageratum conyzoides, offering new avenues for addressing drug-related health challenges..

2. Materials and methods

Sample collection and preparation: *Ageratum conyzoides* was collected from an abandoned farmland located at the back of the ETF building, Abuja campus, University of Port Harcourt, Nigeria. The plant parts were identified and authenticated by a taxonomist from the Plant Science and Biotechnology Department at the University of Port Harcourt. The leaves were carefully separated and air-dried for 7 days to preserve their botanical properties. Subsequently, the dried leaves were finely ground into a powder using an electric grinder, resulting in a total weight of 319.25 grams. Next, the powdered sample was macerated in distilled water for 72 hours to extract its bioactive constituents. Following maceration, the extract was filtered to remove particulate matter, yielding an aqueous extract. The aqueous extract was then concentrated using a rotary evaporator to obtain a concentrated solution of the plant material. Finally, the concentrated extract was stored in a refrigerator in preparation for further in vivo investigation of its pharmacological properties and potential therapeutic effects.

Drugs and Chemicals: Paracetamol and Silymarin tablets were obtained from an accredited pharmacy store in Port Harcourt, Nigeria. All other chemicals used in the experiment were of analytical grade and sourced from Sigma Aldrich Chemical Company in the USA.

Experimental Protocol: A total of 27 Wistar strain rats, weighing between 95 to 180g, were acquired from Mark Bam Ltd. Rats of similar weights were grouped together, with groups 1, 2, and 3 consisting of five rats each, while groups 4, 5, and 6 had four rats each. The rats were administered paracetamol and plant concentrate through oral gavage. The treatment groups were as follows:

Group 1 (Normal Control): Rats received only feed and water.

Group 2 (Overdose Control): Rats received feed, water, and 1000mg/kg paracetamol.

Group 3 (Drug Control): Rats received the same as group 2 plus 25mg/kg silymarin.

Groups 4, 5, and 6: Rats received the same as Group 2 plus 200mg/kg, 400mg/kg, and 600mg/kg of ACE extract respectively.

After 21 days of treatment, rats were deprived of food and water for at least 18 hours, then weighed and sedated. Blood samples were collected from the jugular vein and placed in lithium heparin bottles for chemistry tests. The experiments were conducted following the guidelines of the ethics committee of the Sciences Faculty at the University of Port Harcourt, Nigeria.

Assay Procedure: The collected blood samples underwent centrifugation at 2800 rpm for 18 minutes. Plasma was isolated, transferred to clean test tubes, and stored at very low temperatures. Activities of alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT), and levels of total

protein, albumin, creatinine, and bilirubin in serum were determined using a chemistry analyzer. Albumin levels were calculated as 60% of the total protein concentration.
Statistical Analysis: The results were presented as mean ± standard deviation (n=3). Statistical significance was determined at $p<0.05$.

3. Results and discussion

Tables 1 and 2 illustrate the protective effects of *Ageratum conyzoides* extract (ACE) on specific biomarkers related to liver and kidney damage in both control and treated animals. An overdose of 1000mg/kg paracetamol significantly increased the activity of ALT, AST, and albumin from 17.33±5.33 u/l, 10.00±1.73 u/l, and 17.09±0.43 g/dl to 25.00±4.00 u/l, 12.00±1.00 u/l, and 18.77±0.04 g/dl, respectively [Fig.2, 3]. Additionally, levels of total protein, total bilirubin, urea, creatinine, and bicarbonate increased from 28.50±0.72 g/100 ml, 8.20±0.61 mg/dl, 13.60±0.06, 9.15±0.48 µmol/l, and 34.22±0.99 mmol/l to 31.29±0.06 g/100 ml, 10.23±0.37 mg/dl, 18.01±0.17, 13.03±2.37 µmol/l, and 37.03±0.51 mmol/l, respectively. Conversely, the activity of ALP and levels of potassium and sodium decreased significantly compared to the normal control group. The observed alterations in serum ALT, AST, bilirubin, and electrolyte levels confirmed the successful induction of hepato-renal damage [3, 6, 7, Rao et al., 1973). Among the experimental drug concentrations, exposure to 600 mg/kg of the aqueous extract of A. conyzoides yielded the most promising results, restoring ALT to 16.33±4.33 u/l, AST to 10.00±1.73 u/l, total bilirubin to 2.09±0.16 mg/dl, urea to 9.11±0.12, creatinine to 12.47±1.92 µmol/l, and bicarbonate to 33.41±0.58 mmol/l compared to the control treated with silymarin. However, ALP, total protein, albumin, potassium, and sodium levels were not fully restored by the administration of the plant extract. Therefore, the anti-inflammatory action of ACE in this study exhibited a dose-dependent pattern [8].

Table 1. Effects of ACE on liver function biomarkers in Wistar albino rats

Treatment	AALT (u/l)	AAST (u/l)	AALP (u/l)	Total protein (g/100ml)	Albumin (g/dl)
Group 1	117.33^{a} ±5.33	110.00^{a}±1.73	669.07^{a}±2.19	228.50^{a}±0.72	117.09^{a}±0.43
Group 2	225.00^{b}±4.00	112.00^{b}±1.00	553.82^{b}±0.35	331.29^{b}±0.06	118.77^{b}±0.04
Group 3	116.67^{a}±2.60	115.00^{c}±1.00	440.87^{c}±0.51	113.62^{d}±0.52	88.17^{d}±0.31
Group 4	115.33^{a}±1.67	99.00^{a}±1.00	664.82^{a}±1.00	336.42^{b}±0.44	221.85^{c}±0.27
Group 5	116.33^{a}±4.33	226.33^{d}±9.67	776.62^{a}±1.13	337.49^{b}±0.06	222.49^{c}±0.04
Group 6	116.33^{a}±4.33	110.00^{a}±1.73	330.17^{d}±0.58	442.62^{c}±1.52	225.57^{c}±0.91

*Values are given as mean ± SD of animals in each group. Values with different superscripts (a, b, c, d) in a column statistically differ significantly $p < 0.05$. (ACE - *Ageratum conyzoides* leaf extract)

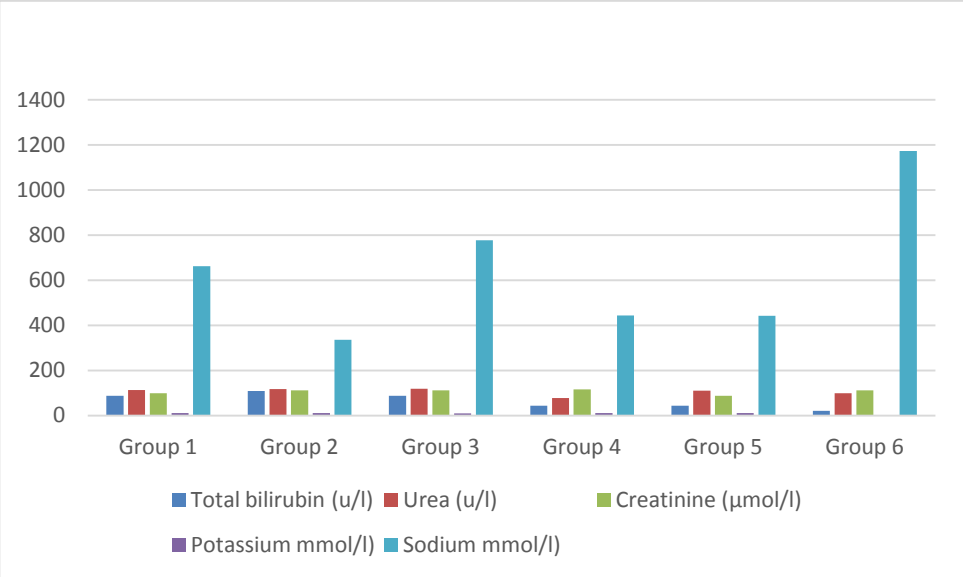

Fig.2. Effects of ACE on liver function biomarkers in Wistar albino rats

Table 2. Effects of ACE on kidney function biomarkers in Wistar albino rats

Treatment	Total bilirubin (u/l)	Urea	Creatinine (µmol/l)	Potassium (mmol/l)	Sodium (mmol/l)	Bicarbonate (mmol/l)
Group 1	88.20^{a}±0.61	113.60^{a}±0.06	99.15^{a}±0.48	11.70^{a}±0.13	662.94^{a}±17.88	334.22^{a}±0.99
Group 2	110.23^{b}±0.37	118.01^{b}±0.17	113.03^{b}±2.37	11.52^{b}±0.18	337.36^{c}±4.32	337.03^{c}±0.51
Group 3	88.69^{a}±0.91	119.12^{b}±0.10	111.64^{a}±0.48	11.09^{c}±0.06	776.92^{b}±2.91	331.74^{b}±0.66
Group 4	44.50^{c}±0.43	77.81^{d}±0.05	117.19^{d}±0.73	11.66^{a}±0.05	444.27^{c}±10.09	224.53^{d}±1.51
Group 5	44.50^{c}±0.73	110.71^{c}±0.22	88.60^{a}±2.38	11.53^{b}±0.16	443.13^{c}±61.21	224.30^{d}±1.39
Group 6	22.09^{c}±0.16	99.11^{c}±0.12	112.47^{a}±1.92	00.91^{c}±0.09	1173.07^{d}±1.2	333.41^{a}±0.58

*Values are given as mean ± SD of animals in each group. Values with different superscripts (a, b, c, d) in a column statistically differ significantly $p < 0.05$. (ACE - Ageratum conyzoides leaf extract)

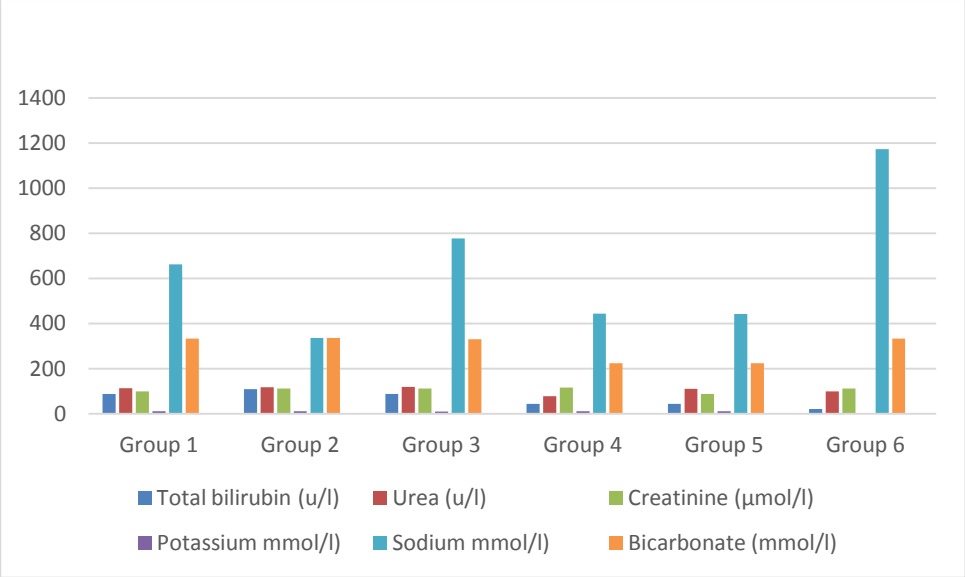

Fig. 3. Effects of ACE on kidney function biomarkers in Wistar albino rats

Qualitative phytochemical screening of ACE revealed high concentration of flavonoids, alkaloids, and tannins while terpenoids, cardiac glycosides and steroids were also present. Qualitative phytochemical screening of *Ageratum conyzoides* leaf extract (ACE) unveiled a rich composition of bioactive compounds, highlighting its potential therapeutic value. The presence of flavonoids, alkaloids, and tannins in high concentrations suggests diverse pharmacological activities such as antioxidant, anti-inflammatory, and antimicrobial properties. Flavonoids are known for their antioxidant effects, which protect cells from oxidative stress and inflammation. Alkaloids exhibit various physiological effects, including analgesic and antimicrobial properties, while tannins contribute to wound healing and possess antimicrobial actions. Additionally, the detection of terpenoids, cardiac glycosides, and steroids in ACE indicates the extract's potential in cardiovascular health and hormone regulation. Terpenoids are associated with anti-inflammatory and anticancer properties, whereas cardiac glycosides can impact heart function positively. Steroids, known for their hormonal effects, may contribute to the overall pharmacological profile of ACE. The comprehensive phytochemical profile of ACE underscores its potential as a natural remedy for various ailments, offering a promising avenue for further research into its therapeutic applications. The synergistic effects of these bioactive compounds warrant exploration into the mechanisms underlying ACE's health-promoting effects, positioning it as a valuable candidate in drug discovery and development. Further studies could elucidate the specific bioactivities and molecular targets of these phytochemicals, paving the way for novel therapeutic interventions based on *Ageratum conyzoides.*

Administration of the aqueous leaf extract of *Ageratum conyzoides* demonstrated significant restoration in the levels of Alanine transaminase, Aspartate transaminase, total bilirubin, urea, creatinine, and bicarbonate compared to the control treated with silymarin. The observations suggest that the aqueous extract of A. conyzoides effectively repaired injured hepatic tissues and restored impaired renal function caused by paracetamol. Therefore, the extract exhibits not only hepatoprotective but also renal-protective properties. Additionally, the effects were dose-dependent, indicating that *Ageratum conyzoides* could be a potent treatment option for paracetamol-induced liver injuries, offering promising therapeutic benefits. The restoration of these liver and kidney markers by *Ageratum conyzoides* highlights its potential as a natural remedy for drug-induced organ damage. The dose-dependent response underscores the importance of

optimizing dosage for therapeutic efficacy. Further investigations could explore the underlying mechanisms of action, such as antioxidant or anti-inflammatory properties, to elucidate the full potential of A. conyzoides in hepatorenal protection. Additionally, studies assessing long-term safety and efficacy in diverse experimental models would provide valuable insights into the translational potential of this herbal remedy for liver and kidney disorders.

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